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Effects of ensiling time on corn silage starch ruminal degradability evaluated in situ or in vitro

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ABSTRACT

Accurate measurements of concentration and ruminal degradability of corn silage starch is necessary for formulation of diets that meet the energy requirements of dairy cows. Five corn silage hybrids ensiled for 0 (unfermented), 30, 60, 120, and 150 d were used to determine the effects of ensiling time on starch degradability of corn silage. In addition, the effects of grind size of silage samples on 7-h in vitro starch degradability and the relationship between in vitro, in situ and near-infrared reflectance spectroscopy (NIRS) starch degradability were studied. In situ disappearance of corn silage starch increased from 0 to 150 d of ensiling, primarily as a result of an increase in the washout or rapidly degraded fraction of starch, particularly during the first 60 d of ensiling. When analyzed in vitro and by NIRS, ensiling time increased corn silage starch degradability either linearly or to a greater extent during the first 2 mo of ensiling. Differences in in situ starch disappearance among corn silage hybrids were apparent during the first 2 mo of ensiling but were attenuated as silages aged. No differences among hybrids were detected using a 7-h in vitro starch digestibility approach. Results from the in vitro subexperiment indicate that 7-h in vitro starch degradability was increased by reducing grind size of corn silage from 4 to 1 mm, regardless of ensiling duration. Fine grinding corn silages samples (i.e., 1-mm sieve) allowed distinguishing low- from medium- and high-starch degradability rated hybrids. Correlations among in situ, in vitro and NIRS measurements for starch degradability were medium to high ($r \geq 0.57$); however, agreement among methods was low (concordance correlation coefficient ≤ 0.15). In conclusion, ensiling time linearly increased degradation rate of corn silage resulting in greater in situ starch disappearance after 150 d of ensiling. Reductions in grind size from 4 to 1 mm resulted in greater in vitro starch degradability, regardless of ensiling duration. Strong correlation but low agreement between starch degradability methods suggest that absolute estimations of corn silage starch degradability will vary, but all methods can be used to assess the effect of ensiling time on starch degradability.
Key words: ensiling time, starch degradability, corn silage

INTRODUCTION

Corn silage (**CS**) is the most used forage in dairy diets throughout the United States due to its relatively high biomass yield and energy density when compared with other forage sources (Jordan and Fourdraine, 1993; Wilkinson et al., 2003). Starch is the main carbohydrate in corn grain serving as the major energy source and constitutes 33% of CS (on average) according to the Dairy One database (https://dairyone.com, accessed Mar. 7, 2022). Thus, having accurate measurements of concentration and ruminal degradability of CS starch (**StD**) is essential for diet formulation (Ferraretto et al., 2013). This is important when also considering that forage, including CS, can represent 40% or more of the total NE_L intake in dairy cows (Hristov et al., 2020).

Although starch in corn grain is considered to be highly digestible in ruminants (over 95% total-tract digestibility; Patton et al., 2012; Moharrery et al., 2014), corn starch granules are encapsulated in a hydrophobic protein matrix that represents one of the principal obstacles for ruminal StD (Kotarski et al., 1992; McAllister et al., 1993). Additionally, the pericarp of the corn grain is also resistant to ruminal degradation (Huntington, 1997). Thus, alterations in the physical and morphological structures of the grain are necessary to facilitate microbial attacks to the granules and access of microbial amylolytic enzymes (Offner

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et al., 2003; Giuberti et al., 2014). Previous reports have shown that the protein matrix surrounding starch granules is solubilized during the silage fermentation process (Hoffman et al., 2011; Der Bedrosian et al., 2012; Ferraretto et al., 2015a,b). However, these reports have used in vitro (**IV**) assays to quantify the effect of ensiling time (**ET**) on StD and data on in situ (**IS**) StD as affected by ET are unavailable. It is argued that in vitro assays to determine StD may not accurately emulate the ruminal environment, including fermentation end products removal, thus the preferred method to measure the kinetics of ruminal StD is the IS procedure (Hvelplund and Weisbjerg, 1998). However, the IS technique is not perfect, and shortcomings (lack of chewing, retention time, particle size effects, physical loss of feed particles through the bag pores that may escape ruminal fermentation, and so on) have been vastly discussed (Stern et al., 1997; NRC, 2001). This has prompted in vitro starch degradability (**IVStD**) procedures using ruminal inoculum to be widely used by commercial feed analyses laboratories. Although reports using the IS procedures have shown increased StD in CS when compared with fresh-chopped corn (Philippeau and Michalet-Doreau, 1998; Peyrat et al., 2014), to our knowledge no studies have evaluated changes in StD of CS with continuous increase of ET.

In addition to ET, alterations in ruminal StD can also be prompted by differences in corn hybrid type. Hybrids with more vitreous endosperms are known to have lower ruminal StD (Philippeau and Michalet-Doreau, 1998; Ngonyamo-Majee et al., 2009; Allen and Ying, 2021). Some authors have reported that differences between corn genotypes prevail throughout the ensiling process (Verbič et al., 1995; Ferraretto et al., 2015b), whereas others have observed that differences in StD disappear with ET (Der Bedrosian et al., 2012). This inconsistency suggests that accounting for the interaction of hybrid and ET on StD is necessary to accurately assess the nutritive value of CS.

Recently, Gleason et al. (2022) reported strong agreement (correlation and concordance correlation coefficient >0.85) between IS and IV assays for concentrate feeds commonly included in dairy diets. The use of near-infrared reflectance spectroscopy (**NIRS**) techniques, which predicts nutrient constituents of feedstuffs based on the absorption or reflection of electromagnetic radiation (Garnsworthy et al., 2000), has garnered attention as it allows for more rapid laboratory feed evaluations. Although the NIRS technique has been used to determine rumen degradation kinetics and nutrient digestibility of cereal grains (Krieg et al., 2017, 2018), studies have reported variability in NDF degradability of forages when using NIRS (Bender et al., 2016; Hristov et al., 2020). Despite this, Krieg et al. (2018) reported moderate to high relationship ($R^2 \geq 0.63$) between in situ StD and StD of cereal and pea grains predicted by NIRS. No consensus currently exists on which of the above-mentioned methods is best; thus further evaluation regarding their applicability for analysis of CS is warranted.

Therefore, the main objective of this study was to evaluate the effects of ET on StD of CS hybrids. Secondary objectives were to: (1) evaluate the effects of grind size of CS samples on 7-h IVStD and (2) describe the relationship between IVStD, StD predicted by NIRS, and IS StD of starch in CS samples. Our hypotheses were that: (1) StD of CS would increase with ET, independently of hybrid, (2) reductions in grind size would increase StD but may mask differences among hybrids, and (3) there will be a reasonable agreement in CS StD among evaluation methods (i.e., in situ, in vitro, and NIRS).

