

**Effects of Feeding Hulless Barley (*Hordeum vulgare L.*) and Supplementing a  
Fibrolytic Enzyme on Production Performance, Nutrient Digestibility, and  
Milk Fatty Acid Composition of Lactating Dairy Cows**

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## **Composition of Lactating Dairy**

Yang Yang

### **ABSTRACT**

The overall objective of this study was to evaluate the effects of feeding hulless barley and supplementing a xylanase enzyme on production performance and nutrient utilization of lactating dairy cows. In study 1, we evaluated production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn and hulless barley in different proportions as the grain source. We hypothesized that a plausible reduction in production performance would be explained by an altered rumen function, which would be reflected in a reduction of the proportion of *de novo* fatty acids in milk fat. The inclusion of hulless barley grain as the energy source in diets for lactating dairy cows resulted in similar production performance and nutrient utilization as corn grain. We concluded that hulless barley is as good as corn grain as an energy source and increasing NDF concentration in hulless barley-based diet is not necessary. In study 2, we evaluated production performance, nutrient digestibility, and milk fatty acid composition of high-producing dairy cows consuming diets containing hulled or hulless barley as the grain source. We hypothesized that rumen function is altered when cows are fed low-forage diets containing barley grains, and this altered rumen function would be reflected in lower production performance and a reduction of fatty acids synthesis in the mammary gland. Contrary to our expectations, feeding hulled barley or hulless barley based diets with different forage to concentrate ratios to lactating dairy cows resulted in similar production performance and nutrient

utilization. We concluded that both hulled or hulless barley grains are good energy sources for sustaining high milk production and there is no need to increase NDF concentration in diet when using barley grain as the grain source. In study 3, we evaluated the effects of supplementing a xylanase enzyme on production performance and nutrient digestibility of lactating dairy cows fed diets containing corn or sorghum silage as the forage source. We hypothesized that supplementing a xylanase enzyme product in diets containing corn or sorghum silage increases NDF digestibility, and production performance of lactating dairy cows would also be improved due to enhanced fiber digestion. Supplementation of xylanase for 19 d did not affect cow performance and nutrient utilization. Supplementation of xylanase may require a longer period of time to show any response in production performance and nutrient digestibility. We concluded that supplementing xylanase to cows fed corn or sorghum silage-based diets did not improve fiber digestion. But for feeding hulled or hulless barley grains to lactating dairy cows, increased NDF concentration in diets is not necessary and hulless barley is good as corn grain for feeding lactating dairy cows as the grain source.

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## **Composition of Lactating Dairy**

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### **ABSTRACT**

(General Audience)

The overall objective of this study was to evaluate the effects of feeding hulless barley and supplementing a xylanase enzyme on production performance and nutrient utilization of lactating dairy cows. Barley starch is fermented faster than corn starch and can possibly reduce ruminal pH. Reduced ruminal pH can compromise cow production performance and cause some health problems. In study 1, we evaluated production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn or hulless barley as the grain source. We hypothesized that a plausible reduction in production performance and milk fat percentage would be reduced by feeding hulless barley as the grain source in the diet. According to our results, the inclusion of hulless barley grain as the energy source in diets for lactating dairy cows resulted in similar production performance and nutrient utilization as corn grain. We concluded that hulless barley is as good as corn grain as an energy source and increasing fiber concentration in hulless barley-based diet is not necessary. In study 2, we evaluated production performance, nutrient digestibility, and milk fatty acid composition of high-producing dairy cows consuming diets containing hulled or hulless barley as the grain source. We hypothesized that rumen function is altered when cows are fed low-forage diets containing barley grains, and this altered rumen function would be reflected in lower production performance and a

reduction of milk fat percentage. Contrary to our expectations, we did not observe any differences in cow production performance among all treatments. We concluded that both hulled or hulless barley grains are good energy sources for sustaining high milk production and there is no need to increase fiber concentration in diet when using barley grain as the grain source. In study 3, we evaluated the effects of supplementing a xylanase enzyme on production performance and nutrient digestibility of lactating dairy cows fed diets containing corn or sorghum silage as the forage source. We hypothesized that supplementing a xylanase enzyme product in diets containing corn or sorghum silage increases fiber digestibility, and production performance of lactating dairy cows would also be improved due to enhanced fiber digestion. Supplementation of xylanase for 19 d did not affect cow production performance and nutrient digestion. The effects of supplementation xylanase may require a longer period time to detect. We concluded that supplementing xylanase to cows fed corn or sorghum silage-based diets did not improve fiber digestion. For feeding hulled or hulless barley grains to lactating dairy cows, increased fiber concentration in diets is not necessary and hulless barley is good as corn grain for feeding lactating dairy cows as the grain source.

## **DEDICATION**

Dedicated to my parents, Jianzhong Yang and Chunlan Bai, who have offered valuable support and encouragement during the past eight years, and to my wife and kids, who have provided precious companionship and love to me.

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## CHAPTER 1 INTRODUCTION

Carbohydrates (**CHO**) are the major energy source for lactating dairy cows. Carbohydrates are classified as structural and non-structural. Non-structural carbohydrates (**NSC**) consist of starch and free sugars, while structural carbohydrates consist of hemicellulose and cellulose (NRC, 2001). Non-structural carbohydrates are measured by enzymatic methods, which generally measure starch, sucrose, and fructans (Smith, 1981). Hemicellulose and cellulose are included in neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**). Neutral detergent fiber consists of hemicellulose, cellulose, lignin, ash, and insoluble protein while ADF does not include hemicellulose.

Moreover, feeds are categorized as roughages and concentrates based on their physical forms and fiber concentrations. Cereal grains, such as barley, corn, sorghum, and wheat, are often used in concentrate feeds for meeting the high-energy requirements of lactating dairy cows during early- or mid-lactation. Cereal grains consist of a pericarp, endosperm, and germ (McAllister and Cheng, 1996). Barley and oat grains also are surrounded by a fibrous husk (McAllister and Cheng, 1996). Due to the fibrous husk, barley grain has lower starch and higher NDF concentrations than corn grain (Yang et al., 1997a; Yang et al., 1997b). Hulless barley is another variety of barley that has a loose husk, which is easily removed during harvest and cleaning (Thomason et al., 2009). Although hulless barley has higher starch and lower fiber concentrations than hulled barley, hulless barley is not commonly used for feeding dairy cows.

The main concern about feeding hulless barley is that barley starch is degraded faster in the rumen than corn and sorghum starches (Herrera-Saldana et al., 1990). Feeding rapid-fermentable starch can produce excessive organic acids in the rumen and lower ruminal pH (Silveira et al., 2007; Mohammed et al. 2010). Reduced ruminal pH may alter the pathways of

biohydrogenation of unsaturated fatty acids, which may lead to milk fat depression by a reduction in *de novo* fatty acids synthesis (Bauman and Griinari, 2003; Daniel et al., 2003). To avoid ruminal acidosis, dietary NDF concentration may need to be increased when rapidly fermentable starch is included in the diet. Current recommendations from NRC (2001) suggests that diets containing barley grain should contain about 34% NDF, however, this recommendation is based on one study which only reported starch sources instead of the starch concentrations in diets (Beauchemin, 1991). In addition, Yang et al (1997a) observed a reduction in dry matter (**DM**) intake and decreased milk production in cows fed low-forage diets containing 50% hulless barley or corn grain as the grain source (DM basis). Contrary to this study, Yang et al (1997b) reported similar DM intake and production performance of cows fed hulless barley as the grain source compared to cows fed corn grain as the grain source. Therefore, the effects of feeding hulless barley to lactating dairy cows are not conclusive.

Also, increased NDF concentrations may negatively relate to dry matter intake and milk production due to increased rumen filling (Allen, 1996). However, feeding diets with improved *in vitro* NDF digestibility increased production performance of lactating dairy cows (Kendall et al., 2008). Xylan is a major structural polysaccharide in hemicellulose, and xylanase is a hydrolytic enzyme that cleaves the  $\beta$ -1,4-linked backbone of xylan (Collins et al., 2005). Xylanase supplementation is commonly used to enhance feed values by improving fiber digestibility for non-ruminant animals, especially for broiler chickens fed diets containing barley, oats, or wheat (Wang and McAllister, 2002). However, limited information exists in the literature to determine the effects of xylanase supplementation on cow production performance.

The purposes of this research were to evaluate the utilization of hulless barley as the grain source by lactating dairy cows and to evaluate the effects of xylanase supplementation on

production performance of cows fed diets with increased NDF concentrations by using different silages and grass hay. Therefore, the objectives were to: 1) evaluate production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn and hulless barley in different proportions as the grain source; 2) evaluate production performance, nutrient digestibility, and milk fatty acid composition of high-producing dairy cows consuming diets containing hulled or hulless barley as the grain source when feeding low- or high-forage diets; 3) evaluate the effects of supplementing a xylanase enzyme product on production performance and nutrient digestibility of lactating dairy cows fed diets containing corn or sorghum silage as the forage source.

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## **CHAPTER 2 LITERATURE REVIEW**

### **2.1 CARBOHYDRATES**

Dietary energy used by the rumen microbes enters the rumen generally in forms of carbohydrates (**CHO**). Carbohydrates are synthesized from carbon dioxide, water, and solar energy (Cheeke and Dierenfeld, 2010). The name CHO is derived from the concept of hydrates of carbon because CHO consist of carbon, hydrogen, and oxygen, with the hydrogen and oxygen in the same proportion as in water:  $C_n(H_2O)_n$  (Cheeke and Dierenfeld, 2010). Monosaccharides are the basic building blocks of the CHO structure, and pentoses and hexoses are the most common monosaccharides encountered in animal nutrition (Cheeke and Dierenfeld, 2010). Carbohydrates can be classified as non-structural carbohydrates (**NSC**) and structural carbohydrates (**SC**). Non-structural carbohydrates consist of starch and free sugars, while SC consist of hemicellulose and cellulose (NRC, 2001). The primary difference between starch and cellulose is that starch is a polymer consisting of  $\alpha$ -D-glucose, while cellulose is a polymer consisting of  $\beta$ -D-glucose (Van Soest, 1994). Starch has greater nutrient availability than cellulose because only microbial enzymes can break down  $\beta$ -1-4 linkages in cellulose and hemicellulose (Kung, 2006), and  $\beta$ -1-4 linkages in cellulose are more stable than the  $\alpha$ -1-4 linkages in starch, and greater molecular stability means that cleavage or degradation of cellulose requires greater energy cost than starch (Van Soest, 1994).

### **2.2 CEREAL GRAINS**

Cereal grains are commonly used in dairy rations to provide energy to lactating dairy cows. Cereal grain consists of an embryo, the endosperm, and the pericarp surrounding the storage endosperm (Zhou et al., 2012). The endosperm comprises more than 80% of the grain weight,

while the embryo and the pericarp comprise about 15% and 3% of the grain weight, respectively (Zhou et al., 2012). All cereal grains have similar anatomical structures, however, there are still some minor differences among them. For example, wheat and corn grains have a naked caryopsis because it is only covered by a fruit coat. On the other hand, barley, oats, and rice have an additional husk surrounding the caryopsis (Zhou et al., 2012). Cereal grains contain 60 to 70% of starch, 10 to 15% of protein, and 3 to 8% of fiber (Saulniera et al., 2007). The composition of cereal grains varies largely and highly depends on grain variety, growing environment, and diseases (Tester et al., 1995). The starchy endosperm constitutes the major portion of the cereal grain and is the major nutrient supplier for embryo development during germination (Zhou et al., 2012). During growth and germination, enzymes released from the pericarp and embryo hydrolyze the endosperm reserves and make the nutrients available for plants to use (Zhou et al., 2012).

### **2.2.1 STARCH IN CEREAL GRAINS**

Starch is a major component of cereal grains and consists of two main polysaccharides, amylose and amylopectin. Both amylose and amylopectin have chains of 1-4 linked  $\alpha$ -D-glucose, however, amylose is essentially linear, and amylopectin is highly branched, containing on average one branch point, which is 1-4-6 linked for every 20 – 25 straight chain residues (Park and Ring, 2001). Amylopectin is the most abundant component of starch (70 to 85% of starch), whereas amylose is the minor component of starch (15 to 30% of starch; Bertoft, 2017). The proportion of amylose varies among cereal grains (Table 2.1) and the quality of the starch granule is affected by its amylopectin and amylose proportions (Van Soest, 1994). For instance, the floury endosperm in corn contains a high proportion of amylose, whereas amylopectin is related to flinty or hardness characteristics, which is more resistant to enzymatic digestion (Van Soest, 1994). The

concentration of amylose and amylopectin also influences the digestion of barley grain. Tang et al. (2002) reported that normal barley starch was hydrolyzed faster than the waxy barley starch, containing greater proportion of amylopectin. For ruminal starch digestion, Foley et al. (2006) reported that the ruminally soluble starch fraction, rate of starch degradation, and in situ effective degradability of waxy barley starch were lower than that of normal barley starch.

Furthermore, starch exists in nature as water-insoluble granules whose form is characteristic of botanical origin (Park and Ring, 2001). The starch granule is also partially crystalline, and the crystallites are formed by short, external chain segments of amylopectin with a degree of polymerization containing 10 to 20 glucosyl units (Bertoft, 2017). There are two forms of crystallites in starch granules. The A form crystallite, found in most cereal starches, consists of starch double helices packed into a monoclinic array, a structural category in which all 3 axes are unequal in length, and two axes are perpendicular to each other (Park and Ring, 2001). The B form crystallite, found in some tuber starches and high amylose cereal starches, consist of starch double helices placed in a hexagonal unit cell with 36 water molecules (Bertoft, 2017). The percentage of starch crystallinity is negatively related to starch hydrolysis because crystalline regions are usually resistant to enzyme hydrolysis (Foley et al., 2006).

## **2.3 HULLESS BARLEY GRAIN**

Barley grain is the one of the most important cereal grains grown in the United States (corn, wheat, barley, and sorghum) and about 66% of the barley is used for feed and by-products (Griffey et al., 2010). Barley has a great adaptability to harsh environmental conditions and can grow on marginal lands (Zhu, 2017). Barley is important for farmers in areas with humid weather and variable rainfall because barley performs better than corn on soil that has less water-holding

capacity (Thomason et al., 2009). In addition, growing barley can improve farm cash flow due to summer harvest and grains sales (Thomason et al., 2009). Also, yields of double-cropped soybeans planted following barley are generally higher than soybeans planted following wheat due to earlier planting (Thomason et al., 2009). Using barley as an alternative grain source for replacing corn grain could have a significant impact in dairy farming systems. For example, drought stress always results in increased corn prices (USDA, 2012a; 2012b) and a reduction in net farm income. Replacing corn grains with other cheaper ingredients, such as barley, sorghum, and wheat is a reasonable solution to reduce increased feed cost. According to the USDA (2016), the price of corn grain has been 18% higher than barley grains for the last 15 years. Therefore, feeding barley grain to lactating dairy cows could reduce the reliance on corn grain and potentially control feed cost when drought stress occurs.

