

In vitro and in situ techniques yield different estimates of ruminal disappearance of barley

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ABSTRACT: Objectives were to compare in vitro and in situ disappearance of dry matter (DM), neutral detergent fiber (NDF), and starch of traditional (unprocessed and rolled) and hulless (unprocessed) barley. Experiment 1: three barley sources were compared using in vitro techniques. The sources were: 1) traditional barley that was not processed, 2) traditional barley processed through a roller mill, and 3) hulless barley that was not processed. For in vitro incubation, each barley source was ground through a 1-mm screen. Ground barley sources were weighed into bags (25 micron porosity) and incubated in ruminal fluid from two steers fed 80% rolled corn for 3, 6, 12, 24, 48, or 72 h. Intact bags were assayed for NDF; remaining bags were opened and the residual was removed and analyzed to determine disappearance of DM and starch. Experiment 2: the barley sources used in Exp. 1 were compared using in situ techniques. For in situ analysis, each barley source was ground in a Wiley mill with no screen to mimic mastication. Artificially masticated samples were weighed into Dacron bags (50 ± 10 micron porosity) and incubated in eight ruminally fistulated steers ($n = 8$) for 3, 6, 12, 24, 48, and 72 h. Residual contents were analyzed to determine in situ disappearance of DM,

NDF, and starch. Data were analyzed using the MIXED procedures of SAS (9.4 SAS Institute, Cary, NC) with repeated measures. DM disappearance was greatest ($P < 0.05$) for hulless barley in vitro and for rolled barley in situ, regardless of time postincubation. For both trials, NDF disappearance was greatest ($P < 0.05$) for hulless barley, regardless of time postincubation. Starch disappearance at all time points was greatest ($P < 0.05$) for rolled barley in situ. Starch disappearance was greater ($P < 0.05$) for hulless barley at 6 h of in vitro incubation compared to rolled and unprocessed barley, whereas starch disappearance in vitro was comparable ($P = 0.60$) between barley sources. When the grains were compared in vitro, minor differences were noted, presumably because barley sources were finely ground prior to incubation. Compared to in vitro estimates, in situ techniques had greater variation in ruminal degradation estimates. Differences observed between in situ and in vitro techniques are driven largely by differences between the procedures. Although laboratory methods are widely used to estimate ruminal degradation, these techniques did not provide comparable estimates of ruminal degradation of barley.

Key Words: barley, cattle, digestibility, hulless barley, processing

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INTRODUCTION

Barley often is fed to animals as an energy source. However, barley grain must be processed to maximize digestion when fed to cattle because its protective hull limits the extent of digestion in the rumen (Holopainen-Mantila, 2015). Improvements in average daily gain and feed efficiency result when processed barley is fed to cattle due to increased total tract organic matter digestibility (Dehghan-banadaky et al., 2007). Dry rolling is one of most common and economical processing methods for barley (Dehghan-banadaky et al., 2007). Even though dry rolling is the cheapest processing method, it is still an expense. Boyles et al. (2001) reported that dry rolling barley costs \$2 to \$5 per ton. In addition to the added expense, processed grains also can negatively affect cattle health and productivity. Production of fine particles and flour during processing increases the surface area of the grain particles. This increased surface area increases the rate of fermentation by rumen microbes (Zhao et al., 2016) and can make ruminal pH more acidic. Excessively acidic ruminal pH is associated with ruminal acidosis, inconsistent intake, and reduced animal performance (Anele et al., 2015).

Hulless barley varieties may improve growth performance when compared with traditional barley varieties because they have less fiber and more starch and protein than traditional barley (Edney et al., 1992). Zinn et al. (1996) reported that compared with traditional barley, cattle fed hulless barley had a 7% greater total tract digestibility, 7% greater net energy value, 9% less feed intake, and 18% less methane production when the barley was fed after being rolled. To our knowledge, the study by Zinn et al. (1996) is novel, being the only trial comparing cattle fed hulless or traditional barley when both sources were either rolled or flaked.

