SHORT COMMUNICATION

Effects of processing, moisture, and storage length on the fermentation profile, particle size, and ruminal disappearance of reconstituted corn grain

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Abstract

Our objective was to examine the effects of processing, moisture, and anaerobic storage length of reconstituted corn grain (RCG) on the fermentation profile, geometric mean particle size (GMPS), and ruminal dry matter disappearance (DMD). Dry corn kernels were ground (hammer mill, 5-mm screen) or rolled, then rehydrated to 30%, 35%, or 40% moisture, and stored for 0, 14, 30, 60, 90, 120, or 180 d in laboratory silos. Rolled corn had an increased GMPS compared with ground corn (2.24 and 1.13 mm, respectively, at ensiling). However, there was a trend for an interaction between processing and moisture concentration to affect particle size, with GMPS increasing with increased moisture concentration, especially in ground corn. Longer storage periods also slightly increased GMPS. Processing, moisture, and storage length interacted to affect the fermentation pattern (two- or three-way interactions). Overall, pH decreased, whereas lactic acid, acetic acid, ethanol, and $\text{NH}_3\text{-N}$ increased with storage length. RCG with 30% moisture had less lactic acid than corn with 35% and 40% moisture, indicating that fermentation might have been curtailed and also due to the clostridial fermentation that converts lactic acid to butyric acid. Ensiling reconstituted ground corn with 30% of moisture led to greater concentrations of ethanol and butyric acid, resulting in greater DM loss than grain rehydrated to 35% or 40% of moisture. Ammonia-N and in situ ruminal DMD were highest for reconstituted ground corn with 35% or 40% of moisture, mainly after 60 d of storage. Therefore, longer storage periods and greater moisture contents did not offset the negative effect of greater particle size on the in situ ruminal DMD of rolled RCG. Nonetheless, RCG should be ensiled with more than 30% moisture and stored for at least 2 mo to improve the ruminal DMD and reduce the formation of ethanol and butyric acid.

Key words: digestibility, fermentation, flint corn, moisture, storage duration

Abbreviations

CP	crude protein
DM	dry matter
DMcorr	dry matter corrected for volatile compounds
DMD	dry matter disappearance
DML	dry matter loss
GMPS	geometric mean particle size
GSD	geometric standard deviation
LAB	lactic acid bacteria
MIC	minimum inhibitory concentration
RCG	reconstituted corn grain

Introduction

Corn grain is the main energy source in dairy and beef feedlot cattle diets. However, the corn grain genotypes cultivated in Brazil are predominantly flint, with a higher proportion of vitreous endosperm than dent hybrids used predominantly in countries with temperate climates (Correa et al., 2002). Compared with dent corn, the endosperm of flint corn has a greater content of prolamins that form the protein matrix embedding starch granules (Philippeau and Michalet-Doreau, 1997; Fernandes, 2014) and hinders the action of ruminal microorganisms and their enzymes in the ruminal environment as well as post-ruminal digestion (McAllister et al., 1993; Ngonyamo-Majee et al., 2008). Processing methods have been developed to overcome this issue and improve grain digestibility with the ultimate goal of improving the growth performance and/or feed efficiency (Owens et al., 1997; Owens and Basalan, 2013).

In the last decade, the popularity of fermented corn as a feed source has rapidly increased in Brazil (Bernardes et al., 2018; Bernardes and Castro, 2019; Daniel et al., 2019). Ensiling of high-moisture or reconstituted corn is a convenient method to improve grain digestibility, especially when compared with other processing methods, such as steam flaking.

Preparation of reconstituted corn grain (RCG) benefits from mechanical processing capable of breaking the pericarp and reducing particle size prior to moisture reconstitution. Although the geometric mean particle size (GMPS) is highly dependent upon the mill set, depending on the hole size and distribution, hammer mills can yield smaller but less uniform particle sizes than roller mills (Rémond et al., 2004). Currently, some farmers are reconstituting and ensiling corn kernels after rolling, due to the greater milling throughput (t/h) in an attempt to reduce labor, time, and energy costs, and because of the availability of rollers mounted in ag baggers. The GMPS has an inverse relationship with starch digestibility in vivo in dry-processed corn grain (Galyean et al., 1979; Rémond et al., 2004; Ahmadi et al., 2020) and high-moisture corn (Ferraretto et al., 2013), but the effect of mill type (grinding or rolling) on the fermentation pattern and ruminal degradation of RCG made from flint corn is not well-established.

In addition to particle size, moisture content and storage length affect the digestibility of corn grain silages. Higher moisture contents (Szasz et al., 2007; Owens and Basalan, 2013) and longer periods of storage (Benton et al., 2005; Fernandes, 2014; da Silva et al., 2019) typically increase starch and dry matter (DM) digestibility (Hoffman et al., 2011). Moreover, the disruption of the protein-matrix during silage fermentation hypothetically could result in a reduction of kernel particle size during long storage periods, increasing the surface area for digestion (Dias Junior et al., 2016; Saylor et al., 2020).

Thus, the objective of this study was to examine the effects of processing (grinding or rolling), moisture content (30%, 35%, or 40% of moisture), and storage length (0 to 180 d) on the fermentation pattern, GMPS, and ruminal DM disappearance (DMD) of RCG. We hypothesized that a longer storage period combined with a greater moisture content may offset the negative effect of larger particle size on the ruminal DMD of rolled corn compared with ground corn.