MATERIALS AND METHODS

All study procedures involving animals were reviewed and approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Corn Silage Hybrids and Analyses

Five CS hybrids included in the 2014 Pennsylvania State University Commercial Silage Hybrid Corn Test Program (https://extension.psu.edu/2021-results-pa-commercial-grain-and-silage-hybrid-corn-tests-report; accessed Apr. 21, 2022) were used in the study. The CS hybrids were as follows: Hubner H5333RC3P, H6191RCSS, and H5222RC3P (Hubner Seed), Masters Choice MC5250 (Masters Choice), and Healthy Herd Genetics 42HFC15 (Healthy Herd Genetics and Nutrition). Three of the hybrids (H5222RC3P, MC5250, and 42HFC15) were rated (by the seed company) as high in StD, whereas the other 2 (H5333RC3P and H6191RCSS) were rated medium to low in StD, respectively. The corn hybrids were grown in Centre County, Pennsylvania, at The Pennsylvania State University's Russell Larson Research Farm. Each hybrid was grown on 3 separate plots. After harvest, plants were ensiled (in triplicate) in sealed 2.5-kg capacity plastic bags. Silages were stored at room temperature (around 23°C) and moved to a −20°C freezer on d 0 (unfermented), 30, 60, 120, and 150 postensiling and stored frozen for at least 30 d before analysis. Samples of the silages were dried at 55°C for 72 h for calculation for dry matter and were then processed for further analyses as described below.

In Situ Starch Disappearance

Dried CS samples were ground through a 4-mm sieve in a Wiley mill (Thomas Scientific) and assessed for ruminal StD using the IS method. Three ruminally-cannulated (10-cm internal diameter cannulas; Bar Diamond Inc.) lactating Holstein cows (average ± SD: DMI = 26.8 ± 1.0 kg/d; milk yield = 39.8 ± 2.9 kg/d; and DIM = 170 ± 10.8 d) were used for the IS incubations. Cows were fed a standard 60% forage (CS, alfalfa haylage, and grass hay) and 40% concentrate feeds (ground corn grain, whole roasted soybeans, canola meal, and a mineral and vitamin premix) ration. Approximately 7 g of silage DM were weighed into 10 × 20–cm nylon bags with 50 ± 10 μm porosity (ANKOM Technology Corp.) and closed with a ziptie after folding. Duplicated bags were sequentially incubated in each cow for 0 (not incubated in the rumen), 4, 8, 12, 24, and 48 h, and removed simultaneously. The 0-h samples were processed in the same way as the incubated samples, excluding the rumen incubation step. Upon removal from the rumen, bags were rinsed 3 times with cold water in a washing machine set to agitate for 6 min each rinse. Further details on the IS procedure can be found in Harper et al. (2017). Silage samples and IS bag residues were analyzed for starch as described in Hall (2009). Ruminal disappearance was calculated based on initial dry weight of the incubated sample, residue dry weight, and starch concentration of initial sample and bag residue. In situ starch disappearance from the nylon bags incubated in the rumen was assumed to be degraded by rumen microorganisms. Degradation curves were fitted to a single, 3-parameter exponential rise to a maximum model (SigmaPlot 13.0; Systat Software):

$$p = a + b \times [1 - \exp(-c \times t)],$$

where p (%) is the degraded fraction of CS starch at time t, a (%) is the washout or rapidly degraded fraction of starch, b (%) is the potentially degradable fraction, and c (%/h) is the rate of degradation of fraction b (Ørskov and McDonald, 1979); $a + b$ was constrained to ≤100%. The IS effective degradability (**ISED**; %) of starch was determined using the following equation (Ørskov and Mcdonald, 1979):

$$\text{ISED} = a + b \times [c \div (c + k)],$$

where a, b, and c are as specified above, and k is the rate of passage, assumed to be 0.06/h, which is the passage rate estimated for concentrate feeds when DMI is at 4% of BW and the diet is 50% forages (NRC, 2001).

In Vitro Starch Degradability

Aliquots of dried CS samples were submitted to 2 commercial feed analyses laboratories: Rock River Laboratories Inc. (Watertown, WI; **LabA**) and Cumberland Valley Analytical Services (Waynesboro, PA; **LabB**) for determination of starch concentration and degradability. Nutrient composition of the CS was analyzed using NIRS procedures; methods can be found at https://rockriverlab.com/pages/Animal-Nutrition-Analysis.php (LabA; accessed Apr. 21, 2022) and at https://www.foragelab.com/Lab-Services/Forage-and-Feed/NIR (LabB; accessed Apr. 21, 2022). Measurements for 7-h StD by NIRS from LabA are from an IS calibration built upon 6-mm ground, 7-h rumen incubated corn grain, silage and other high cornstarch feeds, whereas NIRS estimations for 7-h StD from LabB are from an IV calibration built upon 4-mm ground, 7-h IV rumen incubated starchy feeds. Furthermore, LabB also analyzed the CS samples for 7-h StD using an IV ruminal incubation procedure (Richards et al., 1995). Separate samples were submitted to the Department of Animal Science, Food and Nutrition (DIANA) at the Università Cattolica del Sacro Cuore, Piacenza, Italy (**LabC**), for analysis of 7-h IVStD according to the method developed by Sveinbjörnsson et al. (2007) and modified by Gallo et al. (2014). Briefly, CS samples (250 mg each) were incubated with 30 mL rumen inoculum in 125-mL glass bottles equipped with rubber stoppers at 39°C for 7-h with continuous agitation (50 rpm). Blanks and internal standards were included, and IVStD was calculated as the ratio between the amounts of starch disappearance occurring after 7-h of incubation, and the amount of starch in the sample before initiating the incubation, after correcting for blanks (Sveinbjörnsson et al., 2007). Following incubation, bottles were submerged into a bath containing ice to cease fermentation and starch in the residues was estimated using a 2-step enzymatic approach (Gallo et al., 2014).

A subexperiment was conducted at LabB to determine the effect of particle size on 7-h IVStD. Aliquots of dried CS samples from 0, 30, and 120 d ET were ground through 4- or 1-mm screens using a Wiley mill (Thomas Scientific) and submitted for analysis of 7-h IVStD by the IV procedure stated in the previous paragraph.

Organic Matter Digestibility Index

The Penn State CS Organic Matter Digestibility Index (**OMDI**) is calculated based on the sum of potentially digestible CP, fatty acids, NDF, and starch and is designed to facilitate CS hybrid selection by dairy farmers and nutritionists. The OMDI is calculated using the following equation: OMD Index (%) = {[(CP − NDF-bound CP) × 0.89] + (fatty acids × 0.75) + (starch × StD ÷ 100) + [(ash-free NDF- lignin) × 30-h NDF degradability ÷ 100)]} ÷ [(CP − NDF-bound CP) + fatty acids + starch + (ash-free NDF − lignin)] × 100 (Penn State Extension, 2022). Crude protein, fatty acids, starch, NDF-bound CP, ash-free NDF and lignin (ash-free) are expressed as % of CS DM; StD, and NDF degradability were obtained from an IV assay from LabB.

