

THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ANIMAL SCIENCE

RUMINAL DISAPPEARANCE OF BARLEY SOURCES USING IN VITRO TECHNIQUES

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SPRING 2019

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Veterinary and Biomedical Sciences
with honors in Animal Science

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ABSTRACT

Even though barley is often used in animal feed, processing of the grain is necessary to break the tough outer hull and expose the nutritious endosperm. Because processing is expensive, a hullless variety of barley was developed. Not much research has been done comparing the digestibility of hullless barley to traditional processed barley, especially in beef cattle. Therefore, the objectives of this study were to compare in vitro disappearance of dry matter (**DM**), neutral detergent fiber (**NDF**), and starch of traditional unprocessed barley, rolled barley, and hullless barley. Each barley source was ground through a 1mm Wiley screen, and subsamples were weighed into F57 filter bags. The samples were incubated in ruminal fluid taken from 2 Angus steers in the DAISY II Incubator for 3, 6, 12, 24, 48, and 72 hours. Residual contents were analyzed to determine in vitro disappearance of DM, NDF, and starch. Data were analyzed using MIXED procedures of SAS (9.4 SAS Inst., Cary, NC) with repeated measures. There was a barley by time interaction for dry matter disappearance. Dry matter disappearance was greatest ($P<0.05$) for hullless, and was similar ($P>0.05$) between unprocessed barley and rolled barley, at all time points except for hour 3. Similarly, there was an interaction with time for NDF disappearance where it was also greatest ($P<0.05$) for hullless barley at all time points when compared to unprocessed barley and rolled barley except for hour 3. NDF disappearance differed ($P<0.05$) between rolled and unprocessed barley at hours 6 and 48, but rolled barley and unprocessed barley did not differ ($P>0.05$) in NDF disappearance at 3, 12, 24, and 72 hours post incubation. There was a barley by time interaction for starch disappearance, as well. Starch disappearance was greatest ($P<0.05$) for hullless barley at 6 hours post incubation when compared to rolled and unprocessed barley. Hullless barley had the greatest ($P<0.05$)

disappearance of both DM and NDF. While unprocessed and rolled barley were not different in DM disappearance, NDF disappearance of unprocessed barley was least ($P<0.05$) and NDF disappearance of rolled barley was intermediate and different ($P<0.05$) than both unprocessed and hulless barley. Mean starch disappearance was comparable ($P=0.60$) between the barley sources. Of the 3 grains tested, hulless barley had the greatest DM and NDF disappearance in vitro. Hulless barley grain also contained 35% less NDF than unprocessed and rolled barley. At 6 hours post incubation, hulless barley had greater starch disappearance than either rolled and unprocessed barley, by 53% and 23% respectively. Based on these results, hulless barley appears to be an acceptable substitute for both whole and processed barley, but more research should be performed on total tract digestibility before making this conclusion.

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ACKNOWLEDGEMENTS

I would like to thank the wonderful Dr. Tara Felix for being such a patient mentor and providing a positive learning environment that allowed me to explore my interests. I would also like to thank Pedro Carvalho for answering my endless questions and teaching me how to perform a number of tasks. If it weren't for Kelsey Shampoe paving the way in the world of thesis writing, it would have taken me much longer to complete mine. I would also like to thank Dr. Staniar for reviewing my thesis and providing insight on how to make it better. Last but not least I would like to thank my family for supporting me in all of my endeavors, helping me reach my goals, and listening to me complain endlessly along the way.

Chapter 1

Review of Literature

1.1 Introduction to Barley

Barley is the 4th largest grain crop in the world, behind corn, rice, and wheat. Barley is grown worldwide with the European Union being the largest producer. The United States is the 7th major producer in the world, with Idaho, Montana, and North Dakota being the major contributing states. In 2017, the United States alone produced 141.9 million bushels of barley resulting in a crop value of \$614.3 million (Barley Profile, 2018). For comparison, corn is the primary grain crop in the United States and 14.6 billion bushels of corn were produced in the United States in 2017 (White and Honig, 2018). There are many benefits to both growing and using barley.

Barley can be grown in a variety of climates, ranging from subarctic to subtropical, making it an attractive crop for farmers all over the world (Barley Profile, 2018). It can fit nicely into crop rotations due to its short growing period. In addition, the short growing period of barley also allows it to produce the most biomass in the shortest amount of time compared to other cereal grains (Clark, 2007). From a soils standpoint, barley works well to manage topsoil and prevent erosion as well as recycle nutrients in the soil and suppress weeds (Clark, 2007). Barley's strong outer hull allows it to survive well during droughts and in saline soils (Edney et al., 1992). Because of barley's toughness in varying climates, Yang et al. (2017) suggested that corn experiencing drought stress should be replaced by barley.

Being one of the top cereal grains in the world, barley has many uses in both human and animal applications. Barley is used primarily for the production of alcoholic beverages through a process known as malting, but it is also used in the preparation of many foods (Edney et al., 1992). Barley is often used in the Northern part of the United States in animal feeds, and it is the main component of concentrates fed to dairy and beef cattle in Western Canada (Anele et al., 2014).