The varieties of barley can be categorized as hulless or hulled. Compared to hulled barley, hulless barley has a loose husk surrounding the kernel of grain that can be easily removed during harvesting and processing (Griffey et al., 2010). Due to the loss of the husks, hulless barley has been reported to contain 8% to 14% greater digestible energy (Bhatti et al., 1979), higher starch, and lower fiber concentration than hulled barley (Shon et al., 2007). Because of higher nutritional values of hulless barley, hulless barley has a high potential for replacing corn grain as the grain source to feed lactating dairy cows.

## **2.4 STARCH METABOLISMS IN DAIRY COWS**

Starch is a major NSC in dairy rations of lactating cows and provides a valuable energy and carbon source for microbial fermentation (Hall and Mertens, 2017). Although protozoa and fungi play important roles in ruminal digestive processes, the fermentation processes are mainly

performed by ruminal bacteria (Huntington, 1997). In general, amylolytic bacteria (starch fermenting bacteria) first attaches to feed particles and secretes endo- and exo-amylases, which hydrolyze both  $\alpha$  1-4 and  $\alpha$  1-6 linkages in starch granules releasing maltooligomers (disaccharides). Next, maltooligomers are transported into the cell and cleaved into individual hexoses by intracellular maltase. Then, the individual hexoses are used for generating ATP (Kotarski et al., 1992). Acetate, propionate and butyrate are the major VFA produced in rumen, and the proportion of VFA is affected by the type and the composition of the diet. Increasing starch proportion in the diet generally changes the acetate to propionate ratio, but the major response to changes in molar proportions is due to changes in propionate productions (Sutton et al., 2003). Murphy et al. (1982) developed VFA stoichiometric parameters separately for forage and concentrate based on the Koong et al. (1975) model and products of fermentation were estimated based on the diet composition, such as non-starch soluble carbohydrate, starch, hemicellulose, cellulose, and protein. According to the Murphy et al. (1982) model, starch fermentation was estimated to yield an acetate to propionate ratio of 4.2:1 and 1.3:1 for high-roughage and high-concentrate diets, respectively. Similarly, Sutton et al. (2003) reported productions of 56.5, 16.8, and 6.5 mol/d for acetate, propionate, and butyrate, respectively, for a diet containing a 60:40 concentrate to forage ratio, and productions of 49.0, 36.2, and 4.8 mol/d for acetate, propionate, and butyrate, respectively, for a diet containing a 90:10 concentrate to forage ratio. In general, increasing availability of fermentable proportion of the diets such as starch and non-starch soluble carbohydrates increases propionate production whereas increasing availability of fiber in the diet increases production of acetate and butyrate in the rumen.

Dairy cows consume large amounts of starch (20-40% on a dry matter basis) and starch digestion is affected by many factors (Patton et al., 2012). Accurate prediction of the amount of

ruminal starch degradation is important for accurate prediction of starch digestion as well as the amount the types of VFA production and absorption (Patton et al., 2012). Starch degradability in the rumen is estimated by the equation generated by Orskov and McDonald (1979), where starch effective ruminal degradability (ERD) (%) = fraction of starch disappearing immediately (a) + fraction of potentially degradable starch (b) × fractional degradation rate of potentially degradable starch (c) / [fractional degradation rate of potentially degradable starch (kd) + fractional passage rate (kp)]. The fractional degradation rate of potentially degradable starch and fractional passage rate are expressed as percentage per hour and the degradation rate for individual starch sources is calculated by in situ incubations using Dacron bags (Patton et al., 2012). The ruminal starch degradability differs among different starch sources and it is also affected by processing methods. Processing methods usually consist of breaking, cracking, grinding, rolling, or pelleting dried grains (Nocek and Tamminga, 1991).

In **Table 2.2**, previous data of in situ starch degradability from different starch sources and processing methods were reported. Overall, the degradation kinetics and effective ruminal degradability of starch were higher for wheat and barley starch than that of corn starch (Herrera-Saldana et al., 2009; Zebeli et al., 2010; Ferraretto et al., 2013) because sorghum and corn have more slowly degradable protein matrix in their endosperms than barley and wheat (Theurer, 1986). McAllister et al. (1993) based on an examination by electron microscopy reported that grinding of cereal grains exposed the endosperm cells to enzymatic digestion, but starch granules still remained within a protein-rich matrix and the protein matrix in horny corn endosperm is extremely resistant to digestion by ruminal microorganisms. Also, there is a significant variability in the characteristics of ruminal degradation among varieties of barley (Foley et al., 2006). Starch effective degradability of waxy barley is about 10% less than that of corn and soluble starch level

of waxy barley is 23% lower than that of corn grain. Foley et al. (2006) observed that the percentage of relative crystallinity of waxy barley was greater than that of normal barley and might be more resistant to enzyme hydrolysis.

Grinding processes generally increase the surface areas for enzymatic digestion. Fine ground corn has 9.6% higher starch effective degradability than untreated corn. Remond et al. (2003) reported ruminal starch degradability linearly decreased from 61.6% to 49.5% when mean particle size of processed corn increased from 0.7 mm to 3.7 mm. Gelatinization is a process that starch undergoes an irreversible order to disorder transition when it is heated in the presence of water (Jenkins and Donald, 1998). Changing the degree of gelatinization of starch granules in corn grains can modify starch degradability. Moreover, the combination of physical processing and application of heat and moisture showed greater benefits than the individual process alone (Theurer, 1986). Ruminal starch degradation of steam-flaked corn was increased by 21% compared to untreated corn grain. The conservation method has been reported to have a positive effect on ruminal starch degradation. High moisture corn has a higher starch effective degradability than dry ground corn (86.8% vs. 67.9%). Also, ruminal starch degradability is positively related to ruminal starch digestibility. Oba and Allen (2003) observed that feeding high moisture corn resulted in 10% and 8% greater ruminal starch digestibility for the high starch diet (31.5% starch and 23.6% NDF on a DM basis) and the low starch diet (21.2% starch and 30.5% NDF on a DM basis), respectively compared to the cows fed diets containing dry ground corn. Similarly, Callison et al. (2001) reported that ruminal digestibility of NFC decreased from 87.0% to 46.5% when mean particle size of processed corn increased from 1.2 mm to 4.8 mm, however, effective starch degradability for different particle size of processed corn was not reported in this study. Overton et al. (1995) fed diets containing 5 different ratios of starch from ground shelled corn and steam-rolled barley,

and reported that starch digested in the rumen was increased when barley starch replaced corn starch in the diet and ruminal starch digestibility was increased linearly as the proportion of barley starch increased in the diet.

The absorption mechanism for volatile fatty acids (VFA) is still not fully researched. However, passive diffusion of nonionized VFA across ruminal epithelium is the most commonly used mechanism (Storm et al., 2012). Also, protein-mediated absorption of ionized VFA has been reported as a potential alternative mechanism to passive diffusion (Storm et al., 2012). The passive diffusion has been estimated to contribute about 29 to 59% of acetate absorption and 25 to 76% of butyrate absorption (Penner et al., 2009).

Storm and Kristensen (2010, 2011) reported that ruminal absorption of VFA can be affected in three ways: 1) intraruminal equilibration of VFA between the site of production and the site of absorption; 2) permeability of absorptive epithelium to VFA; 3) removal of VFA with the blood from the serosal side of the epithelium. In sheep and calves, about 10 to 20% of the VFA escaped from the rumen (Dijkstra et al., 1993). In lactating dairy cows, about 20 to 35% of the VFA produced in the rumen passed into omasum and lower gut (Dijkstra et al., 1993). Higher passage of VFA from the rumen in lactating dairy cows is due to greater liquid volume and higher passage rate in the rumen compared to sheep and calves (Dijkstra et al., 1993).

In addition to ruminal digestion, enzymatic digestion in the small intestine of ruminants is similar to other species (Huntington, 1997). Kreikemeier et al. (1990), by identified enzymes present in pancreatic secretions and the lining of the small intestine, detected that cattle have the capacity to digest starch, lactose, and microbial storage CHO in small intestine. On average, about 5 to 20% of dietary starch is digested postruminally, and the most of postruminal starch digestion occurs in the small intestine (Huntington, 1997). In addition to undegraded dietary starch,

microbial glycogen, a storage compound from ruminal microbes, also can pass from the rumen to lower digestive tracts. In the small intestine, the pancreas secretes  $\alpha$ -amylase to hydrolyze amylose and amylopectin into  $\alpha$ -limit dextrins and linear oligosaccharides of two to three glucose units (Gray, 1992; Harmon, 1993). These smaller polysaccharides are degraded by enzymes, such as maltase and isomaltase, in brush border. Then, glucose is transferred from the lumen of the small intestine to the bloodstream by two routes: active transport and paracellular diffusion (Huntington, 1997). For active transport, the sodium-glucose transporter in the intestine (SGLT1) transports 1 glucose molecule and 2 Na<sup>+</sup> in each of its cycles (Huntington, 1997).

Intestinal starch digestion is more energetically efficient than ruminal starch fermentation because absorption and metabolism of glucose has higher efficiency than fermentation and absorption of organic acids (Huntington, 1997). According to the potential benefit of starch energy as simulation directly as glucose, increased starch digestion in the small intestine may spare amino acid utilization in the gut and liver (Reynolds et al., 1994). Nocek and Tamminga (1991) reported a positive relationship, showing that, as more starch escaped from the rumen, intestinal starch digestion also increased. Increasing mean particle size of corn grain may increase duodenal starch flow and may increase intestinal starch digestibility. Callison et al. (2001) observed numerical increase in duodenal flow of NSC for cows fed the diet containing medium-ground corn (2.6 mm) and the digestibility of NFC in small intestine was 13% higher for the diet containing medium-ground corn compared to other treatments. Compare with cow fed diets containing high moisture corn, Oba and Allen (2003) reported that feeding dry ground corn resulted in 2 kg/d greater duodenal starch flow for cows fed high starch diets and 0.5 kg/d greater duodenal starch flow for cows fed low starch diets, but intestinal starch digestibility was similar among treatments. Moreover, feeding highly fermentable starch sources decreases duodenal starch flow. Overton et

al. (1995) reported that duodenal starch flow decreased linearly as the proportion of barley starch increased in the diet.

Although starch is almost completely digested, some factors, such as processing type and conservation method, can affect total-track starch digestion (Oba and Allen, 2003). The dairy NRC (2001) also included a “processing adjustment factor” to describe the effect of processing on starch digestibility. Hoffman et al. (2012) reported that particle size is a primary parameter for corn grain digestion and the fermentation that happens during storage significantly reduces particle size by granule and protein matrix disruption. Ferraretto et al. (2013) reported that in vivo starch digestibility decreased by 15.5% when mean particle size increased from 0.75 mm to 3.75 mm. In addition to reducing particle size of corn grain, the chopping of corn silage is intended to maintain fiber particle size, however, broken corn kernels in corn silage had higher in vivo starch digestibility than those of unprocessed corn kernels (99.3 vs. 95.1; Bal et al., 2000).

## **2.5 CONCERNS ABOUT FEEDING HULLESS BARLEY**

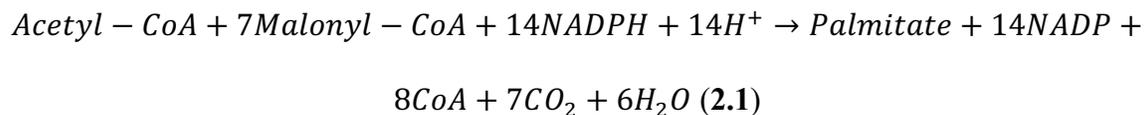
### **2.5.1 SUBACUTE RUMINAL ACIDOSIS**

Several studies have reported that barley starch has greater ruminal degradability and digestibility than corn grain (Herrera-Saldana et al., 1990; Zebeli et al., 2010; Ferraretto et al., 2013). Because of the greater ruminal degradability of barley and wheat starches than starch from corn grain (Herrera-Saldana et al., 1990; Zebeli et al., 2010; Ferraretto et al., 2013), feeding barley or wheat grains has a higher risk of lowering ruminal pH, which can lead to ruminal acidosis (Nocek and Tamminga, 1991). Increased starch availability and a faster rate of starch fermentation in the rumen can alter milk fat percentage, ruminal pH, and ruminal volatile fatty acids profile (NRC, 2001). Also, feeding highly fermentable diets can result in an accumulation of organic acids

in the rumen and reduced rumen buffering (Plaizier et al., 2009). Increased amounts of organic acids in the rumen significantly decrease ruminal pH. A prolonged low ruminal pH can lead to subacute ruminal acidosis (**SARA**) and production losses caused by milk fat depression (**MFD**) and decreased dry matter (**DM**) intake (Nocek, 1997). Current definitions of SARA are based on ruminal fluid pH, measured after collection of rumen fluid (Plaizier et al., 2008). The current guidelines suggest that the risk of SARA increases when ruminal pH is lower than 5.6 for more than 3 h/d or lower than 5.8 for more than 5 to 6 h/d (Humer et al., 2017).

### **2.5.2 DE NOVO FATTY ACID SYNTHESIS AND MILK FAT DEPRESSION**

Inhibition of de novo fatty acid (**FA**) synthesis is one consequence of SARA. Fatty acids in milk are synthesized from preformed FA and *de novo* synthesis in the mammary epithelial cells (Bauman and Griinari, 2003). For milk fat synthesis, short-chain (4 – 8 carbons) and medium-chain (10 – 14 carbons) fatty acids are exclusively from *de novo* synthesis, whereas long-chain (> 16 carbons) fatty acids are obtained from the uptake of preformed FA (Bauman and Griinari, 2003). In ruminants, acetate produced by carbohydrates fermentation in the rumen and  $\beta$ -hydroxybutyrate produced by the rumen epithelium are the major substrates for *de novo* FA synthesis (Lalotitis et al., 2010). The overall reaction catalyzed by fatty acid synthase can be summarized in equation 2.1.



To start the synthesis, acetate is converted to pyruvate, then transformed to acetyl-CoA by acyl-CoA synthetase short-chain family (Lalotitis et al., 2010). Then, acetyl-CoA, the principal building block of fatty acids, adds two carbons derived from malonyl-CoA to build a fatty-acyl

chain each time (Lalotis et al., 2010). This process also requires nicotinamide adenine dinucleotide dehydrogenase (NADPH), which is generated from pentose phosphate pathway and oxidation of isocitrate by isocitrate dehydrogenase (Lalotis et al., 2010). The synthesis is terminated by transacylase and the maximum number of carbons can reach up to 16 carbons and about 50% C16 FA is synthesized from de novo FA synthesis (Knudsen and Grunnet, 1982).

When SARA occurs, there are no clinical signs of illness. However, SARA has been reported to be associated with diarrhea and milk fat depression (Plaizier et al., 2009; Humer et al., 2017). Milk fat depression is a reduction in milk fat yield of up to 50% without any change in milk yield or yield of other milk components. Milk fat depression is observed in ruminants fed highly fermentable diets or diets high in unsaturated oil. During MFD, yields of FA of all chain lengths decreases, but *de novo* fatty acid synthesis is decreased to a greater degree (Bauman and Griinari, 2003).

