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Technical note: In situ ruminal starch disappearance kinetics of hull-less barley, hulled barley, and corn grains

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ABSTRACT

The objective of this study was to compare ruminal starch disappearance rates of hull-less barley, hulled barley, and corn grains. Five different genotypes were used for each of the 2 barley types. In addition, each of these genotypes was grown in 2 different locations and years, resulting 10 independent barley samples for each of the 2 barley grain types. Five different genotypes of corn grain were obtained from a commercial seed company. After being ground to pass through a 4-mm screen of a cutter mill, 3.6 g of each grain was placed into a porous bag, which was then incubated in the rumen of 2 ruminally cannulated cows for 0, 4, 8, 12, 24, and 48 h. Corn grains had greater instant ruminal starch disappearances than barley grains (22.4 and 8.2%, respectively). Instant ruminal starch disappearances did not differ between hulled and hull-less barley grains. Ruminal starch fractional disappearance rates were greatest for hulled barley grains, moderate for hull-less barley grains, and lowest for corn grains (15.3, 13.9, and 7.1%/h, respectively). Ruminal starch half-life was shortest for hulled and hull-less barley grains (4.4 h) and longest for corn grains (6.6 h). Ruminal starch half-life did not differ between hulled barley and hull-less barley grains. In conclusion, using a holistic experimental design and statistical analysis, this study showed that starch from hull-less barley grains has a ruminal half-life similar to that of hulled barley grains and shorter than that of corn grains.
Key words: hull-less barley, hulless barley, in situ, starch half-life

Technical Note

Cereal grains, such as corn, sorghum, barley, wheat, and oats, are included as an energy source in rations for lactating dairy cows. Cereal grains contain high concentrations of starch, a component that is almost completely and uniformly digested in the gastrointestinal tract when processed adequately (Van Soest, 1994; Ferreira and Mertens, 2005; Yang et al., 2017). However, differences exist for ruminal starch disappearance rates among cereal grains. For instance, starch degradability in the rumen is greater for wheat and hulled barley than for corn and sorghum (Herrera-Saldana et al., 1990).

A few studies have determined the ruminal disappearance rates of starch from hull-less barley (Yang et al., 1997, 2013). Yang et al. (1997) measured the in situ ruminal starch disappearance rate of hulled barley, hull-less barley, and corn grains, and reported that starch from hull-less barley disappeared faster than starch from corn but slower than starch from hulled barley. Yang et al. (2013) compared the in situ ruminal starch disappearance rate of 4 genotypes of hull-less barley against 1 genotype of hulled barley, and reported an in situ ruminal starch disappearance rate for hull-less barley that was similar to or slower than that of hulled barley. From these studies, it could be concluded that starch from hull-less barley disappears at slower rates than starch from hulled barley. From the perspective of experimental design, however, these 2 studies (Yang et al., 1997, 2013) lacked the true sense of replication, because a single or only a few hull-less barley grain sources were compared with a single hulled barley grain source. Because of the lack of actual replication, making a broad conclusion contrasting the starch disappearance rates of hulled and hull-less barley grains is not reasonable. This flaw is also applicable to the study of Herrera-Saldana et al. (1990), in which single grain sources were compared with each other without using independent sources of each grain as actual replicates.

In this study, we hypothesized that starch from hull-less barley disappears in the rumen more slowly than starch from hulled barley grains and faster than starch from corn grain. Therefore, our objective was to compare ruminal starch disappearance rates of hull-less barley, hulled barley, and corn grains. To actually com-

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pare the ruminal starch disappearance rates of these grains, multiple and independent sources were used as random and independent replicates for each grain type.