Material and Methods**Preparing and ensiling reconstituted corn**

A blend of corn kernels (11% of moisture; 9.14% crude protein [CP], and 3.52% water-soluble carbohydrates, DM basis) with flint endosperm ($78.6\% \pm 4.2\%$ of vitreousness determined by manual dissection; Dombrink-Kurtzman and Bietz, 1993) was obtained from a local cooperative (Cocamar Cooperativa Agroindustrial, Maringá, Brazil), homogenized, and divided into two batches. One batch was ground using a hammer mill (3,550 rpm) with a uniform 5-mm screen (Máquinas Pereira, Londrina, Brazil). The other batch was processed in a roller mill mounted in an ag bagger (SEGU30, Multiagro, Porto Alegre, Brazil). The single pair of corrugated rolls had a differential speed of 35% and a 1-mm gap between rolls. Immediately before processing, each mill was cleaned with compressed air.

Grain from each mill was divided into 12 piles of 6 kg (total of 24 piles) and rehydrated to achieve 30%, 35%, or 40% of moisture. Four piles from each processing method were rehydrated homogenously using a watering can and manual mixing to a given target moisture content. From each pile, seven nylon-polyethylene vacuum pouches (33×45 cm, 160 μ m thick) were filled with rehydrated grain (800 g/bag). The pouches were vacuum sealed using a chamber vacuum sealer (TM280, TecMaq, São Paulo, Brazil), weighed, and stored for 0, 14, 30, 60, 90, 120, and 180 d at room temperature (14 to 32 °C) (one bag per storage period from each pile). A total of 168 silos were prepared, as a result of the combination of two processing methods by three moisture contents by seven storage periods, with four repetitions, in a split-split-plot design.

After each storage period was reached, mini silos were weighed, opened, and sampled. One subsample was used to prepare an aqueous extract (1:10) by diluting silage with distilled water (Kung et al., 1984). Another subsample was dried at 55 °C for 72 h in a forced ventilation oven. The DM content was thereafter corrected for the loss of volatile compounds (DMcorr) according to Weissbach (2009). The DM loss (DML) corrected for volatiles was assessed by the difference between the DM mass at ensiling and DMcorr mass at an opening in each silo.

In situ DMD test

Approximately, 5 g of the dried sample from each silo (not ground after the processing described above) were weighed into polyfilament polyester in situ bags (10×20 cm; 50 μ m porosity; Ankom Technology, Macedon, NY, USA) and incubated (without soaking before incubation) for 12 h in the rumen ventral sac of two rumen-cannulated non-lactating cows (168 bags per cow) fed a ration (~12% CP) containing 80% corn silage and 20% concentrates (DM basis). One sample from each mini silo was incubated in each animal. Immediately after retrieval, all bags were submerged in cold water (0 °C) for 5 min and washed in a washing machine (three cycles, followed by a final spin). Washed bags were dried in an air-forced oven at 55 °C for 72 h

and weighed to calculate ruminal DMD. The DMD values were not corrected for the loss of particles potentially rinsed through the bag pore, assuming that small particles (i.e., <50 µm) would be completely digested within 12 h of incubation.

Laboratory analysis

For the aqueous extract, pH was recorded with a pH meter (DM22 Digimed, São Paulo, Brazil); the concentrations of fermentation end products were determined in the supernatant fluid after centrifugation (10,000 × g, 15 min), and total lactic acid (D plus L isomers; Pryce, 1969) and ammonia (Chaney and Marbach, 1962) were analyzed by colorimetric methods using a VersaMax Tunable microplate reader (VWR, Pennsylvania, USA). Acetic acid, butyric acid, and ethanol were determined by gas chromatography (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm inner diameter, 0.25 µm crossbond carbowax polyethylene glycol).

Kernel particle size distribution was measured in dried samples using a horizontal shaker (Ro-Tap; Solotest, São Paulo, Brazil), with three rubber balls (13 mm) as a flow agent in each sieve. The sieves had square holes with sides of 8.00, 4.75, 4.00, 2.00, 1.18, 0.60, 0.30 mm, and a bottom pan. Approximately, 120 g of dry sample was shaken for 10 min. The GMPS and geometric standard deviation (GSD) were calculated according to ASABE (2008; method S319.4). The surface area and particles per gram were calculated according to Baker and Herrman (2002). The proportion of particles passing through a 4.75-mm sieve was also calculated. The recovery of particles after 180 d of storage was calculated for each sieve assuming the same DML across the sieves (Supplemental Table S1).

Statistical analysis

Statistical analysis was performed using the MIXED procedure of SAS (v. 9.4; SAS Institute Inc., Cary, NC). The model included the fixed effects of processing (ground or rolled), moisture (30%, 35%, or 40%), storage length (0, 14, 30, 60, 90, 120, or 180 d) and their interactions. Repetition × processing and repetition × moisture (processing) were defined as error terms to test the effects in the main plot and subplot, respectively. Residual error was used to test the effects in the sub-subplot. The in situ ruminal DMD was analyzed with the same model but including the random effect of animal. A sub-dataset with data of particle stratification on days 0 and 180 was also analyzed. Data of particle recovery at 180 d of storage were analyzed with the main model without the effect of time and its interactions (Supplemental Table S1).

Results

Results of the statistical analysis of the fermentation profile, particle size, and in situ ruminal DMD results are shown in Table 1. Rolled corn had higher GMPS and lower surface area compared with ground corn at ensiling and after 180 d of storage (Table 2). Rolled and ground RCG had a lower surface area at 180 d of storage, but there was a greater reduction of surface area in the ground than in rolled RCG ($P = 0.02$ for interaction of processing and storage). The GMPS slightly increased with moisture content, especially in the ground corn. Longer storage periods also led to slightly greater GMPS ($P < 0.01$; Figure 1).