Teter et al. (1990) discovered that lactating mice was not able to incorporate plasma-derived *trans* fatty acids into milk fat and feeding partially hydrogenated vegetable oil containing *trans* fatty acids could result in milk fat depression. Wonsil et al. (1994) reported that milk fat percentage by lactating cows reduced linearly with the amount of *trans* 18:1 passing to the duodenum and the concentration of *trans* 18:1 in milk fat. Bauman and Griinari (2003) developed the “biohydrogenation theory” that suggests that diet-induced MFD is associated with an inhibition of mammary milk fat synthesis by specific FA intermediates produced from altered ruminal fermentation. Metabolism of linoleic acid in the rumen generally produces *trans*-11 C18:1 and *cis*-9, *trans*-11 conjugated linoleic acid (CLA), however, ruminal biohydrogenation pathways are dynamic and allow the production of a wide range of positional and geometric isomers as well as modified FA (Harvatine et al., 2009). These isomers can be absorbed and used for milk fat

synthesis. Also, increased concentration of *trans*-C18:1 and CLA isomers in milk fat can work as hallmarks for detecting diet-induced MFD (Harvatine et al., 2009). Dietary factors, such as high carbohydrate fermentability, high oil, and monensin, can modify ruminal FA metabolism by some complex effects and result in altered ruminal microbial populations, which may lead to altered poly-unsaturated FA biohydrogenation (**Figure 2.1**). The outflow of altered biohydrogenation intermediates (*trans*-10 C18:1 and *trans*-10, *cis*-12 CLA) affects milk fat composition and yield. Bauman and Griinari (2001) reported that increased *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA in duodenal flow and milk indicates altered FA metabolism in the rumen. *Trans* FA intermediates generated by altered ruminal biohydrogenation of unsaturated FA can reduce mammary capacity for milk fat synthesis (Ma et al., 2015) by gene regulation and the reduction in key enzymes used in pathways of milk fat synthesis (Bauman et al., 2006).

## **2.6 BALANCING DIETS FOR HULLESS BARLEY STARCH AND FIBER CONCENTRATION**

Feeding adequate proportions of fiber is very important for preventing ruminal acidosis (Hall, 2002). The NRC (2001) recommends that diets based on barley starch should contain about 34% NDF, but this recommendation was based on only one study (Beauchemin, 1991). In this study, Beauchemin (1991) suggested that increased concentrations of NDF, with allowance for higher proportions of NDF from concentrate feeds, should be used for formulating barley-based diets for lactating dairy cows, although starch concentrations of diets were not reported.

In **Table 2.3**, effects of feeding hulless or hulled barley with different starch and NDF concentrations are reported. Yang et al (1997a) reported that cows fed hulless barley diets containing 50% hulless barley (dry matter basis) with 28.7% NDF resulted in a reduction in DM

intake and decreased milk production compared to cows fed diets containing 50% corn grain (dry matter basis) with 27.7% NDF. In addition to this study, Beauchemin et al. (1997) observed a reduction in DMI for cows fed low forage diet containing hulless barley as the grain source compared to cows fed low forage diets containing hulled barley or corn grain as the grain source, however, milk fat percentage and yield were not affected by grain sources. Contrary to previous studies (Beauchemin, 1991; Beauchemin et al., 1997; Yang et al., 1997a), Beauchemin et al. (1999) did not observe low ruminal pH when cows were fed hulless barley-based diet with 35% starch and 26% NDF. Also, Yang et al. (1997b) reported that cows fed hulless barley-based diets resulted in similar DM intake and milk production compared to cows fed corn-based diets. Based on previous studies, there is limited information to set up the NDF recommendation for hulless barley-based diets.

In addition to increasing NDF concentration of diet, including an adequate amount of physically effective fiber is important for preventing SARA. The concept of physically effective fiber (peNDF) was developed to describe the minimum fiber requirements of dairy cows using a combination of physical and chemical characteristics of fiber (Hall and Mertens, 2017). Physically effective fiber is related to physical effectiveness, the ability of a dairy diet to maintain chewing activity, and rumen health (Humer et al., 2017). Adequate amounts of dietary peNDF provide necessary physical effectiveness to maintain ruminal health and normal ruminal functions. However, increased amounts of physically effective fiber also have been reported to increase the diet particle size, which has the potential to decrease intake and digestibility by increasing ruminal fill and fiber passage rate in the rumen (Hall and Mertens, 2017). Therefore, determination of optimal concentration of peNDF is important for dairy cows to reduce the risk of SARA and increase the efficiency of feed utilization (Zebeli et al., 2010). Zebeli et al. (2010) reported that the

ratio of 1.45 between peNDF to ruminally degradable starch from grains should be reached to maintain ruminal health, but this ratio was calculated using data from corn grain- based studies and it may not be used for setting up recommendations for barley grains.

## **2.7 STRUCTURAL CARBOHYDRATES METABOLISM IN DAIRY COWS**

Structural carbohydrates consist primarily of hemicellulose and cellulose. In dairy feeding systems, hemicellulose and cellulose are included in NDF and ADF. Neutral detergent fiber consists of hemicellulose, cellulose, lignin, ash, and insoluble CP while ADF does not include hemicellulose. Compared to cellulose, hemicellulose consists of a loose matrix constructed by simple sugars, such as arabinose, galactose, and ribose, interwoven with short chains of xylose to form the structure of the matrix (Van Soest, 1994). Fibrolytic bacteria use hemicellulose and cellulose for generating energy. The whole process starts by bacteria attaching themselves to the feed particles, however, the metabolism of structural carbohydrates has a lag phase in which little or no degradation occurs because fiber digestion will not be maximized until microbes and their enzymes are attached or in close proximity to fiber digestion site (Allen and Mertens, 1988). Then, the bacteria start to secrete enzymes, which can degrade the fiber by hydrolyzing  $\beta$ -1-4 bonds to sugars that are subsequently fermented. The primary enzyme involved in this step is cellulase, which is able to degrade the  $\beta$ -1-4 linkages of the straight glucose chains (Krause et al., 2003). The primary products produced by fermentation of structural CHO are acetate, propionate and butyrate. The major fibrolytic bacteria consists of *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* (White et al., 1993). White (1991) reviewed the biochemistry and genetic of microbial enzymes involved in fiber hydrolysis, and summarized that the cellulosic portion of fiber is mainly degraded by *Fibrobacter succinogenes*, *Ruminococcus albus*, and

*Ruminococcus flavefaciens*. Most strains of *Butyrivibrio fibrisolvens* are xylanolytic and primarily degrade the xylan proportion of fiber (White, 1991), and degradation of cellulose by pure cultures of *Butyrivibrio fibrisolvens* generally much less than that by other fibrolytic bacteria (Berger et al., 1989). For fiber digestion, attachment is very important for fibrolytic bacteria to degrade fiber. Bacteria usually attach at epidermal damage sites, where carbohydrates are more accessible (McAllister et al., 1994). Cellulosomes, membrane bound multi-enzyme complexes, have been reported to be used by *Ruminococcus flavefaciens* and *Ruminococcus albus* to attach to the fiber source and degrade cellulose (Wang and McAllister, 2002). Koike et al. (2003), examined the binding kinetics of bacteria to plant tissues, and reported that *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* began binding to hay stems after 5 minutes and reached to a maximum at 24 h with  $10^9$  cells per gram of dry matter for *Fibrobacter succinogenes* and  $10^7$  cells per gram of dry matter for *Ruminococcus flavefaciens*. Jun et al. (2003) reported that 7 endoglucanases, 3 xylanases, a cellodextrinase, and an  $\alpha$ -glucuronidase are produced by *Fibrobacter succinogenes*. Saluzzi et al. (2006) reported that cultivation *Ruminococcus flavefaciens* strain 17 on ryegrass resulted in significant increase in dry matter degradation. *Ruminococcus flavefaciens* possesses a wide range of fiber degrading enzyme specificities, such as cellulases, xylanases, pectinases, and esterases (Saluzzi et al., 2006).

### **2.7.1 FEEDING XYLANASE TO LACTATING COWS**

Feeding adequate proportions of fiber is very important for maintaining ruminal health for lactating dairy cows fed high grain diet. Increased starch availability and a faster rate of starch fermentation in the rumen can alter milk fat percentage, ruminal pH, and ruminal volatile fatty acids profile due to reduced ruminal pH (NRC, 2001). Adequate fiber will increase rumination

time and will simulate more production of saliva which can buffer the rumen fluid (Allen, 1997). However, increased fiber concentration is negatively related to DMI and milk production due to the increased rumen fill (Allen, 1996). Van Soest (1965) reported DMI decreases with forage NDF content increases in the diet. Dado and Allen (1995) reported that DMI of lactating cows in early lactation was decreased by insertion of inert fill (22.2 L) into reticulorumen. The effect of fill caused by forage NDF content on DMI may gradually diminish as digestibility increases (Allen, 1996). Feeding exogenous fibrolytic enzyme is a feasible strategy to improve production performance by increasing fiber digestibility by dairy cows.

Xylan is a major structural polysaccharide in hemicellulose and accounts for the second most abundant polysaccharide in nature (Collins et al., 2005). Xylan, located in the secondary cell wall of plants, is commonly found in hardwood from angiosperms and softwoods from gymnosperms (Collins et al., 2005). Generally, xylan is a highly branched hetero-polysaccharide, which can vary in structure between different plant species and its backbone chain of 1-4 linked  $\beta$ -D xylopyranosyl units can be substituted to different degrees with glucuronopyranosyl,  $\alpha$ -L-arabinofuranosyl, 4-O-methyl-D-glucuronopyranosyl,  $\alpha$ -L-arabinofuranosyl, acetyl, and feruloyl groups (Collins et al., 2005). Due to the complex structure of xylan, the degradation of xylan requires a large variety of enzymes to react cooperatively.

Xylanases are hydrolytic enzymes that randomly cleave the  $\beta$ -1-4 backbone of the polysaccharide xylan. Nowadays, most commercial xylanases are produced by *Trichoderma*, *Bacillum*, *Aspergillus*, *Penicillium*, and *Talaromyces spp* (Lial et al., 2000), and are used commercially in the pulp, paper, food, and animal feed industries (Lial et al., 2000). In animal feeds, supplemental xylanase is used to enhance the digestibility of wheat by poultry and swine. For lactating dairy cows, xylanase is always included in exogenous fibrolytic enzyme products.

Exogenous fibrolytic enzyme products commonly contain a combination of cellulase and xylanase.

The effects of feeding fibrolytic enzymes to lactating dairy cows have been evaluated in several studies (Schingoethe et al., 1999; Rode et al., 1999; Kung et al., 2002; Colombatto et al., 2003; Eun et al., 2007; Romero et al., 2016; Arriola et al., 2011) but the results are not consistent. Previous studies reported that adding exogenous fibrolytic enzyme products improved fiber digestibility both in vitro (Colombatto et al., 2003; Eun et al., 2007) and in vivo (Arriola et al., 2011). Rode et al. (1999) supplemented a combination of cellulase and xylanase, at a rate of 1.3 g/kg of dry matter of TMR per cow per day and observed about 8%, 9%, and 7% increases in total-tract digestibility of DM, NDF, and CP, respectively. Consequently, milk yield increased 4 kg per cow per day, however, this increase was not statistically significant. Refat et al. (2017) supplemented xylanase and cellulase to cows fed barley silage-based diets and reported increased total-tract NDF digestibility (53.4% vs. 47.3%) for cows supplemented 0.75 mL/kg of DM of the mixture of cellulase and xylanase, and the cows supplemented with fibrolytic enzymes resulted in 2.5 kg increase of FCM (38.9 kg/d vs. 36.5 kg/d). Contrary to these studies, Kung Jr. et al. (2000) sprayed liquid enzymes (carboxymethyl cellulase and xylanase) onto forages, and the cows fed enzyme treated forages resulted in similar production performance compared to the cows fed untreated forages. Moreover, Arriola et al. (2001) evaluated the effect of feeding fibrolytic enzyme (3.4 mg/g of DM) to lactating dairy cows fed diets containing different forage to concentrate ratios and reported similar production performance and nutrient utilization among treatments.

## 2.7 Conclusions

Carbohydrates (**CHO**) are the major energy source for lactating dairy cows. Carbohydrates are classified as structural and non-structural. Non-structural carbohydrates (**NSC**) consist of starch and free sugars, while structural carbohydrates consist of hemicellulose and cellulose (NRC, 2001). Non-structural carbohydrates are measured by enzymatic methods, which generally measure starch, sucrose, and fructans (Smith, 1981). Hemicellulose and cellulose are included in neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**). Neutral detergent fiber consists of hemicellulose, cellulose, lignin, ash, and insoluble protein while ADF does not include hemicellulose. Barley grain is an important source of starch, however, barley grain contains less starch and more fiber than corn grain. Hulless barley has a high potential to replace corn grain as the energy source because hulless barley has higher starch and lower fiber concentrations than hulled barley. But barley starch is degraded faster in the rumen than corn starch (Herrera-Saldana et al., 1990). Feeding fast fermentable starch can lead to a reduction of ruminal pH because of an accumulation of organic acids in the rumen. Reduced ruminal pH may change the pathways of biohydrogenation of unsaturated fatty acids and result in milk fat depression. According to NRC (2001), diets containing barley grain should have at least 34% NDF. Few previous studies evaluated the use of hulless barley as a grain source in diets for high-producing cows and observed similar or compromised lactation performance compared to cows fed corn-based diets (Beauchemin et al., 1997; Yang et al., 1997a, 1997b). However, feeding 43.1 to 54.6% grain on a dry matter basis is not common in dairy feeding systems, and results from previous studies were variable (Beauchemin et al., 1991; Beauchemin et al., 1997; Yang et al., 1997a, 1997b;). Therefore, the effects of feeding hulless barley to high-producing dairy cows are not conclusive and further investigation is necessary. Moreover, if increasing NDF concentration is necessary when feeding

diets containing rapid fermentable starch, DMI and lactation performance may be negatively affected by increased NDF concentration in diet. Feeding xylanase may promote lactation performance by enhanced fiber digestion. But there is insufficient information exists in the literature to determine the effects of xylanase supplementation on lactation performance of lactating dairy cows. Therefore, in this project utilization of hulless barley as the grain source by lactating dairy cows and effects of xylanase supplementation on lactation performance will be evaluated.

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**Table 2.1** Amylose content and structure in selected starches.

Source	Amylose, %	N <sub>branched</sub> <sup>2</sup> , %	NC <sub>branched</sub> <sup>3</sup>
Wheat	17-34	26-44	12.9-20.7
Barley	22-27	21-45	6.1-13.8
Oat	18-29	n/a <sup>4</sup>	n/a
Maize	20-28	44-48	5.3-5.4

<sup>1</sup> Adapted from Bertoft, 2017

<sup>2</sup> Molar fraction of branched amylose molecules

<sup>3</sup> Average number of chains in branched molecules.

