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Short communication: Effect of washing method, grinding size, and the determination of an indigestible fraction on in situ degradation of starch in mature corn grain

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ABSTRACT

The objectives of this study were to determine (1) the effect of grinding size (1, 2, 4, and 6 mm) to determine effective ruminal disappearance (ERD); (2) the most adequate method to estimate the rapidly degradable fraction (A); (3) a time point to measure the indigestible fraction (C); and (4) the viability of using fewer time points to estimate starch fractional disappearance rate (k_d) of mature corn grain. Fraction A was determined by rinsing in a bucket or washing machine, rumen immersion followed by bucket or washing machine, and water immersion for 30 min followed by bucket or washing machine. Ruminal in situ incubations were performed at 48, 72, 96, and 120 h to determine fraction C, and at 0 (washing machine), 3, 6, 12, 18, 24, and 48 h to determine the kinetics of starch disappearance. Models were used with either 2 or 3 pools and k_d was determined by the linear slope of the log-transformed bag residues as a proportion of incubated samples over time. The ERD was calculated as A + B [$k_d/(k_d + kp)$], where kp is the ruminal fractional passage rate = 16.0% h^{-1}. Data were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC) with the fixed effects of run (for fraction A analysis only) method (either washing or model), grinding size, and method by grinding size interaction, with cow as a random effect. Correlation between estimates calculated using all time points or combinations of 2 and 3 time points were determined using PROC CORR. Fraction A was reduced as grinding size increased, but was not altered by washing method. Samples ground at 6 mm had greater fraction C than other grinding sizes at 48, 72, or 96 h, but not at 120 h. Model affected the slowly degradable fraction (B) values solely, but the difference was minor (0.5 percentage units). Greater fractions B and C but reduced k_d and ERD were observed as grinding size increased. Based on correlation analysis the 2-pool model, incubation times of 0, 3, and 48 h were suitable to evaluate ruminal starch degradation kinetics in mature corn. Ruminal in situ incubation at 120 h highlighted the lack of a fraction C of starch (0.13% of starch). Washing method did not affect determination of fraction A of starch. Ruminal in situ incubations of 0, 3, and 48 h for starch degradation kinetics using a 2-pool model were adequate for mature ground corn, but 120 h of incubation is suggested to confirm the existence or absence of a fraction C. Grinding size affected starch degradation kinetics and fraction A determination.

Key words: soluble fraction, effective ruminal degradation, fractional degradation rate, starch

Short Communication

Starch is a major energy source for both ruminant animals and ruminal microorganisms (Moharrery et al., 2014). However, ruminal in vivo starch digestibility varies from 25 to 95% of intake, which in turn may alter lactation performance and feed efficiency by dairy cows (Firkins et al., 2001; Ferraretto et al., 2013). Nevertheless, it is difficult to account for this variation in nutritional models, as a standard method established to evaluate the ruminal disappearance of starch has not been defined (Peyrat et al., 2014). Considering that predicting total-tract starch digestibility from fecal starch concentration is reliable (Fredin et al., 2014), an accurate estimation of ruminal starch digestibility would provide useful information for ration formulation, as it alters efficiency of energy usage, ruminal microbial synthesis, and the occurrence of ruminal acidosis (Krause et al., 2002; Firkins et al., 2006). Furthermore, more accurate predictions to rank feedstuffs would benefit various industry sectors.

The ruminal in situ digestibility method is one of the standard procedures used for determination of ruminal starch digestibility through the measurement of nutrient disappearance from bags containing feed samples and

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incubated in the rumen across various time points (Seifried et al., 2016). With the use of several time points, we could determine the soluble or rapidly degradable fraction (fraction A), the slowly degradable fraction (fraction B), and the indigestible fraction (fraction C); however, this procedure is not standardized with amount and length of time points varying substantially (Dias Junior et al., 2016; Seifried et al., 2016; Dieho et al., 2017). The ruminal in situ technique may also be affected by other factors. For example, the sample particle size may interfere with the estimation of ruminal degradation due to the potential loss of small particles through the pores of incubation bags (Philippeau and Michalet-Doreau 1997), and grinding size varies across laboratories. Furthermore, different methodologies applied during bag washing can affect the estimation of fraction A. Moreover, the interaction between grinding size and washing method or time points to determine fractions A and C, respectively, is undefined and warrants further investigation.