An interaction among processing method, moisture, and storage length affected fermentation pattern, with either two- or three-way interactions. Overall, pH tended to decrease, whereas lactic acid, acetic acid, and ethanol increased during the storage period (Figure 2). The majority of changes in the concentration of fermentation products occurred within the first month, except for acetic acid, which continued to increase throughout the storage period. Compared with grain rehydrated to 35% or 40% moisture, silages with 30% moisture had lower concentrations of lactic acid, greater concentrations of ethanol and butyric acid, and higher DML corrected for volatiles than grain rehydrated to 35% or 40% moisture, especially for the ground corn. Rolled RCG had lower DML corrected for volatiles than ground RCG, regardless of moisture content. Respective values of DML corrected and uncorrected for volatiles at 180 d of storage were as follows: rolled-30% moisture 3.04 and 4.68%; rolled-35% moisture 2.76 and 4.45%; rolled-40% moisture 3.08 and 4.78%; ground-30% moisture 5.73 and 8.97%; ground-35% moisture 4.88 and 7.46%; and ground-40% moisture 4.35 and 6.72%.

Ammonia-N concentration continuously increased with storage length and tended to have higher values in ground corn silage with 35% or 40% of moisture, mainly after 60 d of storage. The DMD in situ at 12 h was only affected ($P < 0.01$) by the main effects of processing, moisture, and duration of storage. Ground grain had higher DMD than rolled grain. Storing for longer periods and increasing the moisture content enhanced the DMD (Figure 3).

Discussion

Grain processing methods to reduce particle size or to alter gelatinization of starch or the protein matrix associated with starch granules have been successfully used on commercial farms to increase the extent of grain digestion. Although the fermentation process is not novel, the ensiling process of RCG

Table 1. Statistical analysis (P -values) of the influence of processing method (P), moisture level (M), and storage length (S) on the fermentation profile, particle size, and ruminal disappearance of rehydrated corn grain silage

Item	P	M	S	P × M	P × S	M × S	P × M × S
GMPS	<0.01	0.03	<0.01	0.19	0.51	0.70	0.66
pH	<0.01	<0.01	<0.01	<0.01	<0.01	0.16	0.28
Lactic acid	0.72	<0.01	<0.01	<0.01	0.51	<0.01	<0.01
Acetic acid	<0.01	0.08	<0.01	<0.01	<0.01	0.72	0.04
Butyric acid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ethanol	<0.01	<0.01	<0.01	0.14	<0.01	0.89	0.99
NH ₃ -N	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.57
DML	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DMD ¹	<0.01	<0.01	<0.01	0.19	0.88	0.20	0.99

¹In situ DMD.

Table 2. Distribution of ground (hammer mill, 5-mm screen) or rolled corn grain particles at ensiling and 180 d of storage (% retained, unless otherwise stated)

Item	At ensiling		180-d storage		SEM	P-value ¹		
	Rolled	Ground	Rolled	Ground		P	S	P × S
Sieve opening, mm								
8.00	0.39	0.02	0.42	0.03	0.057	<0.01	0.54	0.61
4.75	6.12	0.40	6.45	0.54	0.406	<0.01	0.13	0.46
2.00	57.3	8.81	61.3	12.3	1.61	<0.01	<0.01	0.62
1.18	23.5	51.1	20.7	53.8	1.79	<0.01	0.95	0.07
0.60	8.57	25.1	7.39	20.3	0.35	<0.01	<0.01	<0.01
0.30	3.27	10.3	2.77	8.43	0.639	<0.01	<0.01	0.03
Pan	0.85	4.27	0.89	4.60	0.694	<0.01	0.67	0.77
Particles passing through a 4.75-mm sieve	93.5	99.6	93.1	99.4	2.54	0.09	0.99	0.92
GMPS ² , mm	2.24	1.13	2.35	1.27	0.041	<0.01	<0.01	0.76
GSD ² , mm	1.49	0.76	1.53	0.92	0.046	<0.01	<0.01	0.13
Particles ³ per gram	151	691	141	463	32.4	<0.01	<0.01	<0.01
Surface area ³ , cm ² /g	19.4	27.8	19.0	25.8	0.35	<0.01	<0.01	0.02

¹Probabilities for processing effect (P), storage effect (S), and interaction of P × S. Probabilities for moisture effect and its interactions were not significant (P ≥ 0.11).

²GMPS and GSD were calculated according to [ASABE \(2008\)](#); method S319.4).

³Surface area and particles per gram were calculated according to [Baker and Herrman \(2002\)](#).

Figure 1. GMPS of rehydrated corn grain silages. Rolled: 30% (○), 35% (□), and 40% (△) of moisture; Ground: 30% (●), 35% (■), and 40% (▲) of moisture. Error bars show the standard error of the mean. The value shown for SEM is the pooled standard error of the mean.

still requires adjustments to optimize nutrient conservation and availability.

Our results show that fermentation was affected by processing, moisture content, storage length, and their interactions. As reported in previous studies ([Fernandes, 2014](#); [da Silva et al., 2019](#)), the concentration of fermentation end products except for ethanol increased throughout storage, but in most cases, there was a marked effect of moisture and processing.