1.2 Barley Structure and Nutrient Composition

Barley grain is composed of a protective outer hull (or husk), the pericarp, and a nutritional endosperm. The innermost part of the barley grain is the endosperm, which is the nutritional storage space for the seed, and makes up 75% of the grain's weight (Holopainen-Mantila, 2015). The pericarp, which consists of a layer of dry, dead cells, lies between the endosperm and outer hull and holds these two parts of the grain together. The outer hull is the second largest component of the grain by weight and is very thick and fibrous (Anele et al., 2014). It acts as a protective layer and prevents water from getting inside the grain.

The nutritional composition of grains, such as barley, is highly dependent on the conditions in which they are grown. Thus, nutrient composition may vary with geographic location, climate, and soil quality (Zhao et al., 2016). Cereal grains, including barley, are usually used as an energy source. One reason that barley is a great source of energy has to do with the large proportion of starch that it contains. Up to 67% of barley's dry matter is comprised of starch (Holopainen-Mantila, 2015). Boyles et al. (2001) stated that the total tract starch digestion of dry, processed barley by cattle is 99.2%. This value is comparable to other grains commonly

used as an energy source in animal feed, such as corn. The NRC for Beef Cattle (NASEM, 2016) reports that barley has 5.7 to 7.1% crude fiber. This fiber comes from barley's protective outer hull, and causes it to supply ample fiber to an animal's diet when fed as the primary energy source. However, because barley is rapidly fermented in the rumen, fiber from barley is not considered an effective source of fiber, that is it generally does not stimulate rumination. It has been suggested, though, that this concentration of crude fiber decreases the roughage inclusion necessary when formulating a diet for cattle. (Boyles et al., 2001).

Barley also has a greater percentage of protein than other grains (NASEM, 2016) which makes it an attractive option for cattle feed. On a dry matter basis, protein concentrations can be as much as 18% (NASEM, 2016), depending on the growing conditions and the variety, and as much as 75% of that protein is digestible (Boyles et al., 2001). Due to its protein concentration, barley may not need to be supplemented with other sources of protein which can greatly reduce the cost of feed (Boyles et al., 2001).

Cereal grains are not known for being good sources of minerals, but many minerals are present in barley. Although lacking in calcium just like corn, barley contains greater potassium concentrations (Bauer et al., 2017) and more phosphorus that is biologically available to the animal than corn (Boyles et al., 2001). Bauer et al. (2017) also states that the concentrations of each trace mineral are greater in barley when compared to the concentrations in corn. In order to account for the insufficient concentrations of minerals, animals fed barley are usually supplemented with calcium and a trace mineral salt (Boyles et al., 2001).

The concentrations of different vitamins present in barley also differs from the concentrations present in other grains. Barley contains thiamine (vitamin B1) and has a significantly greater concentration of riboflavin (vitamin B2) than some other grains (Granda et

al., 2018). Barley also has a greater concentration of vitamin E than other grains which is beneficial because vitamin E is an antioxidant and can also help preserve barley for longer periods of time (Do et al., 2015). Barley also lacks β -carotene which is the provitamin to vitamin A (Boyles et al., 2001). The lack of some vitamins in barley can be corrected with supplementation. It is important that all of the nutrients in barley grain are accessible to the ruminal microbes for digestion. One of the ways to increase accessibility to these nutrients is through processing.

1.3 Barley Processing

In order to use barley as a cattle feed, it must be processed to expose the nutritious endosperm found within the tough hull. Because the hull and pericarp of the grain are resistant to bacterial attachment (Zhao et al., 2016), the digestibility of processed barley is more than 62% greater than the digestibility of whole barley (Bauer et al., 2017). It is imperative that traditional barley is processed in order to receive the full nutritional value from the grain, but processing can be very expensive. In 2001, the average cost to process barley was between \$2 to \$5 per ton (Boyles et al., 2001) depending on the method used. Processing costs are an important consideration because feed costs can be as much as 70% of the cost to raise beef cattle (Hershorin et al., 2018).

There are many different ways to process barley in order to increase access and utilization of its nutrients. Chemical processing can help break down barley's fibrous hull, exposing the endosperm. Chemicals that have been investigated include sodium hydroxide or enzymes (Dehghan-banadaky et al., 2007). Barley can also be soaked in ammonia or urea to

increase ruminal digestibility (Mathison et al., 1989). Treating barley with aldehydes allows more of the grain's protein to bypass the microbes in the rumen and be absorbed in the intestines (Faichney and Davies, 1973).

Another way to process barley is through the combination of heat and physical pressure. Steam rolling and steam flaking both manipulate the temperature and amount of moisture in the grain before rolling the barley to physically break it apart while also reducing the amount of fine particles produced. These techniques require a lot of energy and equipment which can be very costly, so many feed producers choose to use cheaper methods (Dehghan-banadaky et al., 2007).

One example of a cheaper method is cold physical processing. Cold physical processing (often just called physical processing) of barley can be accomplished through a few different techniques. Grinding and dry rolling are the most common ways to physically process grains, and both methods are used to process barley (Zhao et al., 2016). Grinding uses a hammer mill to physically break the grain into pieces, whereas dry rolling uses rotating rollers of different sizes to break the hull and pericarps to expose the endosperm of the grain (Dehghan-banadaky et al., 2007). Dry rolling tends to be faster than grinding, and it requires less energy (Boyles et al., 2001). Grinding also produces smaller particles which makes dry rolling a better option for many cattle producers (Zhao et al., 2016).