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc.). Effects of hybrid and ET on CS nutrient composition and IS starch disappearance and IV starch degradability were analyzed using the GLIMMIX procedure. The model contained hybrid, ET, and hybrid × ET interaction and orthogonal polynomial contrasts were used to evaluate linear and quadratic effects of ET. Nutrient composition data were also analyzed separately using the GLIMMIX procedure to test for the effect of laboratory. The model contained hybrid, ET, and laboratory. Effects of grind size on CS IVStD from LabB were analyzed using the GLIMMIX procedure. The model contained grind size, starch degradability rating of CS hybrid, ET, and ET × grind size and starch degradability rating × grind size interactions. Ensiling time × starch degradability rating interaction was tested in the previous model but was nonsignificant ($P > 0.50$) for all variables and was removed from the final models. The CORR procedure was used to derive Pearson and concordance correlation coefficients (Lin, 1989) for StD data obtained using IS, IV, and NIRS methods. Plotting and curve-fitting of data for figures was performed using SigmaPlot 13.0 (Systat Sofware). Statistical differences were considered at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$ and data are presented as least squares means.

RESULTS AND DISCUSSION

Nutrient Analyses

Chemical changes occurring to the plant material during ensiling are abrupt during the initial days of ensiling and appear to reach a steadier state after a few weeks of fermentation (Kung et al., 2018). Thus, quadratic effects of ET on nutrient composition and degradability of CS observed in the current study were caused by greater rates of change during initial months of ensiling. Dry matter of the CS samples analyzed was on average 36% and was not affected by hybrid or ET (Table 1). Mean starch concentration of the silages tended to be greater ($P = 0.09$) at LabA when compared with LabB (35.2 and 34.4% of DM, respectively). There was no effect of ET on starch concentration, however, H619RCSS had the lowest ($P \leq 0.02$; data not shown) starch when compared with the other CS hybrids used in the experiment. Compared with unfermented CS, CP decreased quadratically ($P \leq 0.001$), being lowest 150 d, and tended to decrease linearly ($P \leq 0.07$) with ET for LabA and LabB, respectively. Similar to starch, H619RCSS had the lowest ($P \leq 0.02$) CP content of the CS hybrids analyzed. Compared with unfermented CS, soluble CP increased quadratically ($P \leq 0.001$) with ET in reports from both commercial laboratories, being highest at 150 d of ensiling. Ammonia-N increased linearly ($P \leq 0.001$) in reports from LabA and quadratically ($P \leq 0.001$) in reports from LabB, being lower at 0 and 30 d of ensiling when compared with fully fermented CS (i.e., 150 d of ET). Concentration of soluble CP was not different among hybrids, but hybrids differed ($P = 0.002$) in ammonia-N concentration for LabA and tended to differ ($P = 0.06$) for LabB. Corn silage samples analyzed in the current experiment had a good overall fermentation profile based on Kung et al. (2018). Ethanol-soluble sugar concentration and pH were greater at 0 and 30 d of ET relative to 150 d silage and quadratically decreased ($P \leq 0.001$) in reports from both commercial laboratories. Compared with CS of 150 d of ET, concentration of lactic acid was lower for unfermented and 30 d CS, but quadratically increased ($P \leq 0.001$) with ET for both commercial laboratories. Furthermore, acetic acid content increased linearly ($P \leq 0.001$) with ET and butyric acid was undetected for all silages for both laboratories. Overall, these data suggest that CS was well preserved for the duration of the experiment. No hybrid × ET interactions were observed for any of the nutrient components reported by the commercial laboratories.

The similar DM concentrations of CS hybrids in the current experiment are indicative of similar maturities at harvest. Overall, starch concentration in the CS used in the current experiment was greater when compared with values in the DairyOne laboratory database for 2014–15 CS (36 vs. 30.7% DM, respectively; https://www.dairyoneservices.com/feedcomposition/; accessed Apr. 21, 2022). A lack of effect of ET on CP content has been previously reported by Young et al. (2012) and Ferraretto et al. (2015b). In contrast, Ferraretto et al. (2015a) reported quadratic increases in CP when silages were ensiled for up to 240 d. The effect of ET on soluble CP and ammonia-N in the current experiment is in agreement with previous reports showing similar changes in these composition variables for high-

Table 1. Effect of hybrid (H)[1] and ensiling time (ET) on corn silage nutrient composition (% of DM or as indicated)

Variable	Days ensiled					SEM	*P*-value[2]	
	0	30	60	120	150		H	ET
DM, % as fed	37.6	35.9	35.5	35.7	35.9	0.01	0.16	0.25
LabA[3]								
CP	7.81	6.98	7.19	7.21	7.13	0.129	0.004	<0.001(Q)
Soluble CP, % CP	49.4	56.2	58.9	62.0	63.8	0.54	0.20	<0.001 (Q)
Starch[4]	34.3	35.9	34.6	35.5	35.6	0.80	0.02	0.54
Ethanol-soluble sugars	5.19	2.77	2.93	2.84	2.91	0.120	0.18	<0.0001(Q)
pH	5.89	5.05	5.09	5.08	5.09	0.028	<0.001	<0.001(Q)
Lactic acid	0.00	3.88	4.24	4.36	4.36	0.105	0.55	<0.001(Q)
Acetic acid	0.17	0.34	0.58	0.61	0.67	0.081	0.44	<0.001 (L)
Ammonia-N	0.04	0.04	0.06	0.06	0.07	0.004	0.002	<0.001(L)
LabB[5]								
CP	7.05	7.08	6.99	6.91	6.79	0.122	0.005	0.07 (L)
Soluble CP, % CP	35.9	51.6	53.9	57.2	59.2	0.55	0.93	<0.001(Q)
Starch[6]	33.5	35.2	34.8	34.5	33.9	0.73	0.007	0.41
Ethanol-soluble sugars	2.86	1.31	1.38	1.47	1.54	0.075	0.44	<0.001(Q)
pH	4.00	3.85	3.85	3.83	3.81	0.012	0.005	<0.001(Q)
Lactic acid	1.65	4.66	4.66	4.77	4.81	0.074	0.11	<0.001(Q)
Acetic acid	0.52	0.69	0.98	1.19	1.25	0.090	0.28	<0.001 (L)
Ammonia-N	0.05	0.09	0.10	0.12	0.0.12	0.025	0.06	<0.001 (Q)

[1]The following hybrids were included in the study: Hubner H5333RC3P, H6191RCSS, and H5222RC3P (Hubner Seed); Masters Choice MC 5250 (Masters Choice); and Healthy Herd Genetics 42HFC15 (Healthy Herd Genetics and Nutrition).