There is a dearth of information comparing the extent of ruminal degradation of hulless barley versus traditional barley for cattle. The hypothesis of this study was that hulless barley may eliminate the need for grain processing because in vitro and in situ disappearance of dry matter (DM), neutral detergent fiber (NDF), and starch should be greater for hulless barley than for traditional unprocessed barley, and should be more similar to rolled barley. Therefore, the objectives of this study were to compare in vitro and in situ disappearance of DM, NDF, and starch of traditional unprocessed barley, rolled barley, and hulless barley.

MATERIALS AND METHODS

All animal procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC protocol #47255). Three barley sources were commercially sourced and tested to determine the difference between in vitro and in situ disappearance of DM, NDF, and starch. The three sources were: 1) traditional barley that was not processed, 2) traditional barley processed through a roller mill, and 3) hulless barley that was not processed.

Initial samples of each barley source were analyzed for DM (method 934.01: AOAC, 1988), starch (Hall, 2009), and NDF and acid detergent fiber (ADF; methods 5 and 6, respectively; Ankom200 Fiber Analyzer, Ankom Technology, Macedon, NY) to determine initial concentrations (Table 1).

Experiment 1: In Vitro

The three barley sources mentioned previously were compared using in vitro techniques. Each barley source was ground through a 1-mm screen on a Wiley mill (Thomas Scientific; Swedesboro, NJ) prior to in vitro incubation. Ground barley sources were weighed into labeled bags (F57 bags with a 25 micron porosity; Ankom Technology). The F57 filter bags were rinsed with acetone in order to remove a surfactant that inhibits microbial digestion. The weight of each bag was recorded and approximately 0.50 g of ground sample was added to each bag. The bags were then heat sealed.

It should be noted that the grinding necessary to obtain a representative sample for the in vitro analysis reduces particle size to great degree than mastication would. Relative differences may still aid in understanding of hulless and traditional barley.

Sealed bags containing the ground samples were incubated in ruminal fluid for 3, 6, 12, 24, 48, or 72 h (method 3 DAISYII Incubator, Ankom Technology). Each Daisy^{II} Incubator digestion jar held 24 sample bags and a blank bag as a correction factor. Residual contents were analyzed to determine in vitro disappearance of DM, NDF, and starch. In order to ensure adequate residual sample for analysis, hours 0, 3, and 6 each had one jar, which contained six bags per sample and one blank. Hour 12 had two jars containing a total of 12 bags per sample and two blanks. Hours 24, 48, and 72 each had four jars each containing a total of 24 bags per sample and four blanks.

Two buffer solutions were made: 1) Buffer A (10 g/L KH_2PO_4 , 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L

Table 1. Analyzed composition of barley sources on a DM basis

	Barley source ¹		
	Hulless	Rolled	Unprocessed
Analyzed composition, % DM basis			
DM	87.43	87.17	87.32
NDF	14.49	22.26	22.45
ADF	1.19	6.38	7.17
Starch	60.56	56.05	57.35

¹Both rolled and unprocessed barley sources were from traditional, hulled barley varieties. The hulless barley source is a different variety, devoid of the outer fibrous hull.

NaCl, 0.1 g/L CaCl₂ · 2H₂O, and 0.5 g/L urea) and 2) Buffer B (15 g/L Na₂CO₃, 1 g/L Na₂S · 9H₂O). Both buffer solutions (A and B) were prewarmed at 39 °C and, in a separate container, 266 mL of solution B was added to 1,330 mL of solution A. The ratio (1:5) was adjusted until the pH was 6.8 at 39 °C. Then, 1,600 mL of the combined buffer solution was added to each of the digestion jars with the samples and put into the Daisy^{II} Incubator. The temperature of the digestion jars was allowed to equilibrate for 20–30 min.