There have been many studies done comparing the feeding of whole barley and processed barley to animals which highlight the benefits of processing barley. Feeding processed barley to beef cattle increased their feed efficiency when compared to whole barley by decreasing dry matter intake (Zinn, 1993) without changing average daily gain (Dehghan-banadaky et al., 2007). Ribeiro et al. (2016) reported that increasing the extent of barley

processing increased net energy gain as much as 5%, likely due to increased total tract organic matter and nutrient digestibility (Dehghan-banadaky et al., 2007).

On the other hand, feeding processed barley can have negative side effects. The fine particles that are created when using physical processing methods can result in inconsistent cattle performance (Anele et al., 2015) and less rumination (Hironaka et al., 1992). Saliva production is affected by the extent to which the barley is processed, and it plays an important role in buffering the rumen and maintaining the proper ruminal pH. Hironaka et al. (1992) reported that saliva production was greatest when animals were fed whole or coarsely ground grains. Processing barley also increased ruminal starch degradation by exposing more of the endosperm, and since barley has a greater rate of ruminal fermentation compared to other grains, together this can lead to an increased risk of ruminal acidosis (Anele et al., 2014) and damage to the rumen wall (Ribeiro et al., 2016). Koenig and Beauchemin (2011) noted that coarser processing of barley can decrease the risk of rumen acidosis by decreasing the digestion rate in the rumen. Hironaka et al. (1992) reported that feeding a coarser diet of barley can lead to healthier rumen conditions measured by a decrease in ruminitis, reduction in clumping of papillae, and an increase in presence of protozoa. Coarser processing also reduced negative effects on the liver, including liver abscesses (Moya et al., 2015). Although processing of barley is important to expose the nutrients of the endosperm to the microbes in the rumen, the risks of feeding processed grains should be considered when choosing an animal feed with grains.

1.4 Hulless Barley

In an attempt to make barley more readily digestible without having to process it, a variety of hulless barley was developed. This hulless barley expresses the recessive *nud* gene which results in the inhibition of the development of the hull and its attachment to the endosperm (Yangcheng et al., 2016). As a result, the hull is loosely attached to the endosperm, and comes off easily when being harvested (Zinn et al., 1996). This saves space in the storage and transportation of the barley (Yangcheng et al., 2016), and also suggests a reduction in the need for processing which would save cattle feeders money.

Hulless barley also differs in nutrient composition when compared to more traditional barley sources. One of the reasons that the hull does not properly attach to the endosperm in hulless barley is because of an increase in β -glucan, which is a mixed linked non starch polysaccharide, in the endosperm (Baidoo et al., 1998). Edney et al. (1992) states that hulless barley has up to 42% reduced neutral detergent fiber and up to 80% less acid detergent fiber compared to traditional barley. Hulless barley has also been shown to have a greater concentration of starch as well when compared to traditional processed barley (Yangcheng et al., 2016). Protein concentrations in hulless barley are greater than protein concentrations in traditional barley sources, but Edney et al. (1992) argues that hulless barley has poorer quality protein than traditional barley because it contains less essential amino acids. Edney et al. (1992) also points out that usually there is a negative correlation between starch and protein concentrations in barley sources, but hulless barley contains significant concentrations of both making it a more attractive option for feed.

Only one study has been done that compared the digestibility of hulless barley to traditional processed barley in beef steers. Zinn et al. (1996) reported a 7% increase in total tract

organic matter digestibility when using hulless barley in the feed of feedlot cattle compared to traditional, processed barley. A 7% increase in net energy value, a 9% decrease in feed intake, and an 18% decrease in methane production were observed when feedlot cattle consumed hulless barley compared to traditional, processed barley (Zinn et al., 1996). Because of the increase in starch digestibility, animals fed hulless barley tended to have a slightly lower ruminal pH (Zinn et al., 1996). Authors suggest this reduction in ruminal pH may be indicative of increased risk of ruminal acidosis.

1.5 Feeding Barley Sources Compared to Other Cereal Grains

Some studies have been performed that compare different barley sources to other cereal grains used in animal feeds, and it could be considered as a possible substitute for some of the more commonly used grains. Barley prices, regardless of variety, are typically 85% of corn prices on a per bushel basis which makes it a cheaper option for animal feed with very similar results (Barley Profile, 2018). Thus, barley has been examined as a replacement grain for corn in dairy feeding operations. Yang et al. (2017) reported 41.3 and 41.2 kg of milk produced per day when dairy cows fed corn were compared to those fed hulless barley, respectively. In the same study, starch digestibility was reported as 97.3 and 97.7 percent for dairy cows fed corn versus those fed hulless barley, respectively (Yang et al., 2017). These similarities occurred even though corn has more starch than barley. Additionally, dairy cows fed corn or hulless barley had similar milk fatty acid composition which suggests rumen metabolism did not differ when either grain was consumed (Yang et al., 2018).