<sup>4</sup> Data not available

**Table 2.2** In situ starch degradability in the rumen from different starch sources and processing methods.

Feedstuffs	Processing method	Starch, % (DM basis)	Degradation <sup>1</sup>		ERD <sup>2</sup>
			a, %	kd, %/h	
Corn grain	Untreated	70.3-78	21.0	6.4	54.4
	Ground		33.8	5.5	67.9
	Cracked		20.0	5.7	58.4
	Steam-rolled		31.2	7.5	68.5
	Steam-flaked		12.7	21.6	80.3
	Pelleted		38.7	5.9	68.5
	High moisture		- <sup>3</sup>	-	86.8
Semi-flint corn	Ground (0.7 mm)	-	18.4	5.6	61.6
	rolled (1.8 mm)	-	8.7	5.7	56.5
	rolled (3.7 mm)	-	1.9	4.0	49.5
Dent Corn	Ground (0.6 mm)	-	24.9	5.4	65.0
	rolled (3.5 mm)	-	0.4	4.15	50.3
Barley grain	Untreated	57.8-74	14.4	11.9	69.3
	Ground		46.0	38.7	85.9
	Cracked		1.0	6.0	50.3
	Steam-rolled		29.5	10.9	70.6
Waxy barley	Untreated	48.3	7.3	7.3	58.9
Wheat grain	Untreated	67.6-77	60.4	32.9	93.9
	By-products		78.2	23.8	93.7

Adapted from Zebeli et al., 2010.

<sup>1</sup> a = fraction of starch disappearing immediately, kd = fractional degradation rate of potentially degradable starch.

<sup>2</sup> ERD = ERD = starch effective degradability (%):  $ERD = a + b \cdot kd / (kd + kp)$ ; where b, slowly disappearing starch fraction (%), and kp, fractional passage rate.

<sup>3</sup> not reported

**Table 2.3** Effects of feeding hulless or hulled barley to lactating dairy cows.

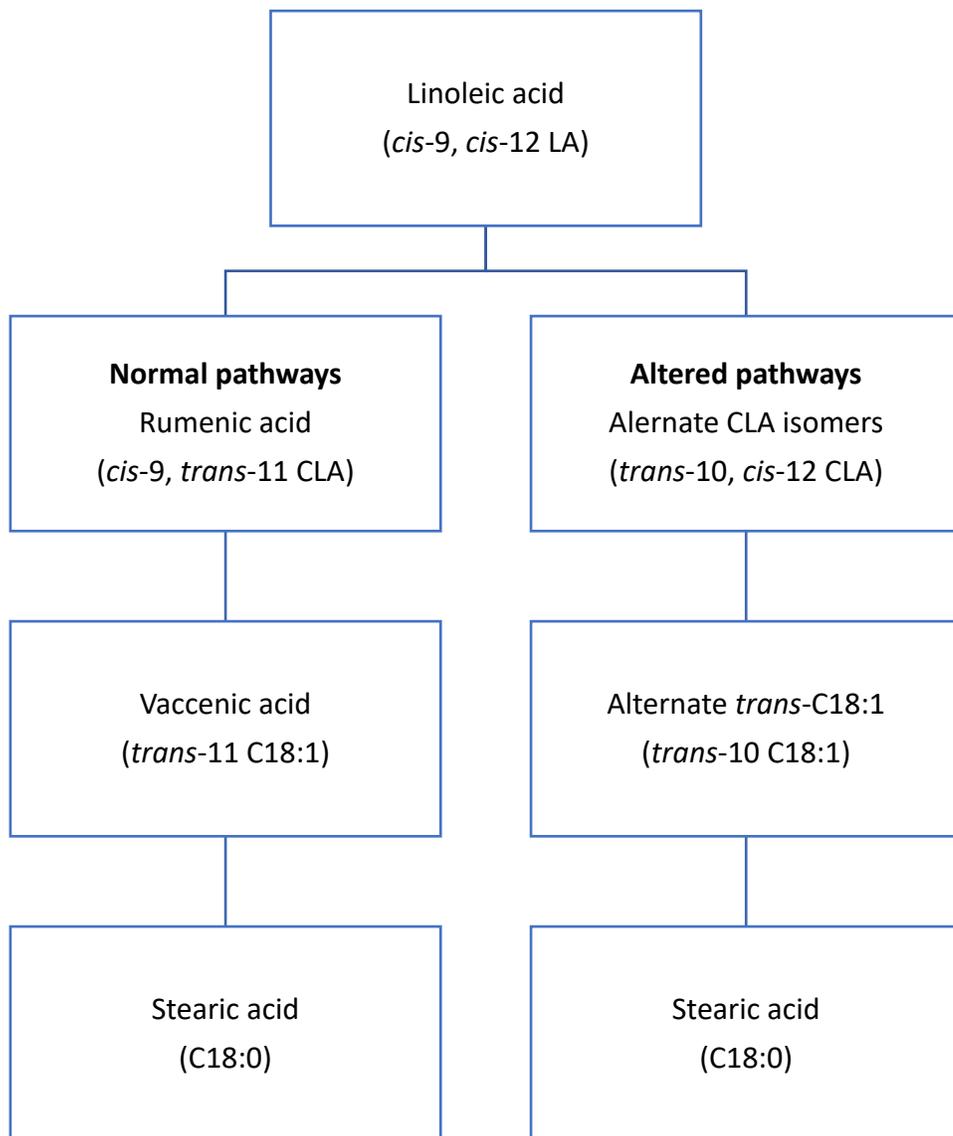
Diet <sup>1</sup> (DM basis), %	Intake, kg/d			Milk Yield, kg/d	Milk Fat, %	Digestibility, %		References
	DMI	Starch	NDF			Starch <sup>2</sup>	NDF <sup>3</sup>	
50% B, 31% starch, and 34.2% NDF	16.9	5.2	5.8	25.3	3.54	95.3	49.8	Yang et al. (1997a)
50% HB, 36% starch, and 29% NDF	17.0	6.1	4.9	25.4	3.68	84.7	49.3	Yang et al. (1997a)
51% B, 31% starch, and 34% NDF	22.4	7.1	7.7	23.7	3.95	93.2	67.3	Yang et al. (1997b)
50% HB, 36% starch, and 29% NDF	22.6	8.6	6.5	22.8	3.93	78.1	67.1	Yang et al. (1997b)
56% HB, 22% starch, and 31% NDF	18.6	4.1	5.8	25.3	3.02	n/a <sup>4</sup>	n/a	Beauchemin et al. (1997)
50% B, 21% starch, and 33% NDF	18.7	4.1	6.2	28.1	2.84	n/a	n/a	Beauchemin et al. (1997)
46% B, 32% starch, and 32% NDF	22.2	7.1	7.1	29.9	3.7	82.7	58.8	Beauchemin et al. (1999)
46% HB, 35% Starch, and 26% NDF	22.1	8.0	5.8	31.0	3.7	92.9	53.3	Beauchemin et al. (1999)

<sup>1</sup> B: hulled barley grain; HB: hulless barley grain

<sup>2</sup> Total tract starch digestibility

<sup>3</sup> Total tract NDF digestibility

<sup>4</sup> Not reported



**Figure 2.1** Pathways of ruminal biohydrogenation of linoleic acid (LA) and conjugated linoleic acid (CLA) under normal and altered ruminal fermentation. Adapted from Harvatine et al. (2009)

# CHAPTER 3 EFFECTS OF FEEDING HULLESS BARLEY ON PRODUCTION PERFORMANCE, MILK FATTY ACID COMPOSITION, AND NUTRIENT DIGESTIBILITY OF LACTATING DAIRY COWS

## 3.1 ABSTRACT

The objectives of this study were to evaluate production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn and hulless barley (cultivar Amaze 10) in different proportions as the grain source. Eight primiparous and 16 multiparous Holstein cows were assigned to 1 of 4 diets in a replicated 4 x 4 Latin square design with 21-d periods. Cows were fed once daily (1200 h) by means of a Calan gate system (American Calan Inc., Northwood, NH). All diets contained ~20% grain (dry matter basis). Treatments consisted of 100% corn (0B), 67% corn and 33% hulless barley (33B), 33% corn and 67% hulless barley (67B), and 100% hulless barley (100B) as the grain sources. Total-tract nutrient digestibility was estimated using lanthanum chloride (LaCl<sub>3</sub>) as an external marker. Dry matter intake differed quadratically among treatments, being lowest for 67B and highest for 0B and 100B. Feeding hulless barley did not affect milk yield, and milk fat concentration differed cubically among treatments. The cubic response was attributed to the higher milk fat concentration observed for the diet containing 67B. Neither the concentrations in milk of protein and lactose nor the yields of protein and lactose differed among treatments. The proportion of *de novo* synthesized fatty acids in milk did not differ among treatments. The apparent total-tract digestibility of dry matter, crude protein and neutral detergent fiber did not differ among treatments. Although a quadratic effect was observed, starch digestibility was minimally affected by treatments. In

conclusion, this study indicates that hulless barley grain is as good as corn grain as an energy source when formulating diets for high-producing dairy cows.

### **3.2 INTRODUCTION**

Cereal grains are a major energy source in diets 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 adequately processed (Nocek and Tamminga, 1991; Ferreira and Mertens, 2005; Ferraretto et al., 2013). Among cereal grains, differences exist for degradation rate of starch in the rumen. For instance, starch degradability in the rumen is greater for wheat and barley than for corn and sorghum (Herrera-Saldana et al., 1990; Overton et al., 1995; Yang et al., 1997b).

Current recommendations from NRC (2001), which are based on diets containing dry ground corn grain, suggest that dietary NDF should be increased when readily available starch sources replace dry ground corn in the diet. Citing a study from Beauchemin (1991), the NRC (2001) established that the NDF requirement for dairy cows should be increased to 34% NDF when feeding diets containing barley as the grain source. For other starch sources, however, insufficient information exists to give specific recommendations (NRC, 2001).

Even though recent in situ and in vitro studies exist (Fellner et al., 2008; Yang et al., 2013a,b), hulless barley is one starch source for which insufficient production performance information exists (Yang et al., 1997a,b) to provide specific dietary recommendations (NRC, 2001). Hulless or “naked” barley differs from traditional hulled barley in that the loose husk covering the caryopsis is removed during combine threshing and cleaning of the grain (Thomason et al., 2009). Due to the loss of the hulls, hulless barley grain can have 10 to 14% less NDF than

hulled barley grain. Yang et al. (1997a) fed low-forage diets containing 50% cereal grains (DM basis) to lactating dairy cows and reported a lower DMI and milk yield when hullless barley grain replaced corn grain. In another study, Yang et al. (1997b) did not observe differences in production performance nor NDF and starch digestibilities when feeding diets containing either corn or hullless barley as the grain source. Based on the limited information, benefits or shortcomings of including hullless barley in diets for high-producing dairy cows are not conclusive (Firkins, 2001).

The objectives of this study were to evaluate production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn and hullless barley in different proportions as the grain source. We hypothesized that a plausible reduction in production performance would be explained by an altered rumen function, which would be reflected in a reduction of the proportion of *de novo* fatty acids in milk fat.

### **3.3 MATERIALS AND METHODS**

#### ***Animals, Housing, and Diets***

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Eight primiparous ( $580 \pm 41$  kg of BW and  $49 \pm 18$  DIM at the beginning of the experiment) and 16 multiparous Holstein cows ( $650 \pm 60$  kg of BW and  $59 \pm 25$  DIM at the beginning of the experiment) were assigned to 1 of 4 diets (Table 1) in a replicated 4 x 4 Latin square design, with 6 independent squares and 21-d periods. Cows were assigned to squares based on parity (1, 2 and  $\geq 3$ ) and DIM. To avoid treatment residual effects, cows were randomly assigned to treatments within squares.

Cows were housed in a 24-stall pen within a free-stall barn. Cows were fed once daily (1200 h) by means of a Calan gate system (American Calan, Inc., Northwood, NH). Cows were trained to find their door for a 2-week period before the beginning of the study.

Treatments consisted of diets containing corn (no identity) and hulless barley grains (cultivar Amaze 10) in different proportions as the grain source (Table 2). Diets contained 100% corn grain (**0B**), 67% corn and 33% hulless barley grains (**33B**), 33% corn and 67% hulless barley grains (**67B**), and 100% hulless barley grain (**100B**). Both cereal grains were ground using a 4.76-mm screen of a hammer mill and incorporated into concentrate pellets at a commercial feed mill (Big Spring Mill, Inc., Elliston, VA). Diets were formulated so the grain provided approximately 50% of the dietary starch and to meet nutrient requirements (NRC, 2001) for a 630-kg lactating dairy cow producing 42 kg of milk/d.

Concentrate pellets were mixed with corn silage, alfalfa hay and soybean hulls (Table 2), and delivered *ad libitum* (~5% refusals) as a TMR. Mixing and delivery was performed using a Calan Data Ranger (American Calan, Inc.). The amount of feed offered and refused was measured daily. Cows were milked twice daily (0100 and 1300 h), and milk weights were automatically recorded at each milking. The average of the daily milk yields and dry matter intake from d 10 to 18 of each period was used for statistical analysis.

### ***Nutrient Digestibility***

Total tract nutrient digestibility was estimated using lanthanum chloride ( $\text{LaCl}_3$ ) as an external marker (Ferreira et al., 2002). A solution of  $\text{LaCl}_3$  (0.8 M  $\text{LaCl}_3$ ) was prepared by reacting lanthanum oxide ( $\text{La}_2\text{O}_3$ ) with HCl as follows: (1) 1 L of distilled water was added to a 4-L Erlenmeyer flask; (2) while stirring, 1,200 mL of concentrated HCl were added very slowly (as this is an exothermic reaction, HCl was added in 2 equal fractions with 30-minute intervals in between); (3) 750 g of  $\text{La}_2\text{O}_3$  were added very gently and slowly (as this is a very exothermic reaction,  $\text{La}_2\text{O}_3$  was added in 3 equal fractions with 45-minute intervals in between); (4) after

apparent solvation, 1 L of distiller water was added and the solution was stirred overnight; (5) after filtering through grade 1 qualitative filter paper (Whatman, GE Healthcare Bio-sciences, Pittsburg, PA) the  $\text{LaCl}_3$  solution was transferred into a 6-L calibrated Erlenmeyer flask, and the solution was raised to volume with distilled water; and (7) after transferring to a carboy, additional 1,272 mL of distilled water was added to the final solution. The procedure was repeated as necessary to obtain enough marker for the whole experiment. To obtain a final dietary concentration of approximately 40 mg/kg of DM, 8.65 kg of the marker solution (density = 1.15 g/mL) were sprayed onto 909 kg of soybean hulls.

Fecal grab samples were collected for each period across 3 consecutive days (starting on day 19) at 6-h intervals skipping sampling times 2 h at the end of each day. Lanthanum concentration was determined in TMR and fecal samples by inductively coupled plasma atomic emission spectroscopy. Samples were prepared as follows (Ferreira et al., 2002): (1) duplicate 2-g samples (TMR and feces) were placed in Pyrex beakers and dry-ashed at 500°C for 6 h (this step was repeated once); (2) the resulting ash was dissolved with 25 mL of concentrated HCl; (3) after 1 h, the dissolved ashes were transferred to a previously tared specimen cup and diluted to 50 g by adding lithium hydroxide (41.7 mM); (4) after an overnight sedimentation, an aliquot of the solution was extracted and analyzed for La concentration.