In this study, the lower moisture content (30%) might have curtailed the proliferation of lactic acid bacteria (LAB), as indicated by the lower final concentration of lactic acid and higher pH, in both ground and rolled RCG. Also, the lower concentration of lactic acid and higher pH were probably in part due to the clostridial fermentation that converts lactic acid to butyric acid ([McDonald et al., 1991](#)). [Mombach et al. \(2019\)](#) also reported the benefits of increasing moisture

content of RCG, with an improved fermentation pattern (low pH associated with less loss of gas and effluent) observed at approximately 38% of moisture. In our study, the concentration of butyric acid was highest in RCG with 30% of moisture compared with other treatments. Although butyric acid is not produced exclusively by Clostridia, the butyric acid concentration is a marker of clostridial development in silages ([McDonald et al., 1991](#)). In well-preserved silages, butyric acid may be absent or only detected in trace concentrations ([Kung et al., 2018](#); [da Silva et al., 2019](#)). In ground RCG with 30% of moisture, the concentration of butyric acid was greatest at the onset of fermentation, whereas in rolled RCG with 30% of moisture, the accumulation of butyric acid was modest and increased along with storage length. Differences in butyric acid production were likely associated with variations in lactic acid concentration and pH, which are capable of suppressing butyric fermentation ([Baird-Parker, 1980](#)). [Lindgren \(1991\)](#) found, under laboratory conditions, minimum inhibitory concentrations (MIC) of undissociated lactic acid of 5 to 10 mM for *Clostridium tyrobutyricum*, the most common species in whole-plant silages. In our study, after day 14 of storage, ground RCG with 30% of moisture contained 36 mM of undissociated lactic acid in silage fluid (calculated from pH = 4.11 and lactic acid = 0.39% DM), which is greater than the MIC found by [Lindgren \(1991\)](#). [Fernandes \(2014\)](#) also observed a butyric fermentation in RCG with 31% to 32% of moisture, even when lactic acid concentrations were approximately 0.6% to 1.1% DM (i.e., 6 to 30 mM), which are also above the MIC reported by [Lindgren \(1991\)](#). The reason why MIC values obtained in controlled laboratory experiments often appear to be low might be associated with the heterogeneous nature of silages, which may contain good silage together with small niches of poorly fermented silage ([Pauly, 1999](#)). Results from the current study underscore that RCG should not be ensiled with low moisture content due to the risk of clostridial toxin development. Recently, [Guizelini et al. \(2019\)](#) reported an outbreak of botulism in feedlot cattle fed contaminated RCG (>1,000 animals died in 4 d). The authors detected a silage sample with high pH (7.4) and botulinum toxin, which is associated with the presence of molds and suggested inadequate storage, likely enabling the

Figure 2. pH (A), lactic acid (B), acetic acid (C), ethanol (D), and butyric acid (E) concentrations, and DML (F) in rehydrated corn grain silages. Rolled: 30% (○), 35% (□), and 40% (△) of moisture; Ground: 30% (●), 35% (■), and 40% (▲) of moisture. Error bars show the standard error of the mean. The value shown for SEM is the pooled standard error of the mean.

development of *Clostridium botulinum* even in the absence of animal waste contamination. While higher moisture contents improve the fermentation of RCG, the addition of excessive amounts of water during grain reconstitution could result in effluent loss and increased silage heterogeneity across the silo face, mainly between the top (drier) to bottom layers (wetter). Thus, the use of 35% moisture may be the most adequate

as supported by data from the present study. This moisture content for reconstituting corn grain will likely differ from the ideal moisture content of high-moisture harvested corn grain. Harvesting corn above 35% grain moisture may reduce grain yield per hectare and compromise the mechanical shelling (kernels near the ear tip will still be soft and when mashed may plug the cylinder or the combine sieves). Recently, we found

Figure 3. Ammonia concentration (A) and in situ ruminal DMD (B) in rehydrated corn grain silages. Rolled: 30% (○), 35% (□), and 40% (△) of moisture; Ground: 30% (●), 35% (■), and 40% (▲) of moisture. Error bars show the standard error of the mean. The value shown for SEM is the pooled standard error of the mean.

no advantage of harvesting high-moisture flint corn at 37% vs. 32% moisture on the in situ DMD and aerobic stability (Santos et al., 2018).

Grinding rather than rolling slightly increased the concentration of acetic acid in RCG, likely because of the greater availability of nutrients for silage fermentation. Overall, RCG had up to 2.5% of acetic acid (DM basis); this is slightly greater than often reported for corn grain silage (Kung et al., 2018). Nonetheless, moderate concentrations of acetic acid are desirable in silages, due to its ability to inhibit the growth of yeast and molds and improve aerobic stability (Danner et al., 2003).

Ground RCG also had higher concentrations of ethanol than rolled RCG, especially in the ground RCG with 30% of moisture. However, the maximum concentration of ethanol found in this study was 0.8% DM, well within the typical range reported for corn grain silage (0.2% to 2% DM; Kung et al., 2018). Although ethanol produced in silages can originate from different microbes (yeasts, Enterobacteria, Clostridia, Bacilli, and heterofermentative LAB; McDonald et al., 1991), Clostridia might have contributed to ethanol formation, at least in ground RCG with 30% of moisture. Independent of source, ethanol production is undesirable in silages, because it is associated with high DM and net energy losses (Daniel and Nussio, 2011).

Ground RCG had higher DML corrected for volatiles than rolled RCG. The highest DML was observed in ground RCG with 30% moisture, again highlighting the risk of ensiling RCG with low moisture content. This silage had the highest concentrations of ethanol, acetic, and butyric acid, which have pathways that are coupled with CO₂ production, heat production, and substrate disappearance (McDonald et al., 1991; Rooke and Hatfield, 2003), which resulted in greater DML corrected for the loss of volatile compounds. Usually, loss of DM exceeds loss of gross energy during silage fermentation (McDonald et al., 1991); therefore, future research involving animals can determine the caloric value of RCG with different proportions of volatile compounds.