[2]Effects of corn silage H and ET (L = linear; Q = quadratic). No H × ET interaction occurred for any of the variables ($P \geq 0.08$); largest SEM presented; n = 74.

[3]LabA = Rock River Laboratories Inc. (Watertown, WI). Analyses were from LabA's Comprehensive Nutrition package.

[4]Starch concentration (% of DM) of the corn silage H, across ET, averaged [mean ± SE; near-infrared reflectance spectroscopy (NIR) analyses by LabA]: 34.7 ± 0.69, 32.3 ± 0.69, 34.4 ± 0.69 for Hubner H5333RC3P, H6191RCSS, and H5222RC3P, respectively (Hubner Seed); 34.1 ± 0.76 for Masters Choice MC 5250 (Masters Choice); and 36.2 ± 0.72 for Healthy Herd Genetics 42HFC15 (Healthy Herd Genetics and Nutrition).

[5]LabB = Cumberland Valley Analytical Services (Waynesboro, PA). Analyses were from LabB's NIR Plus Option package.

[6]Starch concentration of the corn silage H, across ET, averaged (mean ± SE; NIR analyses by LabB): 35.9 ± 0.77, 33.2 ± 0.76, 35.3 ± 0.81 for Hubner H5333RC3P, H6191RCSS, and H5222RC3P, respectively (Hubner Seed); 34.4 ± 0.77 for Masters Choice MC 5250 (Masters Choice); and 36.9 ± 0.81 for Healthy Herd Genetics 42HFC15 (Healthy Herd Genetics and Nutrition).

moisture corn and CS (Hoffman et al., 2011; Ferraretto et al. 2015b).

Effect of Ensiling Time on In Situ Starch Disappearance and In Vitro Starch Degradability. In situ starch disappearance data for the CS analyzed in this study are shown in Table 2. Overall, ET quadratically ($P \leq 0.001$) increased IS starch fraction *a* of CS, being highest at 150 d of ET when compared with unfermented CS. Conversely, ET quadratically decreased ($P \leq 0.001$) fraction *b* for all CS hybrids, being lowest at 150 d of ET when compared with unfermented CS. Ensiling time linearly ($P \leq 0.001$) increased IS starch degradation rate of CS resulting in at least a 2-fold increase for 150 d silage when compared with unfermented CS (i.e., 0 d).

In agreement with the original rankings made by the seed companies, H5222RC3P had a greater ($P \leq 0.001$) concentration of IS starch fraction *a* when compared with the other hybrids during the first 120 d of ensiling, whereas no effect of hybrid on fraction *a* was observed at 150 d of ensiling. We did not observe an overall effect of hybrid or hybrid × ET interaction for IS starch degradation rate. The ISED of starch was different ($P = 0.002$) among hybrids, but no hybrid × ET interaction occurred for this variable. However, likely driven by the increases in IS starch fraction *a* and degradation rate, ET increased quadratically ($P \leq 0.001$) ISED of starch, following an exponential relationship: IS StD, % = 83.23 + 11.44 × [1-exp(−0.024 × ET, d)]. Figure 1 further illustrates the effect of ET, indicating an approximately 10 percentage unit increase of overall IS starch disappearance in 150 d. When averaged across ET, overall ISED of starch measured in the current experiment was high for all CS hybrids evaluated (ISED ≥89%; Table 2). In concordance with seed companies' StD ratings, H5222RC3P had the greatest and H6191RCSS the lowest ($P = 0.002$) mean ISED for unfermented CS. However, differences in ISED among hybrids disappeared after 60 d of ensiling. Starch degradability values estimated by the commercial laboratories using NIRS and IV procedures are also presented in Table 2. Average 7-h StD across ET analyzed by NIRS was similar ($P = 0.81$; data not presented in tables) for LabA and LabB. Both commercial laboratories reported ET increased 7-h StD linearly ($P \leq 0.001$; LabA) or to a greater

Table 2. Starch degradability of corn silages ensiled for 0 to 150 d[1]

Method	Starch degradability variable and corn silage (H)	n[2]	ET, d					SEM[3]	P-value[4]	
			0	30	60	120	150		H	ET
In situ	Washout or rapidly degraded (fraction a), %								<0.001	<0.001 (Q)
	42HFC15	222	59.4[b]	69.9[ab]	78.6[a]	79.8[a]	75.4	2.26		
	H5222RC3P	222	68.9[a]	73.3[a]	76.8[a]	76.9[ab]	80.3	1.85		
	H5333RC3P	222	58.6[b]	61.3[c]	72.9[ab]	73.7[b]	78.7	1.85		
	H6191RCSS	222	58.4[b]	62.2[bc]	70.6[b]	73.9[b]	77.2	1.85		
	MC5250	222	59.3[b]	67.3[b]	69.6[b]	73.7[b]	75.9	1.85		
	SEM		2.61	2.61	2.92	2.61	2.61			
	P-value		0.006	<0.001	0.01	0.09	0.10			
	Potentially degradable (fraction b), %	222	39.1	33.2	26.1	24.3	22.4	0.86	<0.001	<0.001(Q)
	Degradation rate (%/h)	218	0.094	0.138	0.131	0.181	0.198	0.0508	0.31	<0.001 (L)
	Effective degradability, %	222	83.1	89.6	91.4	93.8	94.7	0.54	0.002	<0.001 (Q)
NIRS	7-h Starch degradability—LabA,	75	68.9	71.5	73.6	77.5	79.9	1.19	0.009	<0.001 (L)
	7-h Starch degradability—LabB	74	65.6	75.7	75.9	76.0	77.9	0.53	0.09	<0.001 (Q)
In vitro	7-h Starch degradability—LabB	74	58.3	65.3	67.4	69.3	69.7	1.13	0.66	0.004 (Q)
	7-h Starch degradability—LabC	68	66.0	66.8	68.0	69.2	69.3	0.66	0.50	<0.001 (L)

[a–c]Means within the same column without a common superscript differ ($P < 0.05$).

[1]Values are model estimates, and associated SE of disappearance curves fit using SigmaPlot 13.0 (Systat Software) to the equation $p = a + b\,[1 - \exp(-c \times t)]$, where p is the degraded fraction (of starch) at time t, a is washout or rapidly degraded fraction, b is the potentially degradable fraction, and c is the rate of degradation of the b fraction (Ørskov and McDonald, 1979). NIRS = near-infrared reflectance spectroscopy. H = corn hybrid; ET = ensiling time. LabA = Rock River Laboratories Inc. (Watertown, WI); LabB = Cumberland Valley Analytical Services (Waynesboro, PA); LabC = Università Cattolica del Sacro Cuore, Piacenza, Italy. See Table 1 for details of silage manufacturers.