Dry matter apparent digestibility (DMD) and nutrient apparent digestibility were calculated as described in equations [3.1] and [3.2], respectively.

$$\text{DMD (\%)} = 100 - \frac{\text{Dietary [La]}_{(\text{mg/g of DM})}}{\text{Fecal [La]}_{(\text{mg/g of DM})}} \times 100, [3.1]$$

$$\text{Nutrient Digestibility (\%)} = 100 - \frac{\text{Dietary [La]}_{(\text{mg/g of DM})}}{\text{Fecal [La]}_{(\text{mg/g of DM})}} \times \frac{\text{Fecal [Nutr]}_{(\text{g/g of DM})}}{\text{Dietary [Nutr]}_{(\text{g/g of DM})}} \times 100, [3.2]$$

Where [La] = La concentration and [Nutr] = nutrient concentration.

### ***Sample Collection and Analysis***

Samples of feed ingredients and feed refusals were collected weekly. All samples were dried in a forced-air oven (55°C) until constant weight, and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Crude protein concentration was calculated as percent N × 6.25 after combustion analysis using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ). The concentration of NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY) with sodium sulfite and α-amylase (Ankom Technology). Starch concentration was determined using the acetate buffer method of Hall (2009) with α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspegillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland). Ash concentration was determined after burning feed samples in a furnace (3 h at 550°C).

Milk samples (a.m. and p.m. milkings) were collected on d 17 and 18 for determination of milk fat, protein, lactose, and milk urea nitrogen concentrations with a CombiFoss FT+ Fourier transform infrared analyzer (Foss, Hillerød, Denmark) by United DHIA (Radford, VA). An additional milk sample (a.m. and p.m. milking) was collected on d 18 to determine milk fatty acid composition. Milk fatty acids were extracted and methylated according to the method of Chouinard et al. (1999). Fatty acid methyl esters were analyzed by gas chromatography (Agilent 6890 N GC) using a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2 μm thickness; Varian, Inc., Palo Alto, CA). The oven temperature was initially set at 80°C, and was increased at 2°C/min to

190°C and maintained for 9 min. Inlet and flame-ionization detector temperatures were 250°C, the split ratio was 100:1, and a 1 µL injection volume was used. The hydrogen carrier gas flow rate was 1 mL/min. Hydrogen flow to the detector was 25 mL/min, airflow was 400 mL/min, and the flow of nitrogen makeup gas was 40 mL/min. Fatty acid peaks were identified by using pure methyl ester standards (Nu-Check Prep Inc., Elysian, MN). A butter reference standard (BCR 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was analyzed at regular intervals to determine recoveries and correction factors for individual fatty acyl composition in milk fat.

### ***Statistical Analysis***

All variables were analyzed using the MIXED procedure of SAS (SAS version 9.3, SAS Institute Inc., Cary, NC). The statistical model included the effects of square (fixed; df = 5), treatment (fixed; df = 3), square by treatment interaction (fixed; df = 15), period (random; df = 3), and cow within square (random; df = 18) and the random residual error. After detecting significant differences ( $P < 0.05$ ), treatments were tested for linear, quadratic and cubic effects. For those variables with significant quadratic effects, the optimum proportion of corn and barley grains was determined with the REG procedure of SAS. The model to quantify the intercept and the coefficients for a quadratic regression model (Broderick and Radloff, 2004) included the proportion of hulless barley ( $x$ ) and the proportion of hulless barley to the power of 2 ( $x^2$ ). The optimal proportion of hulless barley was determined solving the first derivative of a quadratic function.

### 3.4 RESULTS AND DISCUSSION

Hulless barley grain contained 3.2% ash, 14.3% CP, 12.6% NDF, and 58.2% starch. Ash and CP concentrations were slightly higher or similar, whereas starch concentration was slightly lower than or similar to values previously reported for hulless barley grains (Griffey et al., 2010; Yang et al., 2013a). Compared with the corn grain used in this study, hulless barley grain had a higher concentration of CP and NDF and a lower concentration of starch (8.8 vs. 14.3% CP, 10.6 vs. 12.6% NDF, and 65.4 vs. 58.2% starch for corn and hulless barley grains, respectively). Differences in nutritional composition of the grains resulted in minor differences in the composition of the diets. The major difference among diets was starch concentration, which ranged from 28.2 to 30.7% (Table 1).

Dry matter intake differed quadratically among treatments ( $P < 0.01$ ), being lowest for 67B and highest for OB or 100B (Table 2). The reasons for this quadratic response are not clear. If we consider that starch fermentation rate in the rumen is faster for barley grains than for corn (Herrera-Saldana et al., 1990; Yang et al., 1997a), and therefore DMI would be lower for diets containing barley grain, we do not find a clear explanation for the higher DMI observed when feeding 100B relative to 33B or 67B. As DMI was greater than 3.9% BW for all treatments, which is greater than DMI previously reported ( $< 3.6\%$  BW; Yang et al., 1997a,b), we consider a negative metabolic effect of treatments on DMI to be unlikely in this study.

Feeding hulless barley did not affect milk yield ( $P < 0.98$ ; Table 2). Different from this study, Yang et al. (1997a) observed a 1.8 kg/d decrease in milk yield for cows fed diets containing hulless barley grain relative to cows fed diets containing corn grain. In another study, Yang et al. (1997b) observed a 1.4 kg/d numerical, but not statistically significant, decrease in milk yield when feeding hulless barley relative to corn. Although the reasons for the different results among studies

are not clear, the dietary starch concentration in this study was substantially lower than in previous studies (Yang et al., 1997a,b). Also, milk production differed substantially between this study (>40 kg/d) and those previously reported ( $\leq 30$  kg/d; Yang et al., 1997a,b). In this study, a higher demand for glucose precursors (e.g., propionate and lactate) could have minimized differences in the metabolism of carbohydrates.

Milk fat concentration differed cubically among treatments ( $P < 0.03$ ; Table 2). The cubic response is attributed to the higher milk fat concentration observed for 67B. Milk fat concentration did not change among the other 3 diets. Firkins et al. (2001) reported an inverse relationship between milk fat concentration and DMI. Accordingly, the high milk fat concentration observed for cows fed the 67B diet in this study could be related to the lower DMI. Neither the concentrations in milk of protein ( $P < 0.33$ ) and lactose ( $P < 0.58$ ) nor the yields of protein ( $P < 0.23$ ) and lactose ( $P < 0.30$ ) differ among treatments. Feeding hulless barley increased MUN concentration linearly ( $P < 0.01$ ). This pattern followed the increasing CP concentrations as hulless barley replaced corn in the diet. Body weight was similar among diets ( $P < 0.71$ ).

Under the assumption that feeding hulless barley grain increases the ruminal fermentability of starch (Herrera-Saldana et al., 1990; Yang et al., 1997a), we hypothesized that the proportion of de novo fatty acids in milk would be diminished when feeding hulless barley grain. Contrary to our hypothesis, the proportion of de novo synthesized fatty acids in milk did not differ among treatments (Table 3). The concentration of a few specific fatty acids, such as C15:0, C16:0, *trans*-12 C18:1, and *trans*-10, *cis*-12 CLA, differed linearly or cubically (Table 3). However, these differences are marginal and have minimum biological implications. For example, the proportion (g/100 g of fatty acid) of *trans*-10, *cis*-12 CLA was substantially lower than values observed for cows infused with *trans*-10, *cis*-12 CLA to induce milk fat depression (Lock et al., 2007; Harvatine

and Bauman, 2011). Based on these observations and on the total milk fat concentrations (Table 2), we disregard the possibility that any of the treatments of this study had a negative effect on de novo milk fatty acid synthesis.

Nutrient utilization did not differ or minimally differed only minimally among treatments (Table 4). The apparent total-tract digestibility of DM ( $P < 0.82$ ), CP ( $P < 0.28$ ), and NDF ( $P < 0.38$ ) did not differ among treatments. The apparent total-tract digestibility of starch increased quadratically, being maximum (97.9%) for 67B. Despite the latter response, the magnitude of the differences (<1%) in starch digestibility is biologically trivial, especially when digestibility of starch was almost complete (>97% starch digestibility). We attribute the almost complete digestibility of starch to the inclusion of both grains into the pelleted concentrate, which ensures an intense processing of the endosperm (Yang et al., 2000). This intense processing could also explain the similar milk production among treatments. In a study in which grains were steam-rolled (Yang et al., 1997a), milk yield and starch digestibility were greater for cows fed diets containing corn grain than for cows fed diets containing hulless barley grain. As starch digestibility in that study was incomplete (<95%), it is possible that differences nutrient utilization among treatments are minimized when cereal grains are intensively processed.

Evaluating the use of hulless barley in diets for lactating dairy cows could have a major impact in dairy farming systems. When drought stress occurs, one immediate effect is an increase in corn prices (USDA, 2012a,b) and a reduction in net farm income due to increased feed costs. For example, corn price increased from \$6.37 per bushel (\$250/Mg) in June 2012 (USDA, 2012a) to \$7.63 per bushel (\$300/Mg) in August 2012 (USDA, 2012b), when drought stress was most extreme. Increased feed prices translate into greater feed costs in dairy farming systems, which may reduce income over feed costs and profitability. When corn prices increase, alternative

feeding strategies, such as increasing the proportion of forage in the diet or increasing the inclusion of by-products, are frequently evaluated for feeding programs (Mullins et al., 2010; Sullivan et al. 2012; Hall and Chase, 2014). Another alternative is replacing corn grain with other less expensive cereal grains, such as barley, oats, sorghum or wheat. According to USDA (2016), the price (\$/bu) of corn grain has been 18% greater than the price of barley for the last 15 yr. As specific densities for corn and hulled barley grains differ (56 and 48 lb/bu, respectively), the difference of grain prices on a per ton basis might be marginal, depending on market situations (Figure 1). A mature market for hulless barley is not established. However, based on this study, the inclusion of hulless barley in diets for dairy cows could reduce feeding costs while sustaining production performance at times of high demand for corn grain.

### **3.5 CONCLUSIONS**

The inclusion of hulless barley grain (cultivar Amaze 10) as an energy source in diets for high-producing dairy cows resulted in similar production performance and nutrient utilization as for corn grain. Also, to optimize FCM yield and feed efficiency, hulless barley should be included as 56.7% of the total grain. Contrary to our expectations, hulless barley did not affect milk fatty acid composition, suggesting that rumen metabolism did not differ when replacing corn with hulless barley. Overall, this study indicates that hulless barley grain is as good as corn grain as an energy source, and that there is no need to increase the concentration of NDF when formulating diets for high-producing dairy cows including hulless barley.

### **3.6 ACKNOWLEDGMENTS**

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**Table 3.1** Ingredient and chemical composition of diets (% DM basis).

	Diet <sup>1</sup>			
	0HB	33HB	67HB	100HB
<b>Ingredients</b>				
Corn silage <sup>2</sup>	36.0	35.9	35.9	35.9
Alfalfa hay <sup>3</sup>	15.3	15.2	15.2	15.2
Corn grain <sup>4</sup>	20.2	13.6	6.7	-
Hulless barley grain <sup>5</sup>	-	6.8	13.9	20.6
Soybean meal	12.7	12.7	12.6	12.6
Soybean hulls	7.7	7.7	7.6	7.6
Corn dry distiller grains with solubles	4.2	4.2	4.2	4.2
EnerGII <sup>6</sup>	0.8	0.8	0.8	0.8
Sodium bicarbonate	1.0	1.0	1.0	1.0
Bentonite	0.9	0.9	0.9	0.9
Salt	0.5	0.5	0.5	0.5
Magnesium oxide	0.2	0.2	0.2	0.2
Trace mineral premix <sup>7</sup>	0.4	0.4	0.4	0.4
Vitamin ADE <sup>8</sup>	0.04	0.04	0.04	0.04
Vitamin E <sup>9</sup>	0.003	0.003	0.003	0.003
Rumensin 90 <sup>10</sup>	0.006	0.006	0.006	0.006
<b>Nutrients</b>				
OM	92.1	91.9	91.8	91.6
CP	17.5	17.8	18.2	18.6
NDF	30.1	30.2	30.4	30.5
Forage NDF	18.4	18.3	18.3	18.2
Starch	30.7	29.9	29.0	28.2

<sup>1</sup> 0B = 100% corn grain; 33B = 67% corn grain and 33% barley grain; 67B = 33% corn grain and 67% barley grain; 100B = 100% barley grain.

<sup>2</sup> Corn silage composition: 36.8% DM, 9.1% CP, 33.7% NDF, and 42.3% starch.

<sup>3</sup> Alfalfa hay composition: 85.3% DM, 22.6% CP, 46.7% NDF, and 3.0% starch.

<sup>4</sup> Corn grain composition: 1.9% ash, 8.8% CP, 10.6% NDF, and 65.4% starch.

<sup>5</sup> Hulless barley grain composition: 3.2% ash, 14.3% CP, 12.6% NDF, and 58.2% starch.

<sup>6</sup> Calcium salts of fatty acids (Virtus Nutrition, LLC, Corcoran, CA).

<sup>7</sup> Contained 22.25% calcium; 7.50% magnesium; 2.75% potassium; 3.90% sulfur; 1.50% manganese; 1.50% zinc; 9,500 ppm iron; 2,500 ppm copper; 200 ppm iodine; 200 ppm cobalt; 66 ppm selenium; 227,273 IU/kg Vitamin A; 136,364 IU/kg Vitamin D3; 636 IU/kg Vitamin E.

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<sup>8</sup> Contained 3,500 IU/kg Vitamin A; 950 IU/kg Vitamin D3; 2,000 IU/g Vitamin E.

<sup>9</sup> Contained 500 IU/g of premix

<sup>10</sup> Contained 200 mg of monensin per gram of product (Elanco Animal Health, Indianapolis, IN).

**Table 3.2** Production performance of dairy cows consuming diets containing different proportions of corn and hulless barley grain.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>			
	0HB	33HB	67HB	100HB		Diet	L	Q	C
DMI, kg/d	26.9	24.8	24.3	25.9	0.88	0.01	0.08	0.01	0.77
Milk yield, kg/d	41.3	41.4	41.5	41.2	1.65	0.98	-	-	-
Milk fat, %	3.43	3.45	3.91	3.52	0.18	0.04	0.24	0.14	0.03
Milk protein, %	2.98	3.07	3.05	2.99	0.06	0.33	-	-	-
Milk lactose, %	4.81	4.84	4.82	4.80	0.04	0.58	-	-	-
Milk fat yield, kg/d	1.39	1.43	1.61	1.38	0.08	0.02	0.53	0.02	0.03
Milk protein yield, kg/d	1.22	1.28	1.26	1.19	0.04	0.23	-	-	-
Milk lactose yield, kg/d	1.98	2.02	2.00	1.93	0.08	0.30	-	-	-
MUN, mg/dL	8.77	8.67	9.74	9.89	0.55	0.04	0.01	0.73	0.21
3.5% FCM, kg/d	40.3	41.3	43.5	40.0	1.53	0.04	0.78	0.02	0.11
Body weight gain, kg/d	0.78	0.81	0.84	0.66	0.24	0.71	-	-	-
Feed efficiency, kg FCM/kg DMI	1.53	1.70	1.81	1.58	0.07	0.01	0.20	0.01	0.18

<sup>1</sup> 0B = 100% corn grain; 33B = 67% corn grain and 33% barley grain; 67B = 33% corn grain and 67% barley grain; 100B = 100% barley grain.