During the fermentation process, proteins, including endosperm prolamins, are partially degraded to amino acids and subsequently deaminated to ammonia and organic acids (Baron et al., 1986; Rooke and Hatfield, 2003; Hoffman et al., 2011). In RCG, proteolytic activity is primarily of bacterial (60%) and kernel enzyme (30%) origin (Junges et al., 2017). As commonly reported for fermented corn grain (Ferraretto et al., 2014; Fernandes, 2014; da Silva et al., 2019), higher NH₃-N concentration was associated with improvements in DMD during storage. Greater values of NH₃-N and DMD were observed in ground RCG with

higher moisture contents. Ferraretto et al. (2014) also reported an inverse relationship of in vitro starch digestibility and DM content in a survey of commercial high-moisture corn samples. The larger surface area caused by grinding and higher moisture content perhaps stimulated proteolysis in RCG. Alternatively, reducing the moisture content impaired proteolysis and decreased the ruminal DMD in RCG with 30% moisture. Even with clostridial development, RCGs with 30% moisture had lower concentrations of NH₃-N, suggesting that clostridia from non-proteolytic groups (e.g., *C. tyrobutyricum* and *C. butyricum*) might have prevailed in those silages (Pahlow et al., 2003).

Compared with ground RCG, rolled RCG had a higher GMPS, which possibly limited the access of proteolytic microorganisms to corn endosperm and consequently, reduced prolamin degradation during storage, as evidenced by the lower values of NH₃-N in these silages. In agreement with the well-known facts that larger particles and a greater presence of prolamins impede starch digestion (McAllister et al., 1993), rolled RCG had lower in situ ruminal DMD than ground RCG. This finding agrees with a short-term study conducted with high-moisture corn (Saylor et al., 2020), which highlighted the greater in situ ruminal starch disappearance observed for finely (1 mm) vs. coarsely (4 mm) ground corn was related to both differences in surface area and extent of fermentation, which could have enhanced the proteolysis of prolamin proteins as determined solely by soluble CP and NH₃-N concentrations. Although changes in ammonia and soluble CP concentrations certainly reflect the proteolysis of corn grain proteins, prolamins comprise less than 50% of the protein fraction in corn grain. Thus, these effects were only partially related to prolamin proteins, and future research should conduct prolamin protein assays to measure its alteration and verify that prolamin degradation during fermentation was affected by GMPS.

Contrary to our expectations, the GMPS slightly increased across the storage period, mainly in ground RCG with 40% of moisture content. This finding contrasts with results recently reported for high-moisture corn (Saylor et al., 2020). In that study, the onset of fermentation (14 vs. 0 d unfermented grain) reduced GMPS in coarsely (4 mm) but not finely (1 mm) ground corn. On the other hand, Oliveira (2020) also observed an increase of GMPS in RCG (35% of moisture) from 0 to 60 d of fermentation. Differences among the current and past studies may be related to the differences in storage duration and particle size. Although this phenomenon is not fully comprehended in grain silages, it may have been caused by two different mechanisms. First, the degradation of the protein matrix and the higher moisture content

may have promoted an aggregation of small particles, which were not completely separated by dry sieving. Shotton and Harb (1966) observed that moderate moisture levels (32% to 55%) increase the cohesion of corn starch particles. In addition, starch molecules, although highly hydrophilic, are surrounded by a hydrophobic protein matrix (Shewry et al., 1995; Hoffman et al., 2011). With protein matrix degradation and a reduction of its water-repellent effect, starch granules underwent a higher exposure to moisture, probably allowing higher cohesion among particles (Shotton and Harb, 1966; Chanvrier et al., 2006). On the other hand, considering that GMPS is a value calculated from the relative prevalence of particles of certain sizes present in a sample, the disappearance of small particles by solubilization or degradation is another possible reason for an increase in GMPS during storage. Among other nutrients, some starch disappearance may occur during silage storage (Miyaji et al., 2017; Ning et al., 2017). In our study, recovery of small particles retained on 1.18-, 0.60-, and 0.30-mm sieves was lower than 100% (Supplemental Table S1), indicating that such particles might have solubilized or degraded during silage fermentation. However, especially for ground RCG, particle recoveries higher than 100% in 2.00- and 4.75-mm sieves (Supplemental Table S1), without a concomitant decrease of particle recovery in upper sieves, suggest that some particles might have aggregated. Therefore, both mechanisms likely contributed to increasing GMPS values in our study.

Meanwhile, in situ ruminal DMD increased during storage regardless of the slight increase in GMPS. If some particles were perhaps agglutinated, they may have been separated after contact with rumen fluid and exposure to rumen contractions, whereas an increase of GMPS during storage can be an artifact of the particle size measurement technique. Nevertheless, the benefit of longer storage periods was much higher than the effect of particle size on DMD. At ensiling, reducing the GMPS by grounding vs. rolling increased DMD by 7% units, whereas storing for 180 d increased DMD by 35% units (average across moisture contents). Therefore, GMPS alone did not fully explain the ruminal degradation of RCG. Hoffman et al. (2012) reported that GMPS alone accounted for only 50% of the fermentation potential of dry or ensiled corn grain. When the authors also included the concentration of prolamins in dry grain or $\text{NH}_3\text{-N}$ in ensiled grain, their model accounted for 84% of the fermentation potential of corn grain. In RCG, the increase in DMD along with the storage period, without a concomitant reduction in GMPS, appears related to the level of proteolytic activity and capability of degrading the protein matrix (Hoffman et al., 2011), as indicated by the higher $\text{NH}_3\text{-N}$ concentration found in the current study. In a previous study from our laboratory, Fernandes (2014) showed a curvilinear reduction in prolamin concentration during the storage of RCG and high-moisture corn, either in dent or flint hybrids. In that study, DMD in situ at 12 h was negatively correlated with prolamin concentration ($r = 0.92$) while the DMD at intercept (prolamin = 0) was 98.4%, indicating that prolamin is an important constrain for starch digestion when grains with different proportion of vitreous endosperm were ground using the same mill and stored either as RCG or high-moisture corn for different periods.