[2]n represents the number of observations used in the statistical analysis.

[3]Largest SEM published in table.

[4]Main effect of corn H, ET, and H × ET interaction. An H × ET interaction ($P \leq 0.01$) occurred for washout and rapidly degraded starch (a) and the potentially degradable starch (b) fractions; no other H × ET interactions were detected ($P \geq 0.15$).

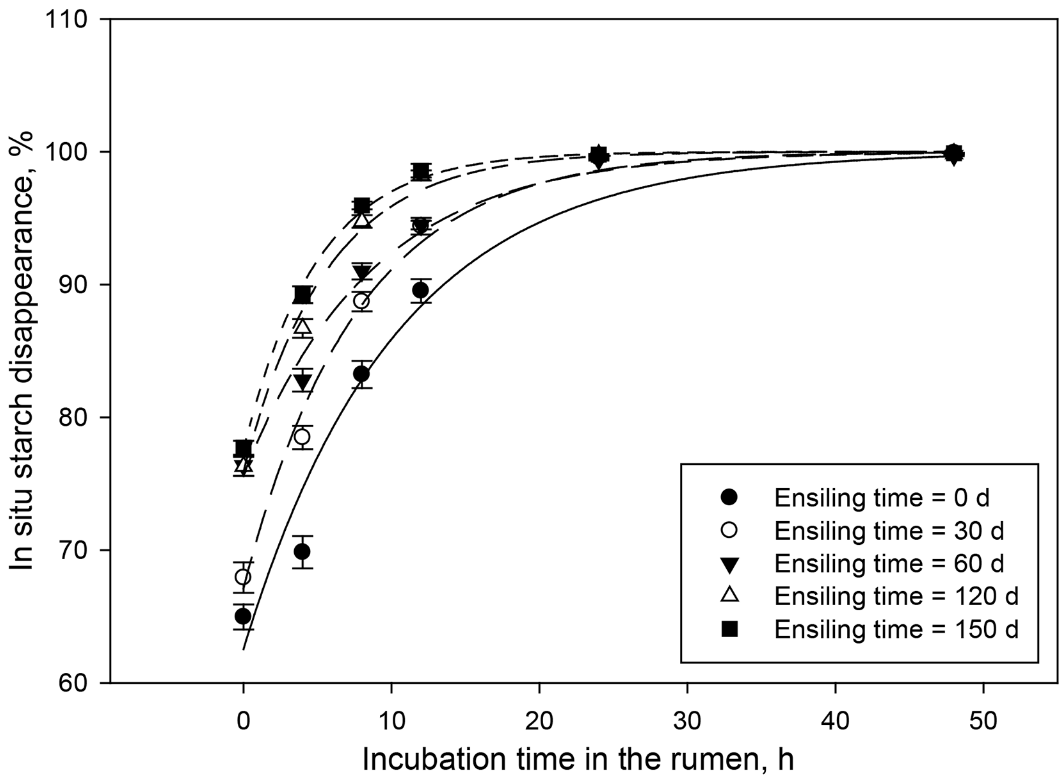

Figure 1. Corn silage in situ total starch disappearance (% of starch). Data are mean ± SE (error bars), averaged across 5 hybrids used in the study. Data were fitted to a single, 3-parameter exponential rise to a maximum model (SigmaPlot 13.0; Systat Software): $p = a + b \times [1 - \exp(-c \times t)]$, where p is the fraction of total starch degraded or disappeared from the in situ bag (%); a is the intercept (washout or rapidly degraded starch), %; b is the potentially degradable starch, %; c is the rate of degradation of fraction b, %/h; and t is incubation time, h. Regression, $P < 0.001$ for all ensiling times; R^2 for all ensiling times = 0.98 to 1.00. Regression lines: solid line = 0-d silage; long-dash line = 30-d silage; medium-dash line = 60-d silage; long-short-dash line = 120-d silage; short-short-dash line = 150-d silage.

extent during the first 2 mo of ensiling ($P \leq 0.001$; LabB). Average 7-h IVStD also tended to be similar ($P = 0.09$) between LabB and LabC. Furthermore, both laboratories reported linear ($P \leq 0.001$; LabC) or quadratic ($P \leq 0.004$; LabB) increases in 7-h IVStD with ET, being highest at 150 d of ET when compared with unfermented CS. Overall, when comparing StD values between methods and across laboratories, StD by NIRS were approximately 7 percentage units greater than IVStD values.

Penn State's OMDI was not different ($P = 0.18$; data not presented in tables) among CS hybrids across ET and no ($P = 0.95$) hybrid × ET interaction occurred. Ensiling time linearly increased ($P = 0.005$) OMDI of CS, and we also observed a trend for a quadratic increase ($P = 0.09$): 62.9, 65.1, and 65.4% at 0, 60, and 150 d, respectively. The OMDI of CS is mainly determined by its starch and NDF degradabilities. Because NDF degradability of the CS samples used in the current study decreased with ET (Hristov et al., 2020), the increase in OMDI in the current experiment can be attributed to increases in StD.

Correlations between IS starch disappearance and laboratory StD analyses were moderate to strong and statistically significant (r $\geq$0.41, $P \leq 0.006$; data not shown in tables). In situ rate of StD was strongly correlated with 7-h StD analyzed by NIRS from LabA, followed by NIRS from LabB (r $\geq$0.60) and was moderately correlated with 7-h IVStD from LabC (r = 0.43). Eight-hour IS starch disappearance was strongly correlated with 7-h StD measured IV and by NIRS from LabB (r $\geq$0.72; Figure 2). However, 8-h IS starch disappearance was only moderately correlated with 7-h StD estimated by NIRS from LabA (r = 0.57) and with 7-h IVStD from LabC (r = 0.42). Furthermore, agreement among 8-h IS starch disappearance and NIRS reports from LabA and LabB was weak (correlation and concordance correlation coefficient = 0.15 for both laboratories; data not shown) and weaker between 8-h IS StD and 7-h IVStD from LabB and LabC (correlation and concordance correlation coefficient = 0.09 and 0.02, respectively). In the current experiment, IVStD was measured assuming a 7-h mean residence time of starch in the rumen, whereas ISED was calculated