<sup>2</sup> *P*-values for linear (L), quadratic (Q) and cubic (C) effects reported only when diets differed.

**Table 3.3** Fatty acid profile (g/100 g of fat) in milk fat from dairy cows consuming diets containing different proportions of corn and hulless barley grain.

Fatty acid	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>			
	0HB	33HB	67HB	100HB		Diet	L	Q	C
C4:0	3.53	3.68	3.57	3.66	0.16	0.51	-	-	-
C6:0	1.95	2.05	2.00	2.05	0.10	0.46	-	-	-
C8:0	1.18	1.25	1.22	1.24	0.07	0.50	-	-	-
C10:0	2.79	2.95	2.91	2.97	0.17	0.48	-	-	-
C12:0	3.40	3.58	3.58	3.58	0.18	0.46	-	-	-
C14:0	10.61	10.84	11.01	10.93	0.27	0.42	-	-	-
C14:1	1.31	1.33	1.32	1.19	0.11	0.26	-	-	-
C15:0	0.94	0.95	1.07	0.99	0.06	0.04	0.09	0.15	0.06
C16:0	29.20	29.49	30.09	30.64	0.68	0.01	0.01	0.60	0.76
C16:1	2.02	1.94	2.03	1.82	0.19	0.59	-	-	-
C17:0	0.47	0.46	0.49	0.49	0.02	0.08	-	-	-
C18:0	7.73	7.57	7.36	7.70	0.30	0.51	-	-	-
C18:1 (trans 6-8)	0.66	0.62	0.58	0.53	0.04	0.01	0.01	0.92	0.90
C18:1 (trans 9)	0.41	0.40	0.37	0.33	0.02	0.01	0.01	0.30	0.84
C18:1 (trans 10)	2.45	2.60	2.10	1.83	0.40	0.10	-	-	-
C18:1 (trans 11)	1.32	1.14	1.14	1.19	0.08	0.10	-	-	-
C18:1 (trans 12)	0.70	0.63	0.61	0.54	0.04	0.01	0.01	0.99	0.29
C18:1 (cis 9)	21.00	20.29	20.22	20.08	0.64	0.43	-	-	-
C18:1 (cis 11)	1.11	1.10	1.15	1.08	0.06	0.42	-	-	-

C18:2 (cis 9, cis 12)	3.40	3.42	3.32	3.34	0.12	0.66	-	-	-
C20:0	0.05	0.06	0.06	0.06	0.02	0.51	-	-	-
C18:3	0.28	0.29	0.30	0.30	0.02	0.10	-	-	-
CLA (cis 9, trans 11)	0.64	0.56	0.58	0.56	0.04	0.08	-	-	-
CLA (trans 10, cis 12)	0.017	0.013	0.018	0.010	0.002	0.01	0.02	0.18	0.01
Unknown	2.82	2.83	2.84	2.79	0.08	0.88	-	-	-
<i>De novo</i> fatty acids <sup>3</sup>	24.76	25.69	25.62	25.61	0.78	0.43	-	-	-

<sup>1</sup> 0B = 100% corn grain; 33B = 67% corn grain and 33% barley grain; 67B = 33% corn grain and 67% barley grain; 100B = 100% barley grain.

<sup>2</sup> *P*-values for linear (L), quadratic (Q) and cubic (C) effects reported only when diets differed.

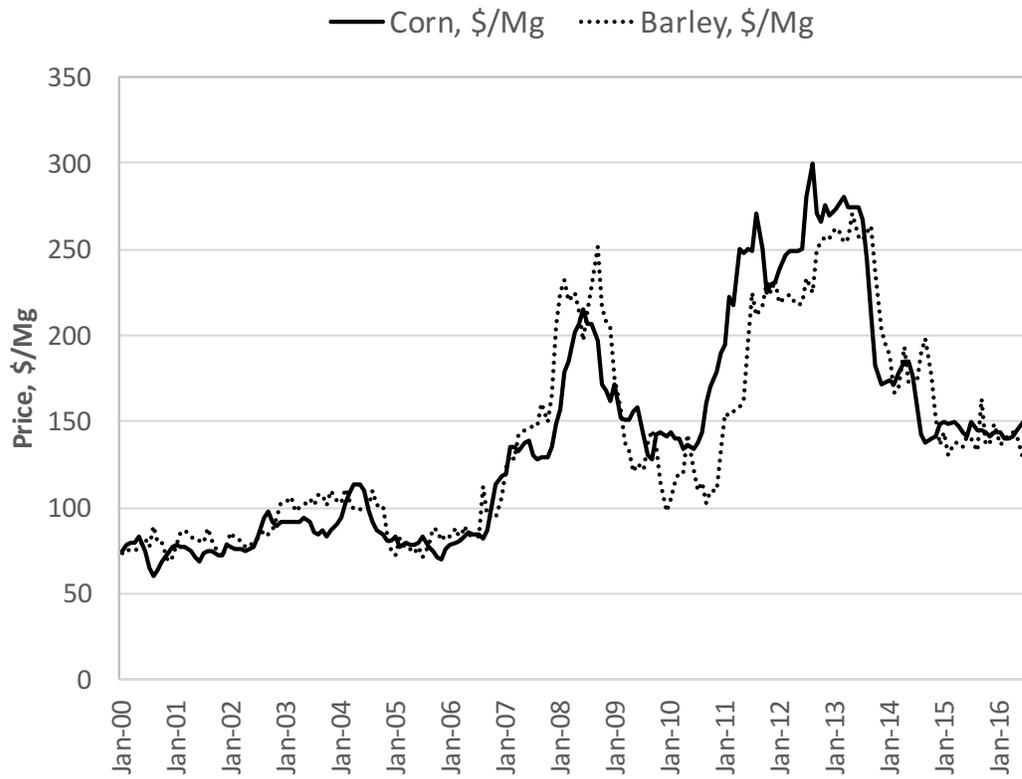
<sup>3</sup> *De novo* fatty acids is the sum of C4:0 to C14:0.

**Table 3.4** Apparent digestibility of dairy cows consuming diets containing different proportions of corn and hulless barley grain.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>			
	0HB	33HB	67HB	100HB		Diet	L	Q	C
DM, %	62.1	61.3	61.1	61.4	1.80	0.82	-	-	-
CP, %	60.3	60.5	61.0	62.4	1.89	0.28	-	-	-
NDF, %	38.7	36.6	35.6	38.3	3.71	0.38	-	-	-
Starch, %	97.3	97.7	97.9	97.7	0.17	0.01	0.01	0.02	0.90

<sup>1</sup> 0B = 100% corn grain; 33B = 67% corn grain and 33% barley grain; 67B = 33% corn grain and 67% barley grain; 100B = 100% barley grain.

<sup>2</sup> *P*-values for linear (L), quadratic (Q) and cubic (C) effects reported only when diets differed.



**Figure 3.1** Market prices for corn and hulled barley grains (adapted from USDA, 2016) from 2000 to 2016.

# **CHAPTER 4 EFFECTS OF FEEDING HULLED AND HULLESS BARLEY WITH LOW- AND HIGH-FORAGE DIETS ON PRODUCTION PERFORMANCE, NUTRIENT DIGESTIBILITY, AND MILK FATTY ACID COMPOSITION OF LACTATING DAIRY COWS**

## **4.1 ABSTRACT**

The objectives of this study were to evaluate production performance, nutrient digestibility, and milk fatty acid composition of high-producing dairy cows consuming diets containing hulled or hulless barley as the grain source when feeding low-forage (**LF**) or high-forage (**HF**) diets. Eight primiparous ( $610 \pm 40$  kg of BW and  $72 \pm 14$  d in milk) and 16 multiparous ( $650 \pm 58$  kg of BW and  $58 \pm 16$  d in milk) Holstein cows were randomly assigned to 1 of 4 diets in a replicated  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of treatments and 21-d periods. Cows were assigned to squares based on parity (1, 2 and  $\geq 3$ ) and days in milk. Diets were formulated to contain on a dry matter basis: (1) 45% forage and hulled barley as the sole grain source, (2) 65% forage and hulled barley as the sole grain source, (3) 45% forage and hulless barley as the sole grain source, and (4) 65% forage and hulless barley as the sole grain source. Dry matter (DM) intake tended to be lower for the diet with 65% forage and hulled barley than for the rest of the diets (24.4 vs. 26.6 kg/d). Neither the type of barley nor the forage-to-concentrate ratio affected milk yield (41.7 kg/d). Barley type did not affect milk fat or protein concentrations. Feeding LF diets decreased milk fat concentration from 3.91 to 3.50%. This decrease was less than anticipated and resulted in a 7% decrease in milk fat yield relative to cows consuming HF diets (1.60 and 1.49 kg/d for HF and LF diets, respectively). Feeding LF diets increased the concentration of C18:1 *trans*-10 in milk fat, suggesting that feeding LF diets may have marginally altered rumen function. In conclusion, LF

diets containing barley grains can marginally decrease milk fat concentration. Overall, and based on the conditions of this study, there is limited evidence to anticipate a dramatic or acute milk fat depression when feeding hulless barley as the grain source in diets for high-producing dairy cows.

## **4.2 INTRODUCTION**

Cereal grains, such as corn, sorghum, barley, wheat and oats, are commonly included in rations for lactating dairy cows as an energy source (Herrera-Saldana et al., 1990). Cereal grains mainly comprise the pericarp that gives protection to the grain, the germ that will become the new plant after germination, and the endosperm that stores starch (McAllister and Cheng, 1996). Using the *in situ* ruminal disappearance technique, Herrera-Saldana et al. (1990) reported different starch disappearance rates among cereal grains: rates were faster for wheat and barley and slower for corn and sorghum. This observation is also supported by *in vivo* studies (Ferraretto et al., 2013).

Feeding rations containing readily fermentable starch can increase the production of lactic acid, therefore reducing ruminal pH (Silveira et al., 2007; Mohammed et al. 2010). Reduced ruminal pH can alter the pathways of fatty acid biohydrogenation by the microbes within the rumen, which may lead to milk fat depression through a reduction of *de novo* fatty acid synthesis within the mammary gland (Bauman and Griinari, 2003; Daniel et al., 2003). As fibrous components ferment slower than NFC, a minimum concentration of dietary NDF is recommended to sustain ruminal and cow health (Mertens, 1997; NRC, 2001). Current recommendations from NRC (2001) suggest that dietary NDF should be increased when readily available starch sources, such as barley, replace dry ground corn in the diet (Beauchemin, 1991).

Yang et al. (2017) evaluated the use of hulless or “naked” barley (Thomason et al., 2009; Griffey et al., 2010) as a grain source for feeding high-producing cows. For their study, the authors

hypothesized that replacing corn with hulless barley would decrease milk fat concentration as a consequence of a lower ruminal pH and a subsequent modification of the pathways of fatty acid biohydrogenation by the rumen microbes within the rumen (Mohammed et al., 2010). Although the dietary NDF concentration was lower than the recommended for diets containing barley grain (30 vs. 34% NDF; Beauchemin, 1991; NRC, 2001), milk yield and milk fat concentration were similar between diets containing 100% corn grain or 100% hulless barley as the grain source. Contrary to their expectations, the concentration of de novo fatty acids was also not affected by grain source (Yang et al., 2017).

Studies evaluating the inclusion of hulless barley as an energy source in diets for lactating dairy cows are limited and have variable results (Beauchemin et al., 1997; Yang et al., 1997a,b; Yang et al. 2017). Under the scope that starch from hulless barley is rapidly fermentable (Yang et al., 1997a), we hypothesized that rumen function is altered when cows are fed low-forage diets containing barley grains and that an altered rumen function would be reflected in lower lactation performance, a reduction of de novo fatty acids in milk fatty acid profile, or a combination of both. Therefore, the objective of this study was to evaluate lactation performance, nutrient digestibility, and milk fatty acid composition of high-producing dairy cows consuming diets containing hulled or hulless barley as the grain source when feeding low-forage (**LF**) or high-forage (**HF**) diets.

## **4.3 MATERIALS AND METHODS**

### *Animals, Housing, and Diets*

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech (Blacksburg). Eight primiparous ( $610 \pm 40$  kg of BW and  $72 \pm 14$  DIM at the beginning of the experiment) and 16 multiparous ( $650 \pm 58$  kg of BW and  $58 \pm 16$  DIM at the

beginning of the experiment) Holstein cows were randomly assigned to 1 of 4 diets (Table 1) in a replicated  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of treatments and 21-d periods. Cows were assigned to squares based on parity (1, 2 and  $\geq 3$ ) and DIM. Cows were housed in a 24-stall pen within a free-stall barn and were fed once daily (1100 h) using a Calan gate system (American Calan, Inc., Northwood, NH). Before beginning the experiment, cows were trained for a 2-wk period to find their door.

Diets (Table 1) were formulated to contain (DM basis): 45% forage and hulled barley as the sole grain source (**LFHD**), 65% forage and hulled barley as the sole grain source (**HFHD**), 45% forage and hulless barley as the sole grain source (**LFHS**), and 65% forage and hulless barley as the sole grain source (**HFHS**). Barley grains were obtained from 2 farms in Virginia (Charlotte and Westmoreland counties for hulled and hulless barley, respectively). Barley cultivars were Thoroughbred (Virginia Crop Improvement Association, Mechanicsville, VA) and Amaze 10 (Virginia Identity Preserved Grains LLC, Charles City, VA) for hulled and hulless, respectively. Both barley grains were ground using a 4.76-mm screen on a hammer mill and incorporated into concentrate pellets at a commercial feed mill (Big Spring Mill, Inc., Elliston, VA). All diets were formulated to meet requirements (NRC, 2001) for a 630-kg lactating dairy cow producing 42 kg of milk per day.

Concentrate pellets were mixed with corn silage and chopped alfalfa hay (Table 1) and delivered ad libitum (~5% refusals) as a TMR. Mixing and feeding was performed using a Calan Data Ranger (American Calan, Inc.). The amount of feed offered and refused was measured daily. Cows were milked twice daily (0100 and 1300 h), and milk weights were automatically recorded at each milking. The averages of daily milk yields and DMI from d 15 to 21 of each period were used for statistical analysis.