Wheat has also been compared to barley as an animal feed for both swine and cattle. Although swine diets containing traditional barley are usually supplemented with enzymes to improve digestibility (Baidoo et al., 1998), Edney et al. (1992) reported that feeding hulless barley to swine resulted in similar performance and digestibility compared to swine fed wheat without the added supplementation. Moya et al. (2015) suggested that replacing barley with wheat in animal diets increased the risk of ruminal acidosis. This suggestion is supported by the fact that cattle fed barley had fewer liver abscesses compared to cattle fed wheat (Moya et al., 2015). Cattle fed barley also had decreased cortisol in their hair compared to cattle fed wheat which is indicative of reduced chronic stress.

One study also suggested that barley would be an appropriate substitute for sorghum in some animal feeds. Boyles et al. (2001) stated that barley has better total tract digestibility for both starch and crude protein in cattle when compared to sorghum. It has also been reported that cattle fed barley had a 5% increase in daily gain compared to cattle fed sorghum, as well as a 9% improvement in feed efficiency (Boyles et al., 2001). Due to the lack of studies regarding the use of hulless barley as cattle feed compared to other grains, it is difficult to make additional comparisons and feeding recommendations.

1.6 Summary

Barley is one of the top cereal grains produced worldwide due to its ability to grow in a range of different climates and because of its many uses. It has a very short growing season and can be used to manage the soil in which it is grown. Human consumption is a major way that

barley is used in the United States, especially in the use of making alcoholic beverages, but barley can also be used as an energy source in animal feeds.

A barley grain is comprised of three layers with the inner endosperm containing the most nutrients. Barley contains a large percentage of starch, making it an excellent source of energy. Barley also has a greater percentage of protein compared to other cereal grains, and it contains a number of vitamins and minerals making it an attractive option for animal feed.

In order to access nutrients, traditional barley must be processed to expose the endosperm. There are many ways to process barley grains, but the most common method of processing is cold physical processing because it is faster and less expensive than other methods.

Processing barley increases digestibility of the grain, but creates fine particles that can lead to negative health side effects including ruminal acidosis. To reduce the need for processing, a new variety of barley has been developed that does not contain the hard, outer hull. Although this hullless barley has a slightly different nutrient composition than traditional barley, a few studies have shown that it is an acceptable substitute, if not a better option, than traditional barley in animal diets.

Barley is not widely used as animal feed in the United States, but some studies have compared it to other cereal grains that are used and suggest it may be a cheaper option in swine (Edney et al., 1992) and dairy (Yang et al., 2017) diets, for example. The current dearth of knowledge regarding the feeding of hullless barley to beef cattle indicates that more research is necessary to study animal performance when fed different sources of barley in order to make proper feeding recommendations regarding cereal grains.

Chapter 2

Ruminal disappearance of barley sources using in vitro techniques

2.1 Introduction

As one of the top cereal grains in the world, barley is used for both human and animal consumption. Barley is often used in animal feed as an energy source due to its large percentage of starch, but the grain must be processed so that cattle can properly access the nutrients found within its protective hull (Holopainen-Mantila, 2015). There are many different ways to process barley, but one of the most common methods is dry rolling. Dry rolling physically breaks up the grain using two rollers and no heat (Dehghan-banadaky et al., 2007). Even though this is the cheapest processing method, processing can still be very expensive. For example, it cost between \$2 to \$5 per ton to process barley in 2001 (Boyles et al., 2001).

Not only is processing expensive, but it can also have negative effects on the health and productivity of the animal consuming the processed grain. During processing, fine particles are created which increases the surface area of the grain. This can lead to an increased rate of fermentation by rumen microbes (Zhao et al., 2016) and result in a drop of ruminal pH. A reduction in ruminal pH can cause ruminal acidosis and inconsistent animal performance (Anele et al., 2015).

A hulless variety of barley was developed in an attempt to reduce the need to process barley. Hulless barley has less fiber and more starch and protein than traditional barley (Edney et al., 1992). Ribeiro et al. (2016) reported that increasing the extent of barley processing

increased net energy gain by as much as 5%, likely due to increased total tract organic matter and nutrient digestibility (Dehghan-banadaky et al., 2007). Researchers have theorized that this increased digestibility would increase feedlot cattle growth performance. One trial has investigated the comparison between feeding feedlot cattle hulless barley or traditional, rolled barley (Zinn et al., 1996). Zinn et al. (1996) reported a 7% increase in total tract digestibility when using hulless barley in the feed of feedlot cattle compared to traditional, processed barley. A 7% increase in net energy value, a 9% decrease in feed intake, and an 18% decrease in methane production were also observed when feedlot cattle consumed hulless barley compared to traditional, processed barley (Zinn et al., 1996). To this authors knowledge, Zinn et al. (1996) is the only research that has compared feeding cattle hulless barley versus traditional barley.

There is a dearth of information regarding the digestibility of hulless barley compared to traditional barley sources for cattle. Therefore, the hypothesis is that the in vitro disappearance of dry matter (**DM**), neutral detergent fiber (**NDF**), and starch would be greater for hulless barley than for traditional unprocessed barley, and may be more similar to rolled barley. The objectives of this study were to compare in vitro disappearance of dry matter (**DM**), neutral detergent fiber (**NDF**), and starch of traditional unprocessed barley, rolled barley, and hulless barley.

2.2 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 tested to determine the difference in in vitro disappearance of dry matter (DM), neutral detergent fiber (NDF), and starch concentrations. The three sources

were 1) unprocessed, traditional barley, 2) processed (rolled), traditional barley, and 3) unprocessed, hulless barley.