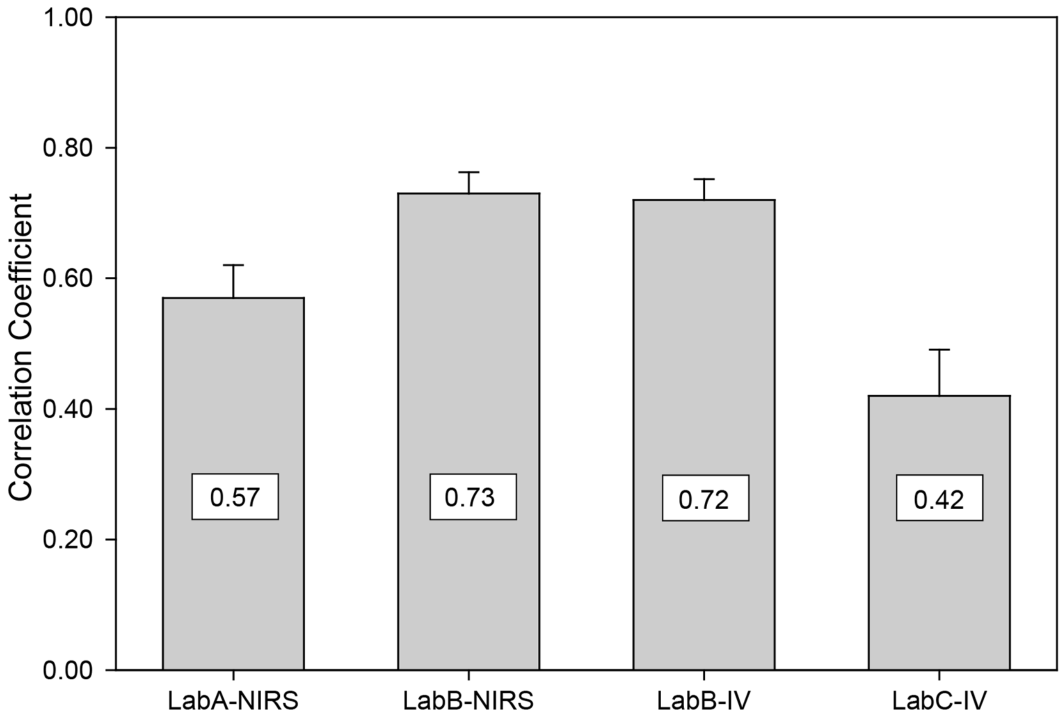

Figure 2. Relationship of 8-h in situ starch disappearance with 7-h starch degradability analyzed by near-infrared reflectance spectroscopy (NIRS) from LabA (Rock River Laboratories Inc., Watertown, WI; n = 73) and LabB (Cumberland Valley Analytical Services, Waynesboro, PA; n = 74) and 7-h starch degradability from in vitro (IV) assays from LabB (n = 74) and LabC (Feed and Food Science and Nutrition Institute, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore Piacenza, Italy; n = 68). Data are Pearson correlation coefficients ± SE (error bars) of 5 corn silage hybrids ensiled for 0, 30, 60, 120, and 150 d. All correlations presented in figure were statistically significant ($P \leq 0.004$).

assuming approximately 16-h ruminal residence time (i.e., 6%/h passage rate). This could have affected the absolute values of StD and consequently the strength of the correlation between methods. Despite this, ISED of starch was strongly correlated with 7-h IVStD and NIRS StD estimations from LabB (r ≥0.74) and was moderately correlated with NIRS from LabA and 7-h IVStD from LabC (r = 0.54 and 0.41, respectively).

The increase in IS starch disappearance with ET observed in the current experiment is in agreement with previous studies where ET increased solubilization of the protein matrix cross-linked to starch granules in corn kernels, allowing for better microbial attachment and enzymatic hydrolysis of starch in the rumen (Hoffman et al., 2011; Der Bedrosian et al., 2012). Offner et al. (2003) reported that fraction *a* comprises 66.8% of starch in CS; these values are similar to IS fraction *a* of CS hybrids at 30 d, but lower than CS at 150 d of ensiling in the current experiment (66.8 and 77.5%, respectively). The washout or rapidly degraded fraction obtained for CS hybrids at 150 d of ensiling in the current experiment was high, which could be partially attributed to the effect of ET (Peyrat et al., 2014). It should be noted that in the current experiment, the rate of IS starch disappearance averaged across ET (14.8%/h) was considerably greater than that reported for CS starch (8.7%/h) in the review by Offner et al. (2003). However, Offner et al. (2003) questioned the ISED data for CS in their meta-analysis because of "*almost linear kinetics*" of IS starch disappearance, whereas starch disappearance in the current experiment followed an exponential relationship (Figure 1). In contrast, IS starch disappearance rate averaged across ET in the present study is similar to ruminal starch digestion rate reported for high-moisture corn (16.8%/h) in low-starch (21% of DM) diets fed to lactating dairy cows (Oba and Allen, 2003). These authors reported that high-moisture corn had a considerably greater ruminal digestion rate when compared with dry-ground corn (22.5 vs. 13.4%/h) and similar results were recently reported by Allen and Ying (2021). The moisture content in CS would likely resemble moisture content in high-moisture corn and could explain the high IS starch disappearance of CS observed in the current experiment. In the meta-analysis by Moharrery et al. (2014), it was estimated that 90.9% of starch in CS was degraded in the rumen, which is similar to the averaged ISED of starch (90.5%) determined in our study.

Although values for ruminal StD and degradation rate of starch of CS are variable in literature, results from the current IS study along with the research cited above indicate that a higher rate of StD in the rumen can be expected with ensiled versus dry corn. However, factors such as assumed rate of passage, CS processing, and ET can affect estimations of IS starch disappearance of CS (Offner et al., 2003; Tothi et al., 2003; Peyrat et al., 2014). As discussed in Moharrery et al. (2014), studies investigating passage rate of whole crop CS are scarce and previous in vivo research estimating the rate of passage of ensiled corn using the rumen evacuation technique has yielded variable results (Oba and Allen, 2003; Taylor and Allen, 2005; Allen and Ying, 2021). It should also be noted that the model proposed by Ørskov and McDonald (1979) assumes that fraction *a* has an infinite rate of degradation and is therefore immediately degraded in the rumen. This assumption does not consider that fraction *a* may in fact escape rumen degradation (Choi et al., 2003). Previous studies using the IS technique have discussed that starch granules can pass through the pores of the nylon bag which may lead to overestimations of rapidly degraded fraction of starch in the rumen (Philippeau and Michalet-Doreau, 1998; Huhtanen and Sveinbjörnsson, 2006), especially when samples are finely ground before incubation (Fernandes et al., 2018). To account for this in the current experiment, CS samples were ground using a 4-mm sieve which has shown to reduce the potential for particle loss through the nylon bag pores and consequent overestimation of fraction *a* (Fernandes et al., 2018). In contrast, separation of the starch granule from the protein matrix during the ensiling process may also increase the chance of starch granules to be washed out from the bag without fermentation and consequently increase fraction *a* and the apparent degradation rate of starch (Tothi et al., 2003; Huhtanen and Sveinbjörnsson, 2006). Together, these potential drawbacks of the IS approach may have contributed to the results from the current experiment.