### ***Nutrient Digestibility***

Total-tract nutrient digestibility was estimated using lanthanum chloride as an external marker as described in Yang et al. (2017). To obtain a final dietary lanthanum concentration of 40 mg/kg of DM, 41 kg of the marker solution (density = 1.15 g/mL; [La] = 102 g/L) was sprayed onto 1,600 kg of soybean meal that was incorporated into the concentrate pellets. Fecal grab samples were collected for each period across 3 consecutive days (starting on d 19) at 6-h intervals, skipping sampling times 2 h at the end of each day. Lanthanum concentration was determined in TMR and fecal samples by inductively coupled plasma atomic emission spectroscopy. Apparent total-tract DM digestibility and apparent total tract nutrient digestibility were calculated using equations [4.1] and [4.2], respectively.

$$\text{DM digestibility (\%)} = 100 - \frac{\text{Dietary [La] (mg/g of DM)}}{\text{Fecal [La] (mg/g of DM)}} \times 100 \quad [4.1]$$

$$\begin{aligned} \text{Nutrient Digestibility (\%)} = \\ 100 - \frac{\text{Dietary [La] (mg/g of DM)}}{\text{Fecal [La] (mg/g of DM)}} \\ \times \frac{\text{Fecal [Nutrient] (mg/g of DM)}}{\text{Dietary [Nutrient] (mg/g of DM)}} \times 100 \quad [4.2] \end{aligned}$$

### ***Sample Collection and Analysis***

Samples of feed ingredients and feed refusals were collected weekly. All samples were dried to constant weight at 55°C in a forced-air oven and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ash concentration was determined after combusting samples in a furnace (Thermolyne 30400, Barnstead International, Dubuque, IA) for 3

h at 600°C (method 942.05; AOAC International, 2016). Crude protein concentration was calculated as percent N  $\times$  6.25 after combustion analysis (method 990.03; AOAC International, 2016) using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ). Ash-free NDF and ADF concentrations were determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY). Sodium sulfite and  $\alpha$ -amylase (Ankom Technology) were included for NDF analysis (Ferreira and Mertens, 2007). Acid detergent fiber and lignin concentrations were determined sequentially. After determining ADF weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a DaisyII Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method of Hall (2009) with  $\alpha$ -amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspegillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland).

Milk samples (a.m. and p.m. milkings) were collected on d 17 and 18 for determination of milk fat, protein, lactose, and MUN concentrations with a CombiFoss FT+ Fourier transform infrared analyzer (Foss, Hillerød, Denmark) by United DHIA (Radford, VA). An additional milk sample (a.m. and p.m. milking) was collected on d 17 to determine milk fatty acid composition. Milk fatty acids were extracted and methylated according to the method of Chouinard et al. (1999). Fatty acid methyl esters were analyzed by gas chromatography (Agilent 6890 N GC; Agilent Technologies, Santa Clara, CA) using a CP-Sil 88 capillary column (100 m  $\times$  0.25 mm i.d. with 0.2- $\mu$ m thickness; Varian, Inc., Palo Alto, CA). The oven temperature was initially set at 80°C and was increased at 2°C/min to 190°C and maintained for 9 min. Inlet and flame-ionization detector temperatures were 250°C, the split ratio was 100:1 and a 1  $\mu$ L injection volume was used. The hydrogen carrier gas flow rate was 1 mL/min. Hydrogen flow to the detector was 25 mL/min, airflow was 400 mL/min, and the flow of nitrogen makeup gas was 40 mL/min. Fatty acid peaks

were identified by using pure methyl ester standards (Nu-Check Prep Inc., Elysian, MN). A butter reference standard (BCR 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was analyzed at regular intervals to determine recoveries and correction factors for individual fatty acyl composition in milk fat.

### ***Statistical Analysis***

All variables were analyzed using the MIXED procedure of SAS (SAS version 9.3, SAS Institute Inc., Cary, NC). The statistical model included the effects of square [fixed; degrees of freedom (df) = 5, treatment (fixed; df = 3), square × treatment interaction (fixed; df = 15), period (random; df = 3), and cow within square (random; df = 18), and the random residual error (df = 51). Orthogonal contrasts were used to test the main effects of dietary forage-to-concentrate ratio (HF vs. LF) and barley type (hulled vs. hulless), and their interaction. Differences between main effects were declared at  $P < 0.05$ , and interactions between main effects were declared at  $P < 0.10$ .

## **4.4 RESULTS AND DISCUSSION**

The nutritional quality of hulled barley was poorer than expected, whereas the nutritional quality of hulless barley was proximate to expected values. Hulled barley contained (DM basis) 2.5% ash, 11.9% CP, 36.0% NDF, and 37.1% starch. The concentration of starch was 16.1 to 27.8% units lower (Yang et al., 1997a; Griffey et al., 2010), whereas the concentration of NDF was 5 to 13.7 percentage units higher than previously reported values for hulled barley grains (Yang et al., 1997a; Fellner et al., 2008). Hulless barley contained 2.7% ash, 13.3% CP, 14.0% NDF, and 53.3% starch. The concentration of starch was 3.1 to 13.5 percentage units lower than previously reported values for hulless barley grains (Yang et al., 1997a; Griffey et al., 2010). However, the

concentration of NDF was 2.9 percentage units higher (Yang et al., 1997a; Yang et al., 2013) and 14.8 percentage units lower (Fellner et al., 2008) than previously reported values for hullless barley grains. Differences in grain quality are likely related to differences in the growing conditions (i.e., soil and environment) between the 2 regions where barley grains were grown (Piedmont and Coastal Plains areas for hulled and hullless barley grains, respectively).

Due to the different compositions of the barley grains and the different forage-to-concentrate ratios, dietary NDF and starch concentrations ranged from 27.8 to 30.8% and 23.7 to 26.5%, respectively (Table 1). As corn silage contained a very high concentration of starch (43.9% starch), the proportion of the total dietary starch provided by the cereal grain ranged from 21.8 to 57.7%.

As the interaction between main effects tended to be significant ( $P < 0.13$ ; Table 2), DMI tended to be lower for HFHD than for the rest of the diets. The reasons for this lower DMI are not clear as a slower digesta flow through the gastrointestinal tract due to greater proportions of forage particles would be expected in both HF diets (Allen, 1996; Manthey et al., 2016), independently of the type of cereal grain. However, as the digestibility of NDF was greater for HFHS than for HFHD (57.9 and 50.6%, respectively; Table 3), it is plausible that a greater NDF digestion rate could have resulted in a faster passage rate and a shorter retention time of the digesta (Oba and Allen, 1999) for the HFHS diet, which would likely explain the differences in DMI between the 2 HF diets.

Neither the type of barley ( $P < 0.75$ ) nor the forage-to-concentrate ratio ( $P < 0.34$ ) affected milk yield (Table 2). As in our previous study (Yang et al., 2017), cows in the current study produced according to the aimed goals when formulating the diets, indicating that feeding barley grains as the only cereal grain source in rations for high-producing cows can sustain milk production without major drawbacks.

Even though the type of barley did not affect milk fat ( $P < 0.23$ ) or protein ( $P < 0.48$ ) concentrations, the fat-to-protein ratio decreased slightly in cows consuming hulless barley ( $P < 0.04$ , Table 2). Alternatively, MUN decreased for cows consuming hulless barley ( $P < 0.01$ ). Although a decrease in fat-to-protein ratio in milk might suggest that rumen function was negatively affected (Slater et al., 2000), the decrease in MUN in milk might alternatively suggest that rumen function was positively-affected by hulless barley (Aguilar et al., 2012). Grain type had no effect on milk fat ( $P < 0.17$ ) or protein ( $P < 0.88$ ) yields.

Feeding LF diets decreased milk fat concentration from 3.91% to 3.50% ( $P < 0.01$ ; Table 2). These milk fat concentrations were similar to those from cows of the same genetic background, subjected to the same management, and fed diets containing 100% of the grain source as corn or hulless barley (Yang et al., 2017). Harvatine (2017) defined diet-induced milk fat depression as a decrease in milk fat yield of up to 50% with no change in milk yield or yield of other milk components. Peterson et al. (2003) defined milk fat depression as a dramatic effect in milk fat concentration and fatty acid composition. To our knowledge, there is not a clear threshold to define what is or is not a dramatic or acute milk fat depression. Peterson et al. (2003) fed diets with very little forage and fiber (16.1% forage and 14.9% NDF) to lactating dairy cows and reported a 27.2% decrease in milk fat yield. Based on our hypothesis, we expected a dramatic milk fat depression (>20% difference in milk fat yield) when feeding LF diets. However, the lower concentration of fat in milk (Table 2) resulted in only a 7% decrease in milk fat yield for cows consuming LF diets relative to cows consuming HF diets (1.49 and 1.60 kg/d, respectively). Therefore, based on the magnitude of this decrease and on the similar concentrations of de novo fatty acids in milk (Table 4), cows fed the LF diets did not show a dramatic or acute milk fat depression (Peterson et al., 2003).

Feeding LF diets increased the concentration of C18:1 *trans*-10 in milk fat ( $P < 0.01$ ; Table 4). High concentrations of C18:1 *trans*-10 in milk fat were associated to reductions in milk fat concentrations in several studies (Grinari et al., 1998; Baumgard et al., 2001; Shingfield et al., 2009; Mohammed et al., 2010). Based on the concentrations of C18:1 *trans*-10 in milk fat observed in this study, feeding LF diets with barley grains may have marginally altered rumen function and therefore increased the production of C18:1 *trans*-10 within the rumen. Mohammed et al. (2010) reported an association between a greater production of C18:1 *trans*-10 within the rumen and lower milk fat concentrations when feeding hulled barley grains in high-starch diets (>35% starch). However, based on the current and a previous study (Yang et al., 2017) in which lower dietary starch concentrations were fed, there is limited evidence to anticipate a substantial or acute milk fat depression as a consequence of an altered rumen function when feeding hullless barley to high-producing dairy cows.

Cows consuming LF diets had greater milk protein concentrations (3.13 vs. 3.07% protein;  $P < 0.01$ ) and yields (1.33 vs. 1.26 kg/d of protein;  $P < 0.01$ ) than cows consuming HF diets. It is plausible that a faster passage rate of digesta in cows consuming LF diets would increase microbial protein flow to the small intestine (Oba and Allen, 2000), therefore increasing MP availability for protein synthesis and secretion.

As an interaction between main effects was observed ( $P < 0.02$ ), cows consuming the HFHS diet had a higher apparent total-tract DM digestibility than the rest of the cows (75.8 and 71.8%, respectively; Table 3). Cows consuming diets containing hullless barley showed greater apparent total-tract CP digestibility than cows consuming diets containing hulled barley (75.5 and 70.1%, respectively), whereas cows consuming HF diets showed higher apparent total-tract CP digestibility than cows consuming LF diets (74.4 and 71.2%, respectively). An interaction between main effects

was observed for apparent total-tract NDF digestibility ( $P < 0.01$ ; Table 3), although the reasons for this interaction are not clear. This interaction was driven by the increased NDF digestibility for HFHS diets (57.9%) and the decreased NDF digestibility for HFHD diets (50.6%). Because the nutritional composition of these diets was almost identical (Table 1), differences in NDF digestibility between HF diets should be related to grain type, such as starch fermentability. However, starch was almost entirely digested in all diets (>99% digestibility), which challenges the latter possibility. Regarding apparent total tract starch digestibility, an interaction between main effects was also observed ( $P < 0.01$ ), although these differences have minimum biological relevance as dietary starch was almost entirely digested.

The current and a previous study (Yang et al., 2017) evaluated the use of hulless barley as a grain source in diets for high-producing cows. Under the scope that NRC (2001) recommends increasing dietary NDF when including barley grain in diets for dairy cows (Beauchemin, 1991), both studies challenged lactation performance by formulating diets with less than 34% dietary NDF (NRC, 2001) while ensuring adequate amounts of physically effective fiber (Mertens, 1997) through the inclusion of chopped alfalfa hay (3 to 5 kg/d). In both studies, high-producing cows performed according to aimed goals, even with LF diets. In addition, no evidence of acute milk fat depression was observed when feeding hulless barley as the grain source in either study. Beauchemin (1991) mentioned a fat-depressing effect of high concentrate barley-based diets. Even though our data seem to conflict with this statement, such conflict is nonexistent because in the current and our previous study (Yang et al., 2017) the inclusion of barley in the diet was always less than 30%, whereas in other studies (Beauchemin, 1991; Beauchemin et al., 1991) the inclusion of barley-based diets with similar NDF concentrations (i.e., 28-31% of dietary NDF) ranged from 43.1 to 54.6% (DM basis). Even though hulled and hulless barley were the only cereal sources in

the current study, their inclusion at less than 30% of the total DM may not necessarily imply these were high concentrate barley-based diets.

## **4.5 CONCLUSIONS**

Feeding hulled or hulless barely as an energy source in diets for high-producing lactating cows resulted in similar milk productions, even when feeding LF diets. However, a decrease in milk fat concentration, which resulted in a 7% decrease in milk fat yield, was observed for cow fed LF diets. In conclusion, based on the minimum effects on milk fatty acid composition, a dramatic or acute milk fat depression as a consequence of an alteration of rumen function when feeding hulless barley to high-producing dairy cows should not be expected when feeding diets containing 30% barley or less as the grain source.

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**Table 4.1** Ingredient and chemical composition of diets (% DM basis).

Ingredients	Diet <sup>1</sup>			
	LFHD	HFHD	LFHS	HFHS
Corn silage <sup>2</sup>	27.3	40.4	27.3	40.4
Alfalfa hay <sup>3</sup>	12.3	18.6	12.3	18.6
Hulled barley grain <sup>4</sup>	29.4	17.6	-	-
Hulless barley grain <sup>5</sup>	-	-	29.4	17.6
Soybean meal	15.8	15.8	15.8	15.8
Soybean meal marked	1.8	1.8	1.8	1.8
Soybean hulls	9.4	1.8	9.4	1.8
EnerGII <sup>6</sup>	0.9	0.9	0.9	0.9
Sodium bicarbonate	1.1	1.1	1.1	1.1
Bentonite	0.9	0.9	0.9	0.9
Salt	0.5	0.5	0.5	0.5
Magnesium oxide	0.2	0.2	0.2	0.2
Trace mineral premix <sup>7</sup>	0.4	0.4	0.4	0.4
Vitamin ADE <sup>8</sup>	0.04	0.04	0.04	0.04
Vitamin E <sup>9</sup>	0.004	0.004	0.004	0.004
Rumensin 90 <sup>10</sup>	0.007	0.007	0.007	0.007
Nutrients				
OM	91.6	92.0	92.3	92.0
CP	16.3	15.9	15.6	15.3
NDF	30.8	27.8	28.4	28.0
Forage NDF	13.2	18.0	12.3	18.1
Non-forage NDF	17.6	9.8	16.1	9.9
Starch	23.7	26.4	26.3	26.5
Grain starch	10.2	5.8	15.2	11.0

<sup>1</sup>LFHD: 45% forage and hulled barley; HFHD: 65% forage and hulled barley; LFHS: 45% forage and hulless barley; HFHS: 65% forage and hulless barley

<sup>2</sup> Corn silage composition: 41.7% DM, 6.0% CP, 31.7% NDF, and 43.9% starch.

<sup>3</sup> Alfalfa hay composition: 90.0% DM, 24.6% CP, 37.8% NDF, and 1.8% starch.

- <sup>4</sup> Hulled barley grain composition: 2.5% ash, 11.9% CP, 36.0% NDF, and 37.1% starch.
- <sup>5</sup> Hulless barley grain composition: 2.7% ash, 13.3% CP, 14.0% NDF, and 53.3% starch.
- <sup>6</sup> Calcium salts of fatty acids (Virtus Nutrition, LLC, Corcoran, CA).
- <sup>7</sup> Contained 22.25% calcium; 7.50% magnesium; 2.75% potassium; 3.90% sulfur; 1.50% manganese; 1.50% zinc; 9,500 ppm iron; 2,500 ppm copper; 200 ppm iodine; 200 ppm cobalt; 66 ppm selenium; 227,273 IU/kg Vitamin A; 136,364 IU/kg Vitamin D3; 636 IU/kg Vitamin E.
- <sup>8</sup> Contained 3,500 IU/kg Vitamin A; 950 IU/kg Vitamin D3; 2,000 IU/g Vitamin E.
- <sup>9</sup> Contained 500 IU/g of premix
- <sup>10</sup> Contained 200 mg of monensin per gram of product (Elanco Animal Health, Indianapolis, IN).