Initial samples of each source were analyzed for DM (method 934.01: AOAC, 1988), starch (Hall, 2009), and NDF and ADF content (ADF; method 5 and 6, respectively; Ankom200 Fiber Analyzer, Ankom Technology, Macedon, NY).

There were a total of 425 F57 filter bags which included the 3 sources and blank bags. Each Daisy^{II} Incubator digestion jar held 24 sample bags and a blank bag for a correction factor. Hours 0, 3, and 6 each had 1 jar which contained 6 bags per sample and 1 blank. Hour 12 had 2 jars containing a total of 12 bags per sample and 2 blanks. Hours 24, 48, and 72 each had 4 jars with each hour containing a total of 24 bags per sample and 4 blanks. More bags were added to the longer incubation times in order to have enough residual to perform the starch analyses.

The F57 filter bags (Ankom Technology) were rinsed with acetone in order to remove a surfactant that inhibits microbial digestion. The weight of each bag was recorded and 0.50 g of the sample was added to each bag. The samples were ground through a 1mm Wiley screen (Thomas Scientific; Swedesboro, NJ) prior to being added to the bags. The grinding necessary for the in vitro methodology inadvertently reduces particle size to smaller than mastication would, however the relative differences that can be assessed will still aid in our understanding of the comparison between hulless barley and traditional barley. The bags were heat sealed and placed into the Daisy^{II} Incubator digestion jars.

Two buffer solutions were made: 1) Buffer A (10g/L KH_2PO_4 , 0.5g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5g/L NaCl, 0.1g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5g/L urea) and 2) Buffer B (15g/L Na_2CO_3 , 1g/L $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$). Both buffer solutions (A and B) were pre-warmed at 39°C, and in a separate container 266 mL of solution B was added to 1330 mL of solution A. The ratio (1:5) was

adjusted until the pH was 6.8 at 39°C. 1600 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 to 30 minutes.

Rumen fluid was taken from 2 Angus steers after being fed an 80% corn-based diet. Two 2L thermos bottles were preheated to 39°C using warm water. The proper collection procedure was used to remove 2L of rumen inoculum and put into 1 thermos. The other thermos held about 2 fistfuls of fibrous mat from the rumen. A blender was preheated to 39°C using warm water before adding the inoculum. It was purged with CO₂ gas and blended at high speed for 30 seconds. The blended digesta was filtered through 4 layers of cheese cloth into a preheated (39°C) 5L flask. The remaining rumen fluid was filtered through 4 cheesecloths into the same 5L flask. The flask was continuously purged with CO₂ gas. 400mL of inoculum was added to each digestion jar containing buffer solution and sample and was purged with CO₂ gas for 30 seconds before the lid was closed.

The digestion jars were incubated in the Daisy^{II} Incubator for 0, 3, 6, 12, 24, 48, and 72 hours. 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 hours and contents were analyzed for starch and non-detergent fiber (NDF). Nutrient compositions were corrected for a 100°C dry matter. Disappearance of each nutrient was calculated using the formula:

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

2.3 Statistical Analysis

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:

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

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

2.4 Results

Hulless barley contained 7% more starch than the traditional barley sources, and the concentrations of NDF and ADF were 35% and 82% less, respectively, in the hulless barley compared to both unprocessed barley and rolled barley (Table 1).

There was a source by hour interaction for DM disappearance (Figure 1). Hulless barley had a greater ($P < 0.05$) percent DM disappearance than unprocessed barley and rolled barley at all time points except hour 3. At 3 hours post-incubation, in vitro DM disappearance of hulless barley was similar ($P > 0.05$) to rolled barley, but in vitro DM disappearance of hulless barley was greater ($P < 0.05$) than unprocessed barley by 17%. The DM disappearance of unprocessed barley and rolled barley were similar ($P > 0.05$) at all time points except for hour 24 where rolled barley was greater ($P < 0.05$) than unprocessed barley by 3%.

There was also a source by hour interaction for NDF disappearance (Figure 2). Hulless barley had a greater ($P<0.05$) percent NDF disappearance than both unprocessed barley and rolled barley at all time points except for hour 3. At 3 hours post-incubation, in vitro NDF disappearance of hulless barley was similar ($P>0.05$) to rolled barley, but in vitro NDF disappearance of hulless barley was greater ($P<0.05$) than unprocessed barley by 15%. 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.

There was a source by hour interaction for starch disappearance (Figure 3). At hour 3, starch disappearance was similar ($P>0.05$) for all barley sources. At 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.

In addition to the interaction of source by hour, there was a main effect of source on DM and NDF disappearance, but not on starch disappearance (Table 2). Hulless barley had the greatest ($P<0.05$) disappearance of both DM and NDF by 12% and 13%, respectively. While

unprocessed and rolled barley were not different in DM disappearance, NDF disappearance of unprocessed barley was least ($P<0.05$) and NDF disappearance of rolled barley was intermediate and different ($P<0.05$) than both unprocessed and hulless barley. There was no effect ($P=0.60$) of barley source on main starch disappearance.