Rumen fluid was taken and mixed from two ruminally fistulated Angus steers that were fed an 80% corn-based diet. Two 2-L thermos bottles were preheated to 39 °C using warm water. Approximately 2 L of rumen fluid was collected from each steer, mixed, and put into one thermos. The other thermos held approximately 500 g of wet particulate matter from the rumen of each steer. Rumen fluid and contents were transported back to the lab immediately after collection so that rumen inoculum was collected within 20 min of its use. The collected solid rumen contents were added to a blender that was preheated to 39 °C using warm water, and the blender was purged with CO₂ gas and blended at high speed for 30 s. The blended digesta was filtered through four layers of cheese cloth into a preheated (39 °C) 5-L flask. The remaining rumen fluid from the additional thermos was filtered through four cheesecloths into the same 5-L flask. The flask was continuously purged with CO₂ gas. This flask of filtered rumen fluid and blended digesta contents made up the “inoculum” for the in vitro preparation. Then, 400 mL of inoculum was added to each digestion jar that already contained the 1,600 mL of the combined buffer solution and the samples. The jar was purged with CO₂ gas for 30 s before the lid was closed.

The digestion jars were incubated in the Daisy^{II} Incubator for 3, 6, 12, 24, 48, and 72 h. When

complete, the jars were removed and the fluid was drained. The bags were rinsed with cold tap water until the water was clear. The bags were then dried at 55 °C for 72 h. Once dried, two of the F57 bags were used for NDF analysis (method 5; Ankom200 Fiber Analyzer). The remaining bags were cut open. The contents were mixed, and subsamples were analyzed for starch (Hall, 2009). Nutrient compositions were corrected for 100 °C DM.

The disappearance of each nutrient was calculated on a DM basis using the formula:

$$\% \text{ loss} = \left[1 - \left(\frac{\text{ending dry sample wt.}}{\text{starting dry sample wt.}} \right) \right] \times 100$$

Data were analyzed using MIXED procedures of SAS (v. 9.4 SAS Institute Inc., Cary, NC) with repeated measures to determine the effects over time. Degrees of freedom were adjusted using the Kenward–Rodger’s adjustment. The compound symmetry covariance structure was determined the best fit based on the Bayesian Information Criterion. The model was:

$$Y_{ijklm} = \mu + c_j + G_k + T_1 + (GT)_{kl} + e_{ijklm}$$

where Y_{ijklm} = responsible variable; μ = the mean; c_j = random effect of sample; G_k = fixed effect of barley source; T_1 = fixed effect of time; $(GT)_{kl}$ = fixed effect of the interaction of barley source and time; and e_{ijklm} = experimental error. Each individual F57 bag was the experimental unit. Significance was declared at $P \leq 0.05$.

Experiment 2: In Situ

The three barley sources mentioned previously were compared using in situ techniques. The traditional, unprocessed barley and hulless barley were each ground twice through a Wiley mill with no screen (Thomas Scientific) to mimic cattle mastication (Dr. Robbi Pritchard, personal communication). Rolled barley was not artificially masticated because it was already processed. Particle size of the artificially masticated traditional and hulless barley sources was determined using the dry sieving procedure from Stark and Kalivoda (2016) except that no flow agent was used. In addition, particle size of the traditional barley, both unprocessed and rolled sources, were determined with the same technique (Table 2). Thus, although barley sources were identical, the key difference between samples incubated in Exp. 1 and 2 lay in the particle size of the grains evaluated due to the difference in techniques.

Table 2. Barley particle size of select whole and artificially masticated samples

	Artificially masticated ¹		Whole samples	
	Hulless ²	Unprocessed	Unprocessed ³	Rolled
Retention on sieve, % DM				
3.35 mm	0.00	18.54	29.09	13.46
2.8 mm	32.24	48.99	56.75	18.30
1.7 mm	64.64	27.15	13.79	33.40
1.18 mm	2.33	2.23	0.23	12.85
0.85 mm	0.41	0.74	0.00	5.98
<0.85 mm	0.25	0.90	0.00	15.54
Geometric mean size, mm	2.40	2.72	2.99	1.66

¹Each barley source was ground twice through a Wiley mill with no screen (Thomas Scientific) to mimic cattle mastication.

²The hulless barley source is a different variety, devoid of the outer fibrous hull.