Based on DMD values observed in this study, it is worth of noting that ensiling markedly increased the fractional rate of disappearance of DM (but not necessarily starch) for RCG. Considering an exponential model with 1-pool and the first-order approach [ruminal disappearance = $kd / (kd + kp)$, where kp is the fractional rate of passage], one may infer that the fractional rate of degradation (kd) increased from 6% to 21%–28%/h in rolled RCG, and from 7% to 29%–60%/h in ground RCG,

from 0 to 180 d of storage, respectively. Although greater starch disappearance is associated with greater total-tract digestibility (Owens et al., 1997), in some cases, a surplus of rumen-degraded starch in the diet can cause hypophagia and limit the intake of metabolizable energy (Owens and Thornton, 1976; Allen, 2020). Hence, altering the processing method, moisture content, and storage length may be used as tools to manipulate the dietary level of rumen-degradable starch. Additional studies on the effects of particle size and moisture content on the fractional passage rate of RCG are warranted to improve the precision of models capable of predicting the proportion of rumen-degraded starch.

Conclusions

In conclusion, contrary to our hypothesis, longer storage and greater moisture content did not offset the lower ruminal disappearance of rolled- compared with ground-corn grain silage. However, the choice between rolling or grinding corn prior to rehydration requires the consideration of farm capabilities, dietary composition, and animal traits. Nonetheless, rehydrated corn grain silage should be ensiled with 35% of moisture and stored for at least 2 mo to improve the ruminal disappearance and reduce the formation of undesirable fermentation end products.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest

The authors declare no real or perceived conflicts of interest.

Literature Cited

- Ahmadi, F., G. R. Ghorbani, A. Sadeghi-Sefidmazgi, M. Heydari, H. Rafiee, and K. A. Beauchemin. 2020. Performance and feeding behavior of dairy cows fed high-concentrate diets containing steam-flaked or ground corn varying in particle size. *J. Dairy Sci.* **103**:3191–3203. doi:10.3168/jds.2019-17344
- Allen, M. S. 2020. Review: Control of feed intake by hepatic oxidation in ruminant animals: integration of homeostasis and homeorhesis. *Animal* **14**(S1):s55–s64. doi:10.1017/S1751731119003215
- ASABE. *Methods of determining and expressing fineness of feed materials by sieving*. St. Joseph (MI): American Society of Agricultural and Biological Engineers; 2008. Standard No.: S319.4; p. 1–8. Available from <https://elibrary.asabe.org/abstract.asp?aid=24485&t=2&redir=&redirType=>. Accessed February 1, 2020.
- Baird-Parker, A. C. 1980. Organic acids. In: Silliker, J. H., editor. *Microbial ecology of foods*. New York (NY): Academic Press; p. 126–135.
- Baker, S., and T. Herrman. 2002. *Evaluating particle size*. Bulletin No. MF-2051. Manhattan (KS): Feed Manufacturing, Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Kansas State University.