Hulled and hull-less barley grains were obtained from the Small Grains Breeding Program of the Department of Crop and Soil Sciences at Virginia Tech (Blacksburg). For each of the 2 barley types (i.e., hulled and hull-less barley), 5 genotypes were used. In addition, each of these genotypes was grown in 2 diverse environments (different locations and years), resulting in a total of 10 independent barley samples for each of the 2 barley grain types. Five genotypes of corn grain were obtained from a commercial seed company (Dupont-Pioneer, Johnston, IA). Each of the 25 grain samples (Table 1) was ground to pass through the 4-mm screen of a Wiley cutter mill (Thomas Scientific, Swedesboro, NJ). For each of the 25 grain samples, 12 porous Dacron bags (R1020, Ankom Technology, Macedon, NY) were filled with 3.6 g (DM basis) of ground material and sealed. Bag dimensions were 10 cm wide and 20 cm long, and bag pore size was 50 ± 10 μm. The sample amount to bag surface ratio was approximately 9 mg/cm^2 (Ferreira and Mertens, 2005). In addition to an empty bag that served as a blank sample, 6 bags of each of the 25 grain samples were placed in 1 of 12 larger net bags (i.e., laundry-type bags). The Institutional Animal Care and Use Committee (IACUC) of Virginia Tech approved all procedures involving dairy cows. At 0930 h on the test day, all bags were simultaneously immersed within the rumen of 2 cannulated cows fed a TMR containing 40% corn silage, 7% alfalfa hay, and 53% concentrate mix (DM basis). Feed was delivered once daily at 0900 h. Bags were incubated for 0, 4, 8, 12, 24, and 48 h. For the 0-h incubation, bags were totally immersed into the liquid phase of the ventro-distal cavity of the rumen and extracted after 10 s. Once the incubations were finished, bags were placed in a bucket with water and ice to stop bacterial fermentation, rinsed 5 times (3-min washing + spinning cycles) using a washing machine (SKY2767, Best Choice Products, Irvine, CA), and dried in a forced-air oven at 55°C for 48 h. Once dried, all bags were weighed, opened, and the residues were reground for starch analysis. Residues were reground to pass through a 0.5-mm screen of a cyclone mill (Udy Corp., Fort Collins, CO) for samples incubated for 0, 4, 8, and 12 h. Due to the small amount of residue remaining after incubation, residues were reground using a mortar and pestle for samples incubated for 24 and 48 h. Starch concentrations were determined using the acetate buffer method of Hall (2009) with α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland).

Disappearance kinetic parameters were estimated using the NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC), and according to equation [1] (Ørskov and McDonald, 1970):

$$\text{Disappearance } (\%) = A + B \times \left(1 - e^{(-k \times T)}\right), \qquad [1]$$

where T is the time of incubation (h), A is the pool of immediately available starch (%) at time $T = 0$, B is the pool of potentially available starch, and k is the fractional disappearance rate (%/h) of pool B. The half-life of grain starch ($\boldsymbol{T_{50}}$), which we defined as the time at which 50% of the initial starch disappeared, was estimated according to equation [2]:

$$T_{50}(\text{h}) = \log_{\left(1-\left(\frac{50-A}{B}\right)\right)} e^{-k}. \qquad [2]$$

After estimating the kinetic parameters for each grain replicate (n = 50), differences in kinetic parameters among grains were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc.). To test our hypothesis, we challenged the null hypothesis H_0: "T_{50} of hulled barley = T_{50} of hull-less barley = T_{50} of corn" and the alternative hypothesis H_A: "H_0 is not accepted." Data were analyzed as a completely randomized design using a model that included the effects of cow (random, df = 1), grain type (fixed, df = 2), and the residual error (random, df = 46). Differences between grain types were tested using the pdiff option in SAS.

Corn grains had greater ($P < 0.01$) instant ruminal starch disappearances than barley grains (22.4 and 8.2%, respectively; Table 2; Figure 1). Instant ruminal starch disappearances did not differ between hulled and hull-less barley grains ($P = 0.35$). Ruminal starch fractional disappearance rates were greatest for hulled barley grains, moderate for hull-less barley grains, and lowest for corn grains (15.1, 13.7, and 6.9%/h, respectively). Ruminal starch half-life was shortest for hulled and hull-less barley grains (4.4 h) and longest for corn grains (6.6 h). Ruminal starch half-life did not differ

Table 1. Descriptive statistics of starch concentration (%) of hulled barley (n = 10), hull-less barley (n = 10), and corn (n = 5) grains

Grain	Average	SD	Minimum	Maximum
Hulled barley	57.6	2.7	50.9	60.3
Hull-less barley	62.8	1.5	60.3	64.9
Corn	70.9	6.3	64.0	80.2