³Both rolled and unprocessed samples were from traditional, hulled barley varieties.

Eight fistulated steers were given free-choice access to an 80% corn-based diet. Dacron bags (10 × 20 cm with a 50 ± 10 micron porosity) were labeled and preweighed. Traditional and hulless barley, each after being artificially masticated, and rolled barley sources were weighed (15 ± 0.2 g) into the Dacron bags with four replicate bags per source for each of the following time points: 3, 6, 12, 24, 48, and 72 h. Bags were tied shut with nylon string and grouped by hour within steers in larger mesh sacs. Weights were inserted in the mesh sacs to ensure submersion in the rumen. Weighted mesh sacs were placed directly in the rumen via the cannula of each steer to achieve 72, 48, 24, 12, 6, and 3 h of incubation over time and all mesh bags within steer were pulled from the rumen simultaneously.

After removal from the rumen, all Dacron bags were immediately placed in cold (~4 °C) tap water. Bags were washed six times to remove debris, or until the rinse water ran clear. Samples were then placed in aluminum pans and dried at 55 °C for 72 h. During drying, bags were rotated every 12 h to ensure uniform drying.

After drying, ties were removed and each bag (contents included) was weighed and recorded. Percentage DM loss was calculated for each bag. Sample contents were composited by animal and hour if the coefficient of variation was less than 10%. Bags with coefficient of variation greater than 10% were excluded from DM calculations and further analysis. Combined samples were then ground through a Wiley mill (1 mm screen; Thomas Scientific) and analyzed for DM, starch, and NDF, as previously described.

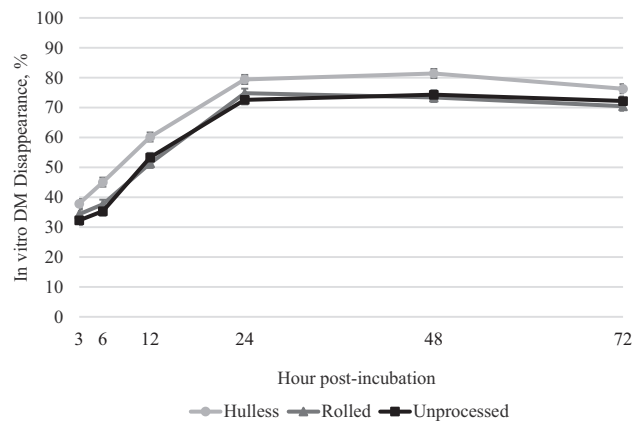


Figure 1. Effect of barley source on in vitro DM disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a source by hour interaction ($P < 0.05$). In addition, there were main effects of both source ($P < 0.05$) and hour ($P < 0.05$). The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 1.524).

Data were analyzed using MIXED procedures of SAS (v. 9.4 SAS Institute Inc., Cary, NC) with repeated measures to determine the effects over time. Steer was the experimental unit, using the model:

$$Y_{ijklm} = \mu + c_j + G_k + T_l + (GT)_{kl} + e_{ijklm}$$

where Y_{ijklm} = response variable; μ = mean; c_j = random effect of calf (or steer); G_k = fixed effect of barley source; T_l = fixed effect of time; $(GT)_{kl}$ = fixed effect of the interaction barley source and time; and e_{ijklm} = experimental error. Significance was declared at $P \leq 0.05$.

RESULTS

Hulless barley contained 7% more starch than the traditional barley sources. In addition, hulless barley had 35% less NDF and 82% less ADF when compared to the mean of unprocessed and rolled barley (Table 1).

Experiment 1: In Vitro

A barley source by hour interaction ($P < 0.05$) for DM disappearance was detected (Fig. 1). Analyzed within interaction times, hulless barley had a greater ($P < 0.05$) percentage DM disappearance than unprocessed barley and rolled barley at all time points except 3 h postincubation. At 3 h postincubation, in vitro DM disappearance of hulless barley was not different ($P > 0.05$) to rolled barley, but in vitro DM disappearance of hulless barley was 17% greater ($P < 0.05$) than unprocessed barley.