The increase in IS starch disappearance with ET was similar among the CS hybrids evaluated in the current experiment. The differences in ISED of starch between CS hybrids observed for unfermented CS (0 d of ensiling) progressively disappeared with ET. This is in agreement with the report by Der Bedrosian et al. (2012), where initial differences in IVStD among hybrid genotypes disappeared after 60 d of ensiling. Similarly, Ferraretto et al. (2015a) did not observe a hybrid effect or hybrid and ET interaction on StD of CS over 240 d of ensiling. Der Bedrosian et al. (2012) suggested that changes in CS StD with ET will vary based on type of hybrid and maturity at harvest. Philippeau and Michalet-Doreau (1998) suggested that initial differences in StD among hybrids tend to be prevalent throughout the process of ensiling. However, ET in their experiment was shorter (90 d), compared with the current experiment (150 d) and that of Der Bedrosian et al. (2012; 360 d) and Ferraretto et al. (2015a; 240 d). Benton et al. (2005) reported that most of the increase in StD of CS or high-moisture corn, compared with dry corn grain, occurs during the initial months of ensiling. Indeed, ISED of CS starch increased by 10% from d 0 to d 60 and by 3.6% from d 60 to d 150 (and only by about 1% from d 120 to d 150). These data suggest that changes in StD of CS are most intensive in the initial stages of silage fermentation but will continue to take place at a slower rate beyond 2 mo of ensiling. Observations from the current experiment would also indicate that allowing extended ET will benefit producers regardless of initial StD differences among CS hybrids. In this regard, routine evaluations of CS StD are recommended, particularly during the first 2 mo of ensiling, as it will facilitate ration formulators to match the dietary starch content and extent and site of starch digestion with the nutrient requirements of the cows.

Average 7-h IVStD values for the CS hybrids used in the current experiment are similar to those reported by Nocek and Tamminga (1991), Ferraretto et al. (2014, 2015b), and average StD data for 2014–15 CS in the DairyOne database (71.5%). Clear differences in StD were observed between NIRS and IV methods in the current experiment, where NIRS procedures resulted in values 7.3 percentage units greater than IVStD. However, average 8-h IS starch disappearance (89.8% ± 6.6) was greater than NIRS estimations by the commercial laboratories. Despite this, the effect of ET on StD was still detectable using NIRS. Clearly, the method used can have a large effect on StD estimates. Differences in StD measurements among laboratories in the current experiment could be attributed to sample processing, methodological differences in the IV assays, and methods used for starch analysis (Stern et al., 1997). In vitro StD can also be largely affected by the rumen inoculum characteristics (Richards et al., 1995; Raffrenato et al., 2022), which usually makes comparisons among laboratories difficult. When related to in vivo ruminal StD, a previous study reported a stronger correlation for IS measurements (r = 0.84) relative to IV estimates (r = 0.76) when starch was determined from residues following an incubation (Weisbjerg et al., 2011). It is argued that IV methods do not adequately simulate the dynamic nature of the rumen environment and results are not representative of in vivo data (Hristov et al., 2012; Hatew et al., 2015). However, it is important to point out that large variability exists among in vivo studies investigating starch concentration and fermentability, thus making it difficult to obtain quality reference data

(Oba and Allen, 2003; Taylor and Allen, 2005; Allen and Ying, 2021). Although IV techniques may still prove useful for comparative analysis, they should not be used to obtain absolute estimations of StD. Interestingly, apart from the large difference reported for unfermented CS, IVStD estimations between LabB and LabC were similar in the present study. In addition, the overall effect of ET on IVStD observed in the current experiment was consistent among laboratories and procedures. Thus, in the absence of rumen-cannulated animals and to reduce labor costs, the IV assays can be used to assess StD and the effect of ET to optimally formulate dairy rations. However, data from the current experiment suggest that variability of IVStD among laboratories is larger for less fermented silages and the approach may not be sensitive enough to detect relative differences in StD among CS hybrids when compared with IS.

A positive relationship between IS and IV methods has been previously reported (Michalet-Doreau et al., 1997) with good agreement between methods when used for ranking of feeds (Herrera-Saldana et al., 1990). More recently, studies have reported moderate to strong and positive correlations between IS StD and IV gas production in cereal grains (r $\geq$0.57; Seifried et al., 2016; Krieg et al., 2017). In contrast, we had expected better agreement between IS, IV, and NIRS methods. Gleason et al. (2022) suggested that accounting for feed nutrient components is necessary to improve agreement between methods given that these could influence procedures differently. Additionally, several factors such as digestibility markers, between animal variation or animal species used can affect data associated with the acquisition of spectra for calibration of NIRS, thus leading to biased prediction values and potentially introducing substantial variation in StD measurements (Cao, 2013; Brogna et al., 2018). Animal species, specific enzyme activity of the rumen inoculum or dietary characteristics could also lead to substantial differences in IV and IS results reported among different laboratories (Stern et al., 1997). Grinding size during samples processing is also a major factor affecting StD measurements (Richards et al., 1995). This can be observed in the current experiment where 8-h IS starch disappearance showed the strongest relationship, relative to StD from other laboratories, with 7-h IV and NIRS StD measurements from LabB, which is likely due to CS samples being processed at the same grind size (4 mm). Collectively, the above-mentioned factors can affect the relationship between StD methods and potentially contributed to the low agreement between 8-h IS starch disappearance and IV and NIRS measurements of StD reported by the commercial laboratories in the current experiment. Correlations among NIRS, IV, and IS StD of CS samples, however, were strong in the current analysis.

Effect of Grind Size on In Vitro Starch Degradability. The IV method was used to test the effect of particle size on StD because it is routinely used by commercial feed analysis laboratories for evaluation of ruminal degradability of feeds. Results (Table 3) show that CS samples ground through a 1-mm sieve had approximately 12 percentage units greater ($P = 0.001$) average 7-h IVStD, independently of ET, when compared with samples ground through a 4-mm sieve (80.9 vs. 69.1%, respectively). In the conditions of this subexperiment, ET did not have a statistically significant effect on 7-h IVStD, although numerical differences were noticeable for both 1- and 4-mm grind sizes. There were no grind size × ET or grind size × CS hybrid interactions on 7-h IVStD. However, 7-h IVStD of CS was greater ($P \leq 0.002$) for the 1-mm grind size samples than the 4-mm samples at each of the ET points (0, 30, and 120 d). No difference in StD ($P = 0.70$) was observed between CS hybrids rated medium- and high-StD by the seed companies. However, the silage hybrid rated low in StD had lower ($P \leq 0.02$) StD when compared with hybrids rated medium- and high-StD, only when CS samples were ground through a 1-mm sieve. Grind size tended to increase ($P = 0.08$) StD for the low-StD hybrids, and increased StD ($P \leq 0.004$) for 1-mm versus 4-mm grind for the medium- and high-StD hybrids.