- Baron, V. S., K. R. Stevenson, and J. G. Buchanan-Smith. 1986. Proteolysis and fermentation of grain-corn ensiled at several moisture levels and under several simulated storage methods. *Can. J. Anim. Sci.* 66:451–461. doi:[10.4141/cjas86-047](https://doi.org/10.4141/cjas86-047)
- Benton, J. R., T. Klopfenstein, and G. E. Erickson. 2005. Effects of corn moisture and length of ensiling on dry matter digestibility and rumen degradable protein. Lincoln (NE): Nebraska Beef Cattle Reports; p. 31–33.
- Bernardes, T. F., M. V. S. Cardoso, and L. M. Lima. 2018. Silage feeding programs on intensive dairy farms. *J. Dairy Sci.* 2:257.
- Bernardes, T., and T. Castro. 2019. Silages and roughage sources in the Brazilian beef feedlots. *J. Anim. Sci.* 97(Suppl. 3):411–411. doi:[10.1093/jas/skz258.815](https://doi.org/10.1093/jas/skz258.815)
- Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8:130–132. doi:[10.1093/clinchem/8.2.130](https://doi.org/10.1093/clinchem/8.2.130)
- Chanhvri, H., G. Della Valle, and D. Lourdin. 2006. Mechanical behaviour of corn flour and starch–zein based materials in the glassy state: a matrix–particle interpretation. *Carbohydr. Polym.* 65:346–356. doi:[10.1016/j.carbpol.2006.01.027](https://doi.org/10.1016/j.carbpol.2006.01.027)
- Correa, C. E., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. *J. Dairy Sci.* 85:3008–3012. doi:[10.3168/jds.S0022-0302\(02\)74386-5](https://doi.org/10.3168/jds.S0022-0302(02)74386-5)
- Daniel, J. L. P., T. F. Bernardes, C. C. Jobim, P. Schmidt, and L. G. Nussio. 2019. Production and utilization of silages in tropical areas with focus on Brazil. *Grass Forage Sci.* 74:188–200. doi:[10.1111/gfs.12417](https://doi.org/10.1111/gfs.12417)
- Daniel, J. L. P., and L. G. Nussio. 2011. Contribution of silage volatile compounds for the animal nutrition. In: Daniel, J. L. P., M. Zopollato, and L. G. Nussio, editors. Proceedings of the 2nd International Symposium on Forage Quality and Conservation (FEALQ); November 16 to 19, 2011; Piracicaba, Brazil:FEALQ; p. 1–24.
- Danner, H., M. Holzer, E. Mayrhuber, and R. Braun. 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.* 69:562–567. doi:[10.1128/aem.69.1.562-567.2003](https://doi.org/10.1128/aem.69.1.562-567.2003)
- Dias Junior, G. S., L. F. Ferraretto, G. G. S. Salvati, L. C. de Resende, P. C. Hoffman, M. N. Pereira, and R. D. Shaver. 2016. Relationship between processing score and kernel-fraction particle size in whole-plant corn silage. *J. Dairy Sci.* 99:2719–2729. doi:[10.3168/jds.2015-10411](https://doi.org/10.3168/jds.2015-10411)
- Dombrink-Kurtzman, M. A., and J. A. Bietz. 1993. Zein composition in hard and soft endosperm of maize. *Cereal Chem.* 70:105–108.
- Fernandes, J. 2014. Influence of genotype, maturity, and storage length on the quality of corn grain silages [master's thesis]. Piracicaba (Brazil): Department of Animal Science, University of São Paulo, ESALQ.
- Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion and milk production by dairy cows through a meta-analysis. *J. Dairy Sci.* 96:533–550. doi:[10.3168/jds.2012-5932](https://doi.org/10.3168/jds.2012-5932)
- Ferraretto, L. F., K. Taysom, D. M. Taysom, R. D. Shaver, and P. C. Hoffman. 2014. Relationships between dry matter content, ensiling, ammonia-nitrogen, and ruminal in vitro starch digestibility in high-moisture corn samples. *J. Dairy Sci.* 97:3221–3227. doi:[10.3168/jds.2013-7680](https://doi.org/10.3168/jds.2013-7680)
- Galyean, M. L., D. G. Wagner, and F. N. Owens. 1979. Corn particle size and site and extent of digestion by steers. *J. Anim. Sci.* 49:204–210. doi:[10.2527/jas1979.491204x](https://doi.org/10.2527/jas1979.491204x)
- Guizelini, C. C., R. A. A. Lemos, J. L. P. de Paula, R. C. Pupin, D. C. Gomes, C. S. L. Barros, D. A. Neves, L. O. B. Alcântara, R. O. S. Silva, F. C. F. Lobato, et al. 2019. Type C botulism outbreak in feedlot cattle fed contaminated corn silage. *Anaerobe* 55:103–106. doi:[10.1016/j.anaerobe.2018.11.003](https://doi.org/10.1016/j.anaerobe.2018.11.003)
- Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblenz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *J. Dairy Sci.* 94:2465–2474. doi:[10.3168/jds.2010-3562](https://doi.org/10.3168/jds.2010-3562)
- Hoffman, P. C., D. R. Mertens, J. Larson, W. K. Coblenz, and R. D. Shaver. 2012. A query for effective mean particle size in dry and high-moisture corns. *J. Dairy Sci.* 95:3467–3477. doi:[10.3168/jds.2011-5126](https://doi.org/10.3168/jds.2011-5126)
- Junges, D., G. Morais, M. H. F. Spoto, P. S. Santos, A. T. Adesogan, L. G. Nussio, and J. L. P. Daniel. 2017. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. *J. Dairy Sci.* 100:9048–9051. doi:[10.1080/10942912.2011.650339](https://doi.org/10.1080/10942912.2011.650339)
- Kung, L., D. B. Grieve, J. W. Thomas, and J. T. Huber. 1984. Added ammonia or microbial inocula for fermentation and nitrogenous compounds of alfalfa ensiled at various percents of dry matter. *J. Dairy Sci.* 67:299–306. doi:[10.3168/jds.S0022-0302\(84\)81302-8](https://doi.org/10.3168/jds.S0022-0302(84)81302-8)
- Kung, L. Jr, R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy Sci.* 101:4020–4033. doi:[10.3168/jds.2017-13909](https://doi.org/10.3168/jds.2017-13909)
- Lindgren, S. 1991. Hygienic problems in conserved forage. In: Pahlow G., and H. Honig, editors. Proceedings of a Conference on Forage Conservation towards 2000. Braunschweig (Germany): Federal Research Center of Agriculture (FAL); p. 177–190.
- McAllister, T. A., R. C. Phillippe, L. M. Rode, and K. J. Cheng. 1993. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. *J. Anim. Sci.* 71:205–212. doi:[10.2527/1993.711205x](https://doi.org/10.2527/1993.711205x)
- McDonald, P., N. Henderson, and S. Heron. 1991. *The biochemistry of silage*. 2nd ed. Marlow (UK): Chalcombe Publications.
- Miyaji, M., H. Matsuyama, and K. Nonaka. 2017. Effect of ensiling process of total mixed ration on fermentation profile, nutrient loss and in situ ruminal degradation characteristics of diet. *Anim. Sci. J.* 88:134–139. doi:[10.1111/asj.12610](https://doi.org/10.1111/asj.12610)
- Mombach, M. A., D. H. Pereira, D. S. Pina, D. C. Bolson, and B. C. Pedreira. 2019. Silage of rehydrated corn grain. *Arq. Bras. Med. Vet. Zootec.* 71:959–966. doi:[10.1590/1678-4162-9676](https://doi.org/10.1590/1678-4162-9676)
- Ngonjamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, and J. G. Lauer. 2008. Relationship between kernel vitreousness and dry matter degradability for diverse corn germplasm. II. Ruminal and post-ruminal degradabilities. *Anim. Feed Sci. Technol.* 142:259–274. doi:[10.1016/j.anifeedsci.2007.09.001](https://doi.org/10.1016/j.anifeedsci.2007.09.001)
- Ning, T., H. Wang, M. Zheng, D. Niu, S. Zuo, and C. Xu. 2017. Effects of microbial enzymes on starch and hemicellulose degradation in total mixed ration silages. *Asian-Australas. J. Anim. Sci.* 30:171–180. doi:[10.5713/ajas.16.0046](https://doi.org/10.5713/ajas.16.0046)
- Oliveira, K. S. 2020. Effect of length of storage and chemical additives on the nutritive value and starch degradability of reconstituted corn grain silage [master's thesis]. Piracicaba (Brazil): Department of Animal Science, University of São Paulo, ESALQ.
- Owens, F., and M. Basalan. 2013. Grain processing: gain and efficiency responses by feedlot cattle. In: Cravey, M., B. Hicks, N. Elam, M. Miller, and T. McCollum, editors. Proceedings of the Plains Nutrition Council Spring Conference. April 18 to 19, 2013; Amarillo (TX): The Plains Nutrition Council; p. 76–100.
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. *J. Anim. Sci.* 75:868–879. doi:[10.2527/1997.753868x](https://doi.org/10.2527/1997.753868x)
- Owens, F. N., and J. H. Thornton. 1976. Moisture content versus intake and energy value of high moisture corn. In: Gill, D., F. Owens, and D. Wagner, editors. Proceedings of the High Moisture Grains Symposium; Stillwater, OK; July 22 to 23, 1976; p. 193–195.
- Pahlow, G., R. E. Muck, F. Driehuis, S. J. W. H. Oud Elferink, and S. F. Spoelstra. 2003. Microbiology of ensiling. Chapter 2. In: Buxton, D. R., R. E. Muck, and J. H. Harrison, editors. *Silage science and technology*. Madison (WI): American Society of Agronomy; p. 31–93.