A barley source by hour interaction ($P < 0.05$) for NDF disappearance also was detected (Fig. 2).

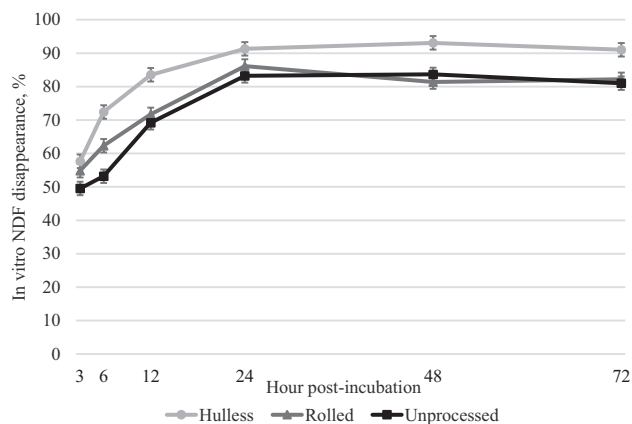


Figure 2. Effect of barley source on in vitro NDF disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a source by hour interaction ($P < 0.05$). In addition, there were main effects of both source ($P < 0.05$) and hour ($P < 0.05$). The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 2.022).

Hulless barley had a greater ($P < 0.05$) percentage NDF disappearance than both unprocessed barley and rolled barley at all time points except for 3 h postincubation. At 3 h postincubation, in vitro NDF disappearance of hulless barley was similar ($P > 0.05$) to rolled barley, but in vitro NDF disappearance of hulless barley was 15% greater ($P < 0.05$) than unprocessed barley. The NDF disappearance of rolled barley was greater ($P < 0.05$) than unprocessed barley by 16% at hour 6, but unprocessed barley had a 5% greater ($P < 0.05$) NDF disappearance than rolled barley at hour 48. Rolled barley and unprocessed barley were similar ($P > 0.05$) in NDF disappearance at all other time points.

A barley source by hour interaction ($P < 0.05$) for starch disappearance also was detected (Fig. 3). At hour 3, starch disappearance was similar ($P > 0.05$) for all barley sources. But, hour 6 starch disappearance was greatest ($P < 0.05$) for hulless barley and least for rolled barley; starch disappearance of unprocessed barley was intermediate and different from both. At hour 12, in vitro starch disappearance of hulless and unprocessed barley were similar ($P > 0.05$). Rolled barley was different ($P < 0.05$) than both and had 4% less in vitro starch disappearance than hulless and unprocessed barley at hour 12. At hours 24 and 48, rolled barley had the greatest ($P < 0.05$) starch disappearance, and hulless barley had the least ($P < 0.05$); starch disappearance of unprocessed barley was intermediate and different from both. At hour 72, in vitro starch disappearance of unprocessed barley and rolled barley were similar ($P > 0.05$), but in vitro starch disappearance of hulless barley was 3% less ($P < 0.05$) than both unprocessed barley and rolled barley.

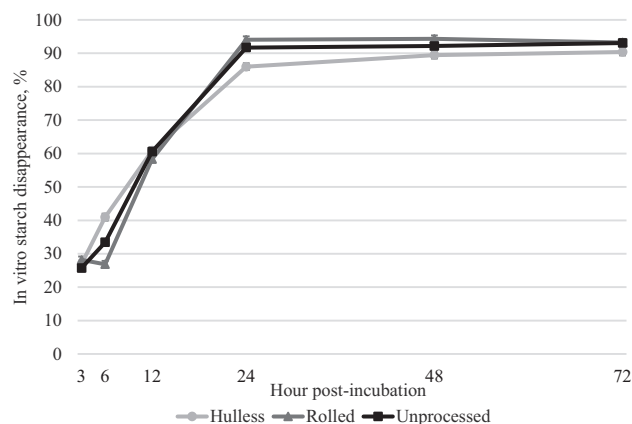


Figure 3. Effect of barley source on in vitro starch disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a significant ($P < 0.05$) hour and source by hour interaction but not a significant ($P > 0.05$) source interaction for in vitro starch disappearance. The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 1.021).