The inverse relationship between grind size and starch degradability is well documented (Bal et al., 2000; Ferreira and Mertens, 2005). Therefore, the increase in 7-h IVStD in the current experiment for samples ground at a 1-mm grind size, when compared with those ground at 4 mm, is not surprising. The likely explanation for this effect is increased surface area, which would enhance microbial adherence and enzymatic access (Blasel et al., 2006; Hoffman et al., 2012; Gallo et al., 2016). Furthermore, the current IV study showed that 7-h StD was decreased by approximately 4 percentage units for every 1-mm increase in grind size. Considering the positive relationship between grind aperture size and mean particle size (Michalet-Doreau and Cerneau, 1991), our results are in agreement with Gallo et al. (2016) who reported a 6.3 percentage unit decrease in 7-h IVStD with each 1-mm increase in mean particle size of corn meal. The lack of effect of ET on 7-h IVStD in the current subexperiment was unexpected because our previous study and reports by Der Bedrosian et al. (2012) and Ferraretto et al. (2014, 2015a) showed that 7-h IVStD of CS clearly increased with ET. Although there were no interactions of ET and grind size, our data indicate that reducing particle size may partially mask the effect of ET on StD of CS; the increase in StD from

Table 3. In vitro starch degradability (% of starch) of corn silage as affected by sample grind size (GS), ensiling time (ET), and hybrid (H)

	ET, d				H[2]				*P*-value[4]	
Grind size	0	30	120	SEM[1]	Low	Medium	High	SEM[3]	ET	H
1-mm	78.9	82.1	81.6	2.15	75.3	84.2	83.1	2.91	0.66	0.11[5]
4-mm	67.0	68.4	71.8	2.04	69.0	69.5	68.7	2.32	0.17	0.95
SEM[6]	3.10	1.31	2.20		2.84	1.57	1.44			
P-value[7]	<0.001	<0.001	0.002		0.08	0.004	<0.001			

[1]Largest SEM published in the table; n = 30; 0 d, n = 10; 30 d, n = 10; 120 d, n = 10.

[2]The corn silage hybrids were as follows: Hubner H5333RC3P, H6191RCSS, and H5222RC3P (Hubner Seed); Masters Choice MC 5250 (Masters Choice); and Healthy Herd Genetics 42HFC15 (Healthy Herd Genetics and Nutrition). Three of the hybrids were rated (by the seed company) as high in starch degradability, whereas the other 2 were rated medium and low in starch degradability.

[3]Largest SEM published in the table; n = 30; low, n = 6; medium, n = 6; high, n = 18 (n represents the number of observations used in the statistical analysis).

[4]Main effect of ET and starch degradability rating of corn H. No ET × GS or starch degradability rating of H × GS ($P \geq 0.13$) interactions were detectable.

[5]We did not observe differences in in vitro starch degradability (IVStD) between corn silage (CS) H rated medium- and high-starch degradability (StD; $P = 0.70$) by the seed companies, but the silage rated low in StD was different when compared with silages rated medium- and high-StD ($P \leq 0.02$).

[6]Largest SEM published in the table; 1-mm, n = 15; 4-mm, n = 15 (n represents the number of observations used in the statistical analysis).

[7]Main effect of GS.

0 to 120 d ET was 4.8% for 4-mm grind and only 2.7% for the 1-mm ground samples. In contrast, data from the current subexperiment suggest that reducing grind size of CS samples is unlikely to mask difference in StD among hybrids and, in addition, can decrease variability in IVStD analysis (M. Michonski, CVAS; personal communication). To account for this effect, incubation time for measurement of IVStD has been reduced from 7h to 4 h in The Pennsylvania State University's Commercial Silage Hybrid Corn Test Program (https://extension.psu.edu/2018-results-pa-commercial-grain-and-silage-hybrid-corn-tests-report#section-13; accessed Jul. 14, 2022) and is used for estimating OMDI. Ferreira and Mertens (2005) noted that reducing grind size for IV fermentation of corn hybrids could negate the effects of their physical characteristics, but earlier research concluded that ruminal IVStD can vary according to sample processing, grinder type and characteristics of the ruminal inoculum (Richards et al., 1995; Raffrenato et al., 2022). Unequivocally, IVStD increases with ET, thus we attribute the lack of statistical effect of ET on IVStD in the current subexperiment to methodological differences mainly related to the reduction in particle size of CS samples.

Although a 1-mm grind size is typically used when evaluating IV NDF degradation in forages, grains are analyzed ground through a 4- or 6-mm sieve (Ferraretto et al., 2015a; Willems et al., 2016). Therefore, to our knowledge, comparisons of 7-h IVStD for samples ground through 1-mm versus 4-mm sieves are nonexistent. In the current subexperiment, the lack of effect of grind size on the lowest StD-rated hybrid when compared with the medium- and high-StD-rated hybrids show a relative concordance with original rankings done by the seed companies; however, differences between medium- and high-StD rated hybrids were not clear at any of the grind sizes used. Hoffman et al. (2012) compared the degradability of starch in high-moisture corn samples processed through multiple sieve sizes and suggested that the IV fermentation potential and StD of the sample is not only related to its physical characteristics but can also be affected by concentration of other nutrients such as CP and NDF. Our hypothesis was that reducing grind size would negate initial differences in StD among hybrids. This, however, was true only for CS rated high and medium in StD. These data would suggest that grinding samples using a 1-mm sieve was successful at discriminating the low-StD-rated hybrid from medium- and high-StD-rated hybrids. However, differences between medium- and high-StD-rated hybrids were not clear.

CONCLUSIONS

In this study, IS disappearance of starch in CS was lowest in unfermented CS but quadratically increased from 0 to 150 d of ET. The quadratic response was primarily driven by increases in the washout or rapidly degraded fraction of starch, particularly during the first 60 d of ensiling. Results from the IV subexperiment showed that 7-h in IVStD was increased by reducing grind size from 4 to 1 mm, regardless of ET. In addition, grinding CS samples through a 1-mm sieve allowed to distinguish low- from medium- and high-StD hybrids. Strong correlation among IS, IV, and NIRS measurements for StD suggests that all methods can be used to obtain relative measurements of StD of CS. However, low agreement among methods indicates that absolute estimations of StD will vary. Overall, both IV and IS techniques can be used to quantify the effect of ET on StD. However, a reduction in grind size may be necessary to identify differences in StD rating among CS hybrids IV.

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