- Pauly, T., 1999. *Heterogeneity and hygienic quality of grass silage* [PhD dissertation]. Uppsala (Sweden): Swedish University of Agricultural Sciences.
- Philippeau, C., and B. Michalet-Doreau. 1997. Influence of genotype and stage of maturity of maize on rate of ruminal starch degradation. *Anim. Feed Sci. Technol.* **68**:25–35. doi:[10.1016/S0377-8401\(97\)00042-4](https://doi.org/10.1016/S0377-8401(97)00042-4)
- Pryce, J. D. 1969. A modification of Barker-Summerson method for determination of lactic acid. *Analyst (Lond.)* **94**:1151–1152. doi:[10.1039/an9699401151](https://doi.org/10.1039/an9699401151)
- Rémond, D., J. I. Cabrera-Estrada, M. Champion, B. Chauveau, R. Coudure, and C. Poncet. 2004. Effect of corn particle size on site and extent of starch digestion in lactating dairy cows. *J. Dairy Sci.* **87**:1389–1399. doi:[10.3168/jds.S0022-0302\(04\)73288-9](https://doi.org/10.3168/jds.S0022-0302(04)73288-9)
- Rooke, J. A., and Hatfield, R. D. 2003. Biochemistry of ensiling. In: Buxton, D. R., R. E. Muck, and J. H. Harrison, editors. *Silage science and technology*. Madison (WI): American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. Publications; p. 95–139.
- Santos, R. M., F. A. Jacovaci, T. Garcia-Diaz, K. C. Scheidt, C. C. Jobim, and J. L. P. Daniel. 2018. Effects of moisture and *L. buchneri* on the conservation and ruminal degradability of high moisture corn and snaplage. In: Gerlach K., and K-H Südekum, editors. *Proceedings of the XVIII International Silage Conference*; July 24 to 26, 2018; Andernach (Germany): OundZ GmbH; p. 498–499.
- Saylor, B. A., F. Casale, H. Sultana, and L. F. Ferraretto. 2020. Effect of microbial inoculation and particle size on fermentation profile, aerobic stability and ruminal in situ starch disappearance of high-moisture corn ensiled for a short period. *J. Dairy Sci.* **103**:379–395. doi:[10.3168/jds.2019-16831](https://doi.org/10.3168/jds.2019-16831)
- Shewry, P. R., J. A. Napier, and A. S. Tatham. 1995. Seed storage proteins: structures and biosynthesis. *Plant Cell* **7**:945–956. doi:[10.1105/tpc.7.7.945](https://doi.org/10.1105/tpc.7.7.945)
- Shotton, E., and N. Harb. 1966. The effect of humidity and temperature on the cohesion of powders. *J. Pharm. Pharmacol.* **18**:175–178. doi:[10.1111/j.2042-7158.1966.tb07844.x](https://doi.org/10.1111/j.2042-7158.1966.tb07844.x)
- da Silva, N. C., C. F. Nascimento, V. M. A. Campos, M. A. P. Alves, F. D. Resende, J. L. P. Daniel, and G. R. Siqueira. 2019. Influence of storage length and inoculation with *Lactobacillus buchneri* on the fermentation, aerobic stability, and ruminal degradability of high-moisture corn and rehydrated corn grain silage. *Anim. Feed. Sci. Tech.* **251**:124–133. doi:[10.1016/j.anifeedsci.2019.03.003](https://doi.org/10.1016/j.anifeedsci.2019.03.003)
- Szasz, J. I., C. W. Hunt, P. A. Szasz, R. A. Weber, F. N. Owens, W. Kezar, and O. A. Turgeon. 2007. Influence of endosperm vitreousness and kernel moisture at harvest on site and extent of digestion of high-moisture corn by feedlot steers. *J. Anim. Sci.* **85**:2214–2221. doi:[10.2527/jas.2006-288](https://doi.org/10.2527/jas.2006-288)
- Weissbach, F. 2009. Correction of dry matter content of silages used as substrate for biogas production. In: Broderick, G., J. Harrison, L. Bocher, F. Contreras-Govea, R. Muck, K. Bolsen, and A. Adesogan, editors. *Proceedings of the 15th International Silage Conference*; July 27 to 29, 2009; Madison, WI. Madison (WI): US Dairy Forage Research Center; p. 483–484.