Experiment 2: In Situ

To provide a general understanding of comparison of relative size differences, particle size differences of the unprocessed barley source are presented as both whole and artificially masticated (Table 2). The retention of particles on screens less than 1.7 mm was 55% greater when the unprocessed barley source was artificially masticated, compared with when that source remained whole. Rolled barley had 15.5% of its DM as fines, particles that were less than 0.85 mm, while other sources had less than 1%, even when artificially masticated.

There was a treatment by hour interaction ($P < 0.05$) for DM disappearance (Fig. 4). In situ DM disappearance was greatest ($P < 0.05$) for rolled barley at all time points when compared with hulless and unprocessed barley. DM disappearance did not differ ($P > 0.05$) between hulless barley and unprocessed barley at hours 3 to 12 postincubation. However, from hour 12 to 72, hulless barley had greater ($P < 0.05$) DM disappearance than unprocessed barley.

In addition to DM disappearance, there was a treatment by hour interaction ($P < 0.05$) for NDF disappearance (Fig. 5). In situ NDF disappearance was greatest ($P < 0.05$) for hulless barley at all time points. Fiber disappearance for rolled barley and unprocessed barley remained comparable ($P > 0.05$) at all time points.

There was also a treatment by hour interaction ($P < 0.05$) for starch disappearance (Fig. 6). Starch disappearance was greatest ($P < 0.05$) for the rolled barley source at all measured time points. Although

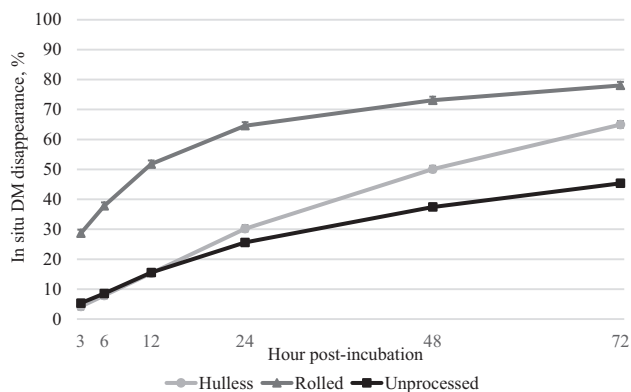


Figure 4. Effect of barley source on in situ DM disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a source by hour interaction ($P < 0.05$). In addition, there were main effects of both source ($P < 0.05$) and hour ($P < 0.05$). The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 1.17).

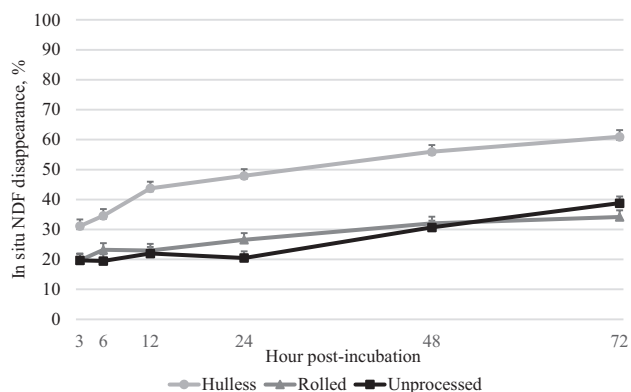


Figure 5. Effect of barley source on in situ NDF disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a source by hour interaction ($P < 0.05$). In addition, there were main effects of both source ($P < 0.05$) and hour ($P < 0.05$). The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 2.22).

starch degradation in hulless barley was more than twice that of unprocessed barley at hour 3, these feeds did not differ ($P > 0.05$) at that time, due to a combination of steer and lab variation. The 2 feeds remained comparable ($P > 0.05$) until hour 72, at which point hulless barley had a 26% greater in situ disappearance ($P < 0.05$) of starch than unprocessed barley.