The lack of a standardized method makes the comparison among feeds analyzed by different research laboratories unfeasible and hinders the ranking of feedstuffs and the establishment of more precise prediction models. Therefore, our experimental objectives were (1) to determine the effect of grinding size on fractions A and C, starch fractional disappearance rate ($\mathbf{k_d}$), and effective ruminal disappearance (**ERD**) in mature ground corn; (2) to evaluate washing methods to estimate fraction A of starch; (3) to determine a time point to measure fraction C of starch; (4) to determine the correlation between starch k_d estimated with various time points; and (5) to measure starch ERD and k_d of various starch sources using the methodology defined by objectives 2, 3, and 4. We hypothesized that grinding size would play a major role in estimating ERD in mature ground corn and that the use of a methodology with few incubation time points to calculate starch ruminal degradation kinetics would yield the same ERD as the prediction performed with all time points.

A batch of 4 kg of dried mature corn was homogenized and allocated into 4 samples using a quartering technique. Briefly, samples were homogenized in a bag and divided into 4 equal subsamples; 2 subsamples were saved for later treatment, whereas the 2 other subsamples were rehomogenized and redivided. The process was repeated until 4 subsamples of 500 g were prepared. Each subsample was assigned to a grinding size and ground to pass 1-, 2-, 4-, or 6-mm sieves in a Wiley mill (A. H. Thomas Scientific, Philadelphia, PA). Each sample (82.4 ± 14.5 g; mean ± SD) was dry sieved using a Tyler Ro-Tap Shaker (model RX-29, W.S. Tyler, Mentor, OH) using a set of 9 sieves (W.S. Tyler) with nominal square apertures of 6.70, 4.75, 3.35, 2.36, 1.70, 1.18, 0.59, 0.20, and 0.15 mm and a pan (ASABE, 2007) to determine particle size distribution. Geometric mean particle size (μm) and surface area (cm^2/g) were calculated using a log normal distribution (Baker and Herrman 2002).

Ruminal in situ procedures were conducted at the University of Florida Dairy Research Unit (Gainesville, FL) under a protocol approved by the University of Florida, Institute of Food and Agricultural Sciences, Animal Care Research Committee. Dacron polyester cloth bags (R1020, 10 × 20 cm and 50 ± 10 micro porosity; Ankom Technology, Macedon, NY) containing 5.04 ± 0.02 g of DM, yielding a ratio of sample mass per bag area of 16.6 mg/cm^2, were used to compare fraction A methods, to determine a time point to measure fraction C, and to estimate k_d of fraction B and ERD.

A comparison of 6 methodologies used to determine fraction A of starch was performed. Three independent runs were conducted per method, and samples from each grinding size were used in duplicate within each run. Each run and method contained a blank bag to allow correction for infiltration of DM into sample bags. Methods were (1) to rinse bags until water was clear (6 ± 2 wash/bag) in a bucket with water at room temperature (approximately 22°C); (2) to rinse in a washing machine using the rinse and spin cycle set with room temperature water for a 30-min cycle (Roper RTW4516F*, Whirlpool Corp., Benton Harbor, MI); (3) to insert bags in the rumen, remove immediately after moistened, soak in cold water (water + ice) for 15 min, and rinse in a bucket until water was clear; (4) to insert bags in the rumen, remove immediately after moistened, soak in cold water for 15 min, and rinse using a washing machine set as described for treatment 2; (5) to submerge bags in water (approximated 22°C; 1,000 mL bag) for 30 min, and rinse it in a bucket until water was clear; and (6) to submerge bags in water (approximately 22°C; 1,000 mL bag) for 30 min, and rinse it using a washing machine set as described for treatment . All 6 methods were performed simultaneously for each run and all bags were dried together in a forced-air oven set at 60°C for 48 h. Residues from duplicates within each grinding size treatment for each fraction A method within run were composited and ground to pass the 1-mm sieve in a cyclone mill (UDY Corporation, Fort Collins CO) and analyzed for starch by an enzymatic method (Hall, 2015) with thermostable α-amylase (Ankom Tchnology, Macedon, NY) and amyloglucosidase (Megazyme E-AMGDF, Bray, Co. Wicklow, Ireland) enzymes.

To define the incubation time for fraction C determination, measurements of ruminal in situ starch