2.5 Discussion

The initial concentrations of nutrients in each of the barley sources were as expected. The 35% and 82% lesser concentrations of both NDF and ADF, respectively, for hulless barley 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, which is similar to the unprocessed barley source used in this experiment. These researchers reported that hulless barley had a 7% greater starch concentration than whole barley. In the present study, starch concentrations of hulless barley analyzed 6% and 8% greater than unprocessed barley and rolled barley, respectively (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 conditions (Yangcheng et al., 2016).

In a study performed by Zinn et al. (1996) comparing feedlot cattle fed either hulless or traditionally whole barley, authors reported that hulless barley had a 7% greater total tract organic matter digestion than the whole barley. Organic matter disappearance was not evaluated in the current study, however, results from the present study confirm a greater percent DM disappearance in the hulless barley compared to the rolled and unprocessed barley at all time

points except hour 3, and organic matter and DM disappearance are highly correlated (Zhao et al., 2015).

Organic matter and DM are just crude pictures of total disappearance. Closer inspection of the individual nutrients is often more telling. In the present study, the 13% greater NDF disappearance for hulless barley compared to the rolled or unprocessed barley is comparable to the results reported by Yang et al (2018). Yang et al. (2018) reported that total tract NDF digestibility of hulless barley was 3% greater than traditional whole barley in dairy cattle. The greater digestibility of OM and NDF previously reported, along with the present in vitro disappearance of DM and NDF disappearance for hulless barley compared to rolled and unprocessed barley may in part be because hulless barley is less fibrous to begin with than the other sources tested. The lack of the outer hull reduces the total fiber concentration of hulless barley when compared to other traditional sources, regardless of processing (Edney et al., 1992).

Perhaps not surprisingly, there were no differences among sources in mean starch disappearance. Zhao et al. (2016) states that the effective digestibility of starch in the rumen increases as the concentration of initial starch increases. All of the barley sources in the present study had similar initial starch concentrations, thus, according to Zhao et al. (2016), they should have similar disappearance. However, the similar percentage of starch disappearance can also be explained by the in vitro procedure.

Dehghan-banadaky et al. (2007) reported that extensive grain processing increases ruminal starch degradation. One important point to note about conducting the in vitro experiment in the present study is that the in vitro procedure requires all of the barley treatments to be ground through a 1mm Wiley screen prior to incubation. Thus, the rolled barley used in this study was technically processed twice. Hironaka et al. (1992) states that feed particle size varies

widely within a single type of processing. This suggests that by processing the rolled barley twice, the particles may have become more uniform, negating potential differences that may have occur if the cattle were simply fed the different barley sources. This sample preparation method may have hindered some differences that could be relevant in in vivo situations; thus, additional research in vivo is warranted.

Despite these challenges, starch disappearance was greatest for hulless barley at 6 hours post feeding, when peak ruminal degradation of starch might be expected in vivo (Van Soest, 1991). Zinn et al. (1996) reported that hulless barley had greater starch solubility than traditional barley which may explain why hulless barley had a 36% greater starch disappearance at hour 6 compared to both rolled and unprocessed barley. The reduction in starch disappearance for hulless barley compared to both unprocessed and rolled barley at hours 24, 48, and 72 could be due to the increased concentrations of beta glucan present in hulless barley relative to other sources as suggested by Yangcheng et al. (2016). However, Grove et al. (2006) states that beta glucans are assumed to be completely digested in the rumen. It is important to note that although hulless barley has less starch disappearance than the other barley sources during the later hours, peak starch digestion should occur between hour 3 and 6, and hulless barley had the greatest disappearance compared to rolled and unprocessed barley at hour 6.

In addition, Zinn et al. (1996) reported that hulless barley has greater post-ruminal digestion of organic matter, starch, and nitrogen when compared to whole barley. However, only ruminal disappearance was tested in the present study. More work is needed to determine total tract digestibility among the sources.

2.6 Conclusion and Implications

The results from the present trial indicate a greater DM and NDF disappearance for hulless barley when compared to both unprocessed and rolled barley. Overall starch disappearance among barley sources were similar, but may be related to the in vitro methodology which required all sources to be ground through a similar sized screen prior to incubation.

Despite the effects that the in vitro methodology may have had on the present study, DM and NDF disappearance were greatest for hulless barley at all time points. In addition, starch disappearance was greatest for hulless barley at 6 hours post feeding. These results suggest hulless barley could be an alternative feed for cattle. When used as an animal feed, reducing grain processing can decrease costs of production and alleviate health risks. Not much research has been done on the digestibility of hulless barley compared to processed barley, especially in beef cattle. Because this study was conducted in vitro, only conclusions about ruminal disappearance can be made from the results. Further digestion in ruminant animals occur in both the small and large intestines, so more research studying total tract digestibility could illuminate how the animal truly uses barley, especially of the hulless variety, for growth performance. Therefore, additional research is needed to determine if hulless barley is an acceptable substitute for the traditional, processed barley in feedlot cattle diets.