DISCUSSION

Newer barley grain varieties have been developed with a greater understanding of animals' needs in mind. Whether these new varieties reduce management inputs is unclear. We tested hulless barley and traditional barley (both unprocessed and rolled) in vitro and these same barley sources, after being artificially masticated, were tested in situ. We evaluated commercially available sources

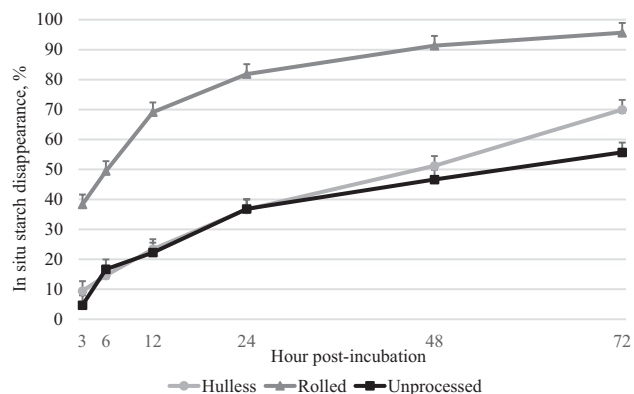


Figure 6. Effect of barley source on in situ starch disappearance over time. Barley sources used in this study were hulless, rolled, and unprocessed. There was a source by hour interaction ($P < 0.05$). In addition, there were main effects of both source ($P < 0.05$) and hour ($P < 0.05$). The error bars reflect the standard error of the mean associated with the interaction of source by hour (SEM = 3.28).

of barley in this experiment. Thus, the traditional, whole barley and the traditional, rolled barley were not the same source. However, nutrient composition of both the whole (unprocessed) and rolled traditional barley sources is similar (Table 1), thus warranting their comparison. We hypothesized that hulless barley could eliminate the need for grain processing because in vitro and in situ disappearance of DM, NDF, and starch should be greater for hulless barley than for traditional unprocessed barley and should be more similar to rolled barley. The research was meant to determine 1) if hulless barley had similar ruminal degradation as processed barley, eliminating the need to process barley prior to feeding, and 2) if in vitro and in situ techniques provided similar estimates of ruminal digestion for barley grain. The presence of barley source by time interactions prohibit statistical comparison among means. However, overall means are discussed where necessary to provide comparison to previous research.

In the present study, in vitro NDF mean disappearance averaged 13% greater and in situ mean ruminal NDF disappearance averaged 77% greater for hulless barley compared with the rolled or unprocessed barley. These results are comparable to the means of total tract digestibility reported when feeding traditional and hulless barley varieties to dairy cows (Yang et al., 2018). Yang et al. (2018) reported that total tract NDF digestibility of hulless barley was 3% greater than for traditional whole barley in dairy cattle. The greater mean digestibility of NDF reported previously, and in the present in vitro and in situ measures for hulless barley, may reflect the lower NDF and ADF of hulless barley compared with traditional barley sources tested.

The lack of the outer hull reduces the total fiber concentration, particularly ADF (cellulose and lignin), of hulless barley when compared with other traditional barley sources, regardless of processing as noted by [Edney et al. \(1992\)](#).

Initial concentrations of nutrients in each of the barley sources were provided to characterize the grains. These decreased concentrations of both NDF and ADF in the initial hulless barley sample can be explained by the absence of its fibrous outer hull and are consistent with the results obtained by [Baidoo et al. \(1998\)](#). [Edney et al. \(1992\)](#) analyzed the nutrient composition of hulless barley and compared it to the nutrient composition of traditional whole barley, similar to the unprocessed barley used in this experiment. These researchers reported that hulless barley had 7% more starch than whole barley versus 6% and 8% greater than unprocessed barley and rolled barley, respectively, in our study ([Table 1](#)). While these differences were not analyzed statistically, differences in starch concentrations and physiochemical properties between barley sources are common and expected due to different growing and fertility conditions ([Yangcheng et al., 2016](#)).

We hypothesized that starch disappearance would be greater for hulless barley than unprocessed barley, due to the lack of the tough outer hull, and comparable to processed, rolled barley. Rather than discussing differences at every time point, it is important to note differences at 3 and 6 h for ruminal starch disappearance. It is often discussed that starch disappearance is expected to peak in grain fed cattle around 6 h postfeeding ([Van Soest, 1994](#)). In addition, [Zinn et al. \(1996\)](#) reported that hulless barley had greater starch solubility than traditional barley. Starch disappearance in the current in vitro test was 36% greater for hulless barley than both unprocessed and rolled barley at 6 h postfeeding; and, there was a 100% greater in situ starch disappearance for hulless barley when compared with whole barley at hour 3. The reason that in vitro starch was greater at hour 6 than hour 3 could be due to the differences in composition and pH of the initial ruminal fluid used because the in vitro initial ruminal fluid was heavily buffered.

In contrast with these early in vitro results, there were no differences among barley sources in mean starch disappearance in vitro. In vitro starch disappearance was about 60% for all barley sources by 12 h postincubation ([Fig. 3](#)). While in situ starch disappearance remained below 60% for the hulless and unprocessed barley sources through 48 h postincubation, rolled barley in situ starch

disappearance reached 40% just 3 h postincubation and approached 100% at 72 h postincubation. The miniscule differences between hulless barley and unprocessed barley in starch degradation in situ do not seem biologically relevant. The differences in starch disappearance between in vitro ([Fig. 3](#)) and in situ ([Fig. 6](#)) techniques are presumably due to steps in the procedures used with each technique.

One of the main differences between techniques is the processing of the grains before incubation. For in vitro analysis, all barley sources were ground through a 1mm Wiley screen. Thus, the particle sizes for all sources were small and uniform. In contrast, for the in situ assays the hulless and unprocessed barley sources were ground through a Wiley mill with no screen twice in an attempt to simulate the comminution of whole barley sources by chewing. While this “artificial mastication” technique has been employed in the past ([Dr. Robbi Pritchard, SDSU; personal communication](#)), it may not accurately simulate the animal’s chewing activity. However, the artificial mastication technique reduced the geometric mean particle size of the barley sources. The fraction of particles on screens less than 1.7 mm was 55% greater when the unprocessed, traditional barley source was artificially masticated, compared with when that source remained whole. When rolled, traditional barley had 15.5% particles (DM basis) that were less than 0.85 mm. Other sources had less than 1% of their particles on this bottom tray, even when artificially masticated. [Dehghan-banadaky et al. \(2007\)](#) reported that extensive grain processing to reduce particle size increases ruminal starch degradation as was evident from a comparison of in vitro and in situ starch degradation in this trial. In vitro analysis obliterated differences among starch disappearance. The presence of fine particles can explain why the processed, rolled barley had the greatest starch disappearance for the in situ trial at all time points.

The current trials focused solely on ruminal degradation techniques. [Zinn et al. \(1996\)](#) reported that postruminal digestion of organic matter, starch, and nitrogen were greater for hulless barley than traditional whole, or unprocessed, barley. Because only ruminal disappearance was tested in the present study, more work is needed to determine total tract digestibility among the sources. Although total tract digestion of starch from barley, regardless of processing, generally exceeds 98%, these results suggest that processing to reduce particle size shifts site of starch digestion back to the rumen.

The comparison of in vitro and in situ degradation of three barley sources in the current study

indicates that results differ markedly. When the grains were compared in vitro, minor differences were noted presumably because the need to intensively process those sources to obtain a representative sample obliterates one factor that limits the rate of ruminal digestion. When compared in situ, an effort was made to “masticate” the hullless and unprocessed sources to determine if hullless barley should be processed prior to feeding. However, the artificial mastication technique, although reducing particle size, may not simulate “complete chewing”. In vivo animal experiments are expensive and labor intensive, so alternative techniques are needed to reduce costs. Inferences about the ruminal degradation of grains based on in situ or in vitro techniques, can yield vastly different results. Thus, all laboratory estimates of digestion must be cross-checked against in vivo measurements before they can be accepted as reliable or dependable.

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