Chapter 3
Tables and Figures

3.1 Tables

Table 1. Chemical composition of barley sources

	Barley Source		
	Hulless	Rolled	Whole
Analyzed Composition, % DM basis			
DM ¹	87.43	87.17	87.32
NDF ²	14.49	22.26	22.45
ADF ³	1.19	6.38	7.17
Starch	60.56	56.05	57.35

¹DM = dry matter

²NDF = neutral detergent fiber

³ADF = acid detergent fiber

Table 2. Effect of barley source on mean in vitro disappearance of DM, NDF, and starch

	Barley Source			SEM	P-value
	Hulless	Rolled	Whole		
In Vitro Disappearance, % DM basis					
DM ¹	63.37 ^a	57.00 ^b	56.60 ^b	0.433	<0.05
NDF ²	81.50 ^a	73.09 ^b	70.70 ^c	0.715	<0.05
Starch	65.77	65.82	66.12	0.275	0.60

¹DM = dry matter

²NDF = neutral detergent fiber

3.2 Figures

Figure 1. Effect of barley source on in vitro dry matter disappearance over time.

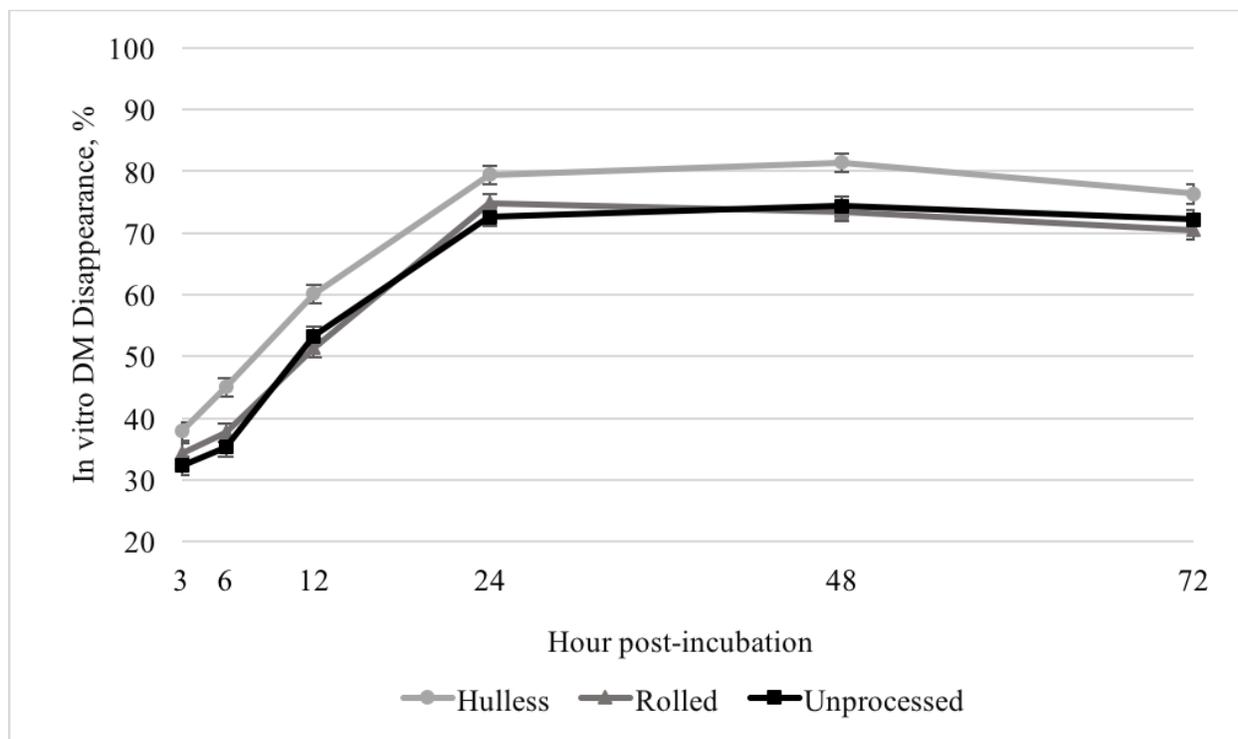


Figure 1. Effect of barley source on in vitro dry matter disappearance over time. There was a source by hour interaction ($P<0.05$). In addition, there were main effects of both source ($P<0.05$) and hour ($P<0.05$). The error bars reflect the SEM associated with the interaction of source by hour (SEM=1.524).

Figure 2. Effect of barley source on in vitro neutral detergent fiber disappearance over time.

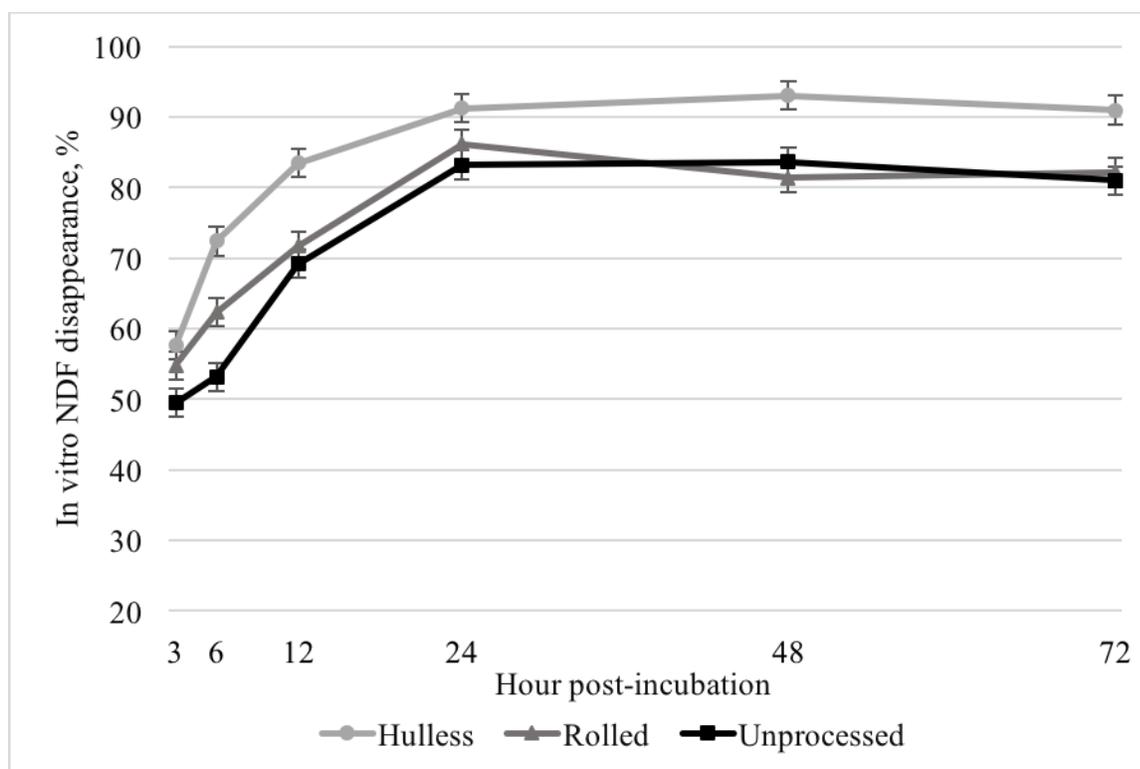


Figure 2. Effect of barley source on in vitro neutral detergent fiber (NDF) disappearance over time. In addition, there were main effects of both source ($P<0.05$) and hour ($P<0.05$). The error bars reflect the SEM associated with the interaction of source by hour (SEM=2.022).

Figure 3. Effect of barley source on in vitro starch disappearance over time.

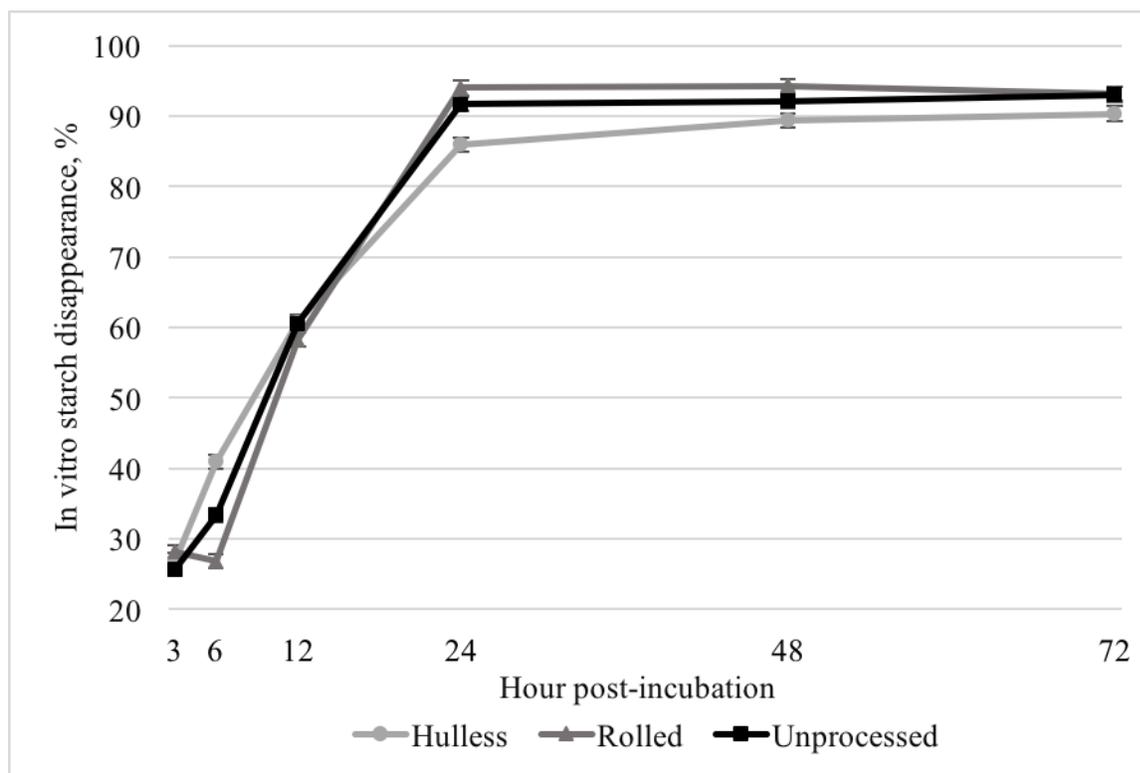


Figure 3. Effect of barley source on in vitro starch disappearance over time. There was a significant ($P < 0.05$) hour and source by hour interaction, but not a significant ($P > 0.05$) source interaction for in vitro starch disappearance. The error bars reflect the SEM associated with the interaction of source by hour (SEM=1.021).

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