**Table 4.2** Production performance of dairy cows consuming diets containing hulled (HD) or hulless (HS) barley grains as the energy source with different forage-to-concentrate ratios.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>		
	LFHD	HFHD	LFHS	HFHS		F	G	F×G
DMI, kg/d	27.1	24.4	26.5	26.3	1.17	0.07	0.47	0.13
Milk yield, kg/d	42.3	41.3	41.7	41.4	1.29	0.34	0.75	0.68
Milk fat, %	3.60	3.92	3.40	3.89	0.20	0.01	0.23	0.40
Milk fat yield, kg/d	1.53	1.63	1.45	1.57	0.10	0.03	0.17	0.81
Milk protein, %	3.11	3.07	3.14	3.07	0.03	0.01	0.48	0.41
Milk protein yield, kg/d	1.32	1.27	1.34	1.25	0.04	0.01	0.88	0.44
Milk lactose, %	4.85	4.85	4.85	4.82	0.02	0.39	0.15	0.16
Milk lactose yield, kg/d	2.08	2.04	2.09	1.98	0.07	0.09	0.60	0.46
MUN, mg/dL	15.1	16.0	13.1	14.8	1.05	0.01	0.01	0.37
Fat to protein ratio	1.18	1.30	1.08	1.26	0.09	0.01	0.04	0.45
Body weight gain, kg/d	0.71	0.63	0.58	0.63	0.21	0.87	0.46	0.49
3.5% FCM, kg/d	43.3	43.9	42.1	42.9	1.59	0.43	0.27	0.93
Feed efficiency, kg FCM/kg DMI	1.61	1.85	1.64	1.76	0.10	0.02	0.74	0.48

<sup>1</sup> LFHD: 45% forage and hulled barley; HFHD: 65% forage and hulled barley; LFHS: 45% forage and hulless barley; HFHS: 65% forage and hulless barley

<sup>2</sup> F: effect of dietary forage-to-concentrate ratio; G: effect of grain type; F × G: interaction between F and G.

**Table 4.3** Apparent total tract nutrient digestibility of dairy cows consuming diets containing hulled (HD) or hulless (HS) barley grains as the energy source with different forage-to-concentrate ratios

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>		
	LFHD	HFHD	LFHS	HFHS		F	G	F×G
DM, %	72.1	71.1	72.1	75.8	1.58	0.17	0.02	0.02
CP, %	69.0	71.1	73.3	77.7	1.45	0.01	0.01	0.27
NDF, %	55.7	50.6	51.3	57.9	2.30	0.66	0.39	0.01
Starch, %	99.3	99.1	99.2	99.4	0.15	0.88	0.23	0.03

<sup>1</sup> LFHD: 45% forage and hulled barley; HFHD: 65% forage and hulled barley; LFHS: 45% forage and hulless barley; HFHS: 65% forage and hulless barley.

<sup>2</sup> F: effect of dietary forage-to-concentrate ratio; G: effect of grain type; F × G: interaction between F and G.

**Table 4.4** Fatty acid profile (g/100 g fat) in milk fat from dairy cows consuming diets containing hulled (HD) or hulless (HS) barley grains as the energy source with different forage-to-concentrate ratios.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>		
	LFHD	HFHD	LFHS	HFHS		F	G	F×G
C4:0	3.01	3.33	2.84	3.43	0.19	0.01	0.69	0.15
C6:0	1.80	1.94	1.69	2.00	0.12	0.01	0.69	0.15
C8:0	1.15	1.19	1.08	1.23	0.07	0.01	0.68	0.15
C10:0	2.98	2.97	2.80	3.05	0.16	0.20	0.66	0.19
C12:0	3.72	3.62	3.56	3.65	0.17	0.99	0.50	0.39
C14:0	10.86	10.88	10.76	10.76	0.21	0.93	0.46	0.96
C14:1	1.71	1.56	1.81	1.36	0.15	0.01	0.45	0.04
C15:0	1.59	1.38	1.64	1.29	0.10	0.01	0.75	0.23
C16:0	34.31	35.02	34.31	34.47	0.71	0.24	0.48	0.49
C16:1	3.02	2.78	3.34	2.44	0.27	0.01	0.96	0.05
C17:0	0.59	0.54	0.61	0.55	0.02	0.01	0.22	0.89
C18:0	4.73	5.27	4.58	5.68	0.26	0.01	0.42	0.10
C18:1 (trans 6-8)	0.42	0.37	0.47	0.35	0.04	0.01	0.56	0.05
C18:1 (trans 9)	0.27	0.25	0.31	0.24	0.03	0.01	0.71	0.19
C18:1 (trans 10)	1.93	1.40	2.38	1.06	0.37	0.01	0.78	0.07
C18:1 (trans 11)	0.61	0.63	0.68	0.62	0.09	0.66	0.40	0.31
C18:1 (trans 12)	0.42	0.40	0.46	0.38	0.03	0.01	0.34	0.03
C18:1 (cis 9)	18.66	19.97	18.59	20.13	0.62	0.05	0.25	0.20
C18:1 (cis 11)	1.25	1.14	1.29	1.10	0.11	0.01	0.91	0.30
C18:2 (cis 9, cis 12)	3.34	2.92	3.15	2.80	0.12	0.01	0.03	0.58
C20:0	0.068	0.072	0.067	0.074	0.005	0.01	0.92	0.60
C18:3	0.34	0.31	0.32	0.31	0.03	0.03	0.20	0.13
CLA <sup>3</sup> (cis 9, trans 11)	0.44	0.40	0.47	0.39	0.06	0.01	0.75	0.30
CLA (trans 10, cis 12)	0.014	0.014	0.017	0.015	0.008	0.84	0.67	0.85
Unknown	2.77	2.65	2.91	2.58	0.09	0.01	0.35	0.01
De novo fatty acids <sup>3</sup>	25.22	25.58	24.54	25.47	0.68	0.16	0.43	0.48

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<sup>1</sup> LFHD: 45% forage and hulled barley; HFHD: 65% forage and hulled barley; LFHS: 45% forage and hulless barley; HFHS: 65% forage and hulless barley.

<sup>2</sup> F: effect of dietary forage-to-concentrate ratio; G: effect of grain type; F × G: interaction between F and G.

<sup>3</sup> Sum of C4:0 to C14:0.

# CHAPTER 5 EFFECTS OF XYLANASE SUPPLEMENTATION ON PRODUCTION PERFORMANCE AND NUTRIENT DIGESTIBILITY IN LACTATING DAIRY COWS FED CORN OR SORGHUM-BASED DIETS

## 5.1 ABSTRACT

The objectives of this study were to evaluate the effects of supplementing a xylanase on production performance and nutrient digestibility of lactating dairy cows fed diets containing corn or sorghum silage as the forage source. Four primiparous (BW  $581 \pm 47$  kg, DIM  $47 \pm 14$  d) and 20 multiparous (BW  $707 \pm 67$  kg, DIM  $51 \pm 14$  d) Holstein cows were randomly assigned to 1 of 4 diets in a replicated  $4 \times 4$  Latin square design with 19-d periods. The treatments were: 1) corn silage-based diet (CORN-XYL), 2) corn silage-based diet with xylanase enzyme (CORN+XYL), 3) sorghum silage-based diet (SORG-XYL), 4) sorghum silage-based diet with xylanase enzyme (SORG+XYL). Xylanase product (Ronozyme WX) was supplemented at a rate of 1.5 g/kg DM. Supplementation of xylanase did not affect production performance of lactating cows. Dry matter intake (28.8 vs. 25.5 kg/cow/day) and milk yield (51.6 vs. 48.9 kg/cow/day) were greater for cows fed CORN diets than for cows fed the SORG diets. Milk fat concentration and yield were greater for SORG than CORN (3.8% vs. 3.3%; 1.81 kg/cow/day vs. 1.68 kg/cow/day). Cows fed CORN diets produced more milk protein and milk lactose and had lower concentrations of milk urea nitrogen (MUN). Feeding CORN diets resulted in greater total digestibility of dry matter (DM), crude protein (CP), and starch than SORG diets. Economically, feeding SORG based diets resulted in cheaper feed cost and increased income over feed cost by \$1.3/cow/day. In conclusion, xylanase supplementation did not affect production performance of lactating dairy cows consuming diets containing corn or sorghum silage as a forage source.

## 5.2 INTRODUCTION

Dairy cows require sufficient NDF in their diets to maintain rumen function and maximize milk production (Oba and Allen, 1999), especially for cows fed the high grain diet. Increased starch availability and a faster rate of starch fermentation in the rumen can alter milk fat percentage, ruminal pH, and ruminal volatile fatty acids profile due to reduced ruminal pH (NRC, 2001). Adequate fiber will increase rumination time and will stimulate more production of saliva which can buffer the rumen fluid (Allen, 1997). However, increased fiber concentration is negatively related to DMI and milk production due to the increased rumen fill (Allen, 1996). Van Soest (1965) reported DMI decreases with forage NDF content increases in the diet. Dado and Allen (1995) reported that DMI of lactating cows in early lactation was decreased by insertion of inert fill (22.2 L) into reticulorumen. The effect of fill caused by forage NDF content on DMI may gradually diminishes as digestibility increases (Allen, 1996). Feeding exogenous fibrolytic enzyme is a feasible strategy to improve production performance by increasing fiber digestibility by dairy cows. Previous studies have reported the positive relationship between NDF digestibility and cow performance (Dado and Allen, 1996; Oba and Allen, 1999; Qiu et al., 2003). Feeding exogenous fibrolytic enzymes is a feasible strategy to enhance NDF digestibility.

The major digestible fiber of forages are hemicellulose and cellulose, which are degraded by the enzymes xylanase and cellulase from bacteria and protozoa in rumen (Schingoethe et al., 1999). Xylanase is a hydrolytic enzyme that cleaves the  $\beta$ -1,4-linked backbone of xylan, the major component of hemicellulose (Collins et al., 2005). Xylanase supplementation is commonly used to enhance feed values for non-ruminant animals, especially for broiler chickens fed diets containing barley, oats or wheat (Wang and McAllister, 2002). Also, previous studies reported that cellulase and xylanase activities remained constant after 6 h of incubation with ruminal fluid (Morgavi et al.,

2000). The remaining enzyme activities after 6 h of incubation with ruminal fluid suggested that cellulase and xylanase were not degraded by the array of protease produced by ruminal microorganisms and supplementing xylanase and cellulase to lactating dairy cows may enhance ruminal fiber digestion by increasing cellulolytic activity in the rumen (Wang and McAllister, 2002).

Previous studies reported that adding exogenous fibrolytic enzyme products improved fiber digestibility in both in vitro (Colombatto et al., 2003; Eun et al., 2007) and in vivo studies (Arriola et al., 2011). Moreover, milk production was increased by supplementing dietary exogenous fibrolytic enzymes, a combination of cellulase and xylanase in previous studies (Schingoethe et al., 1999; Rode et al., 1999; Kung et al., 2000; Peters et al., 2015; Romero et al., 2016), but similar results were not detected by others (Kung et al., 2002; Arriola et al., 2011). This inconsistency may be caused by enzyme activity, application dosage, composition of enzymes, wrong enzyme designations, and the lactation stage of dairy cows (Arriola et al., 2017).

Studies evaluating the effects of adding only a xylanase product in dairy diets containing different silage and grain sources are limited. Under the assumption that adding exogenous fibrolytic enzyme products increase NDF digestibility, we hypothesized that supplementing a xylanase enzyme product in diets containing corn or sorghum silage would increase NDF digestibility, and production performance of lactating dairy cows would be improved due to enhanced fiber digestion. Therefore, the objective of this study was to evaluate the effects of supplementing a xylanase enzyme product on production performance and nutrient digestibility of dairy lactating cows fed diets containing corn or sorghum silage as the forage source.

## **5.3 MATERIALS AND METHODS**

### ***Animals, Housing, and Diets***

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Four primiparous (581±47 kg BW and 47±14 DIM at the beginning of the experiment) and 20 multiparous (707±67 kg BW and 51±14 DIM at the beginning of the experiment) Holstein cows were randomly assigned to 1 of 4 diets (Table 1) in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments and 19-d periods. Sorghum used in this study was BMR forage sorghum (SS 2010 BDF; Southern States Cooperative, Hillsville, VA). Treatments consisted of: 1) corn silage-based diet (**CORN-XYL**), 2) corn silage-based diet plus xylanase (**CORN+XYL**), 3) sorghum silage-based diet (**SORG-XYL**), and 4) sorghum silage-based diet plus xylanase (**SORG+XYL**). Diets were formulated to contain (DM basis) about 33% silage (corn or sorghum), 8% grass hay, and 59% concentrate. Xylanase (Ronozyme WX; DSM Nutritional Products, Parsippany, NJ) was supplemented at a rate of 1.5 g product/kg DM. Concentrate pellets were prepared at a commercial feed mill (Big Spring Mill, Inc., Elliston, VA). All diets were formulated to meet requirements (NRC, 2001) for a 630-kg lactating dairy cow producing 42 kg of milk per day.

Cows were assigned to squares based on parity (1, 2, and ≥ 3) and were housed in a 24-stall pen within a free-stall barn and were fed once daily (1100 h) by means of a Calan gate system (American Calan, Inc., Northwood, NH). Cows were trained for 2 weeks to locate their doors before the beginning of the experiment.

Concentrate pellets were mixed with either corn or sorghum silage and chopped grass hay (Table 1), and the ration was delivered ad libitum (~5% refusals) as a total mixed ration. Mixing and feeding was performed using a Calan Data Ranger (American Calan, Inc.). The amount of feed offered and refused was measured daily. Cows were milked twice daily (0100 and 1300 h), and milk

weights were automatically recorded at each milking. The averages of daily milk yields and DMI from d 13 to 19 of each period were used for statistical analysis.

### ***Nutrient Digestibility***

Total tract nutrient digestibility was estimated using lanthanum chloride as an external marker as described in Yang et al. (2017). To obtain a final dietary lanthanum concentration of 40 mg/kg DM, 42 kg of the marker solution (density = 1.15 g/mL; [La] = 102 g/L) were sprayed onto 1,750 kg of soybean meal that was incorporated into the concentrate pellets. Fecal grab samples were collected for each period across 3 consecutive days (starting on d 17) at 6-h intervals skipping sampling times 2 h at the end of each day. Lanthanum concentration was determined in TMR and fecal samples by inductively coupled plasma atomic emission spectroscopy. Apparent total tract DM digestibility and apparent total tract nutrient digestibility were calculated using equations [1] and [2], respectively.

$$\text{DMD}_{(\%)} = 100 - \frac{\text{Dietary [La]}_{(\text{mg/g DM})}}{\text{Fecal [La]}_{(\text{mg/g DM})}} \times 100 \quad [1]$$

$$\text{Nutrient Digestibility}_{(\%)} = 100 - \frac{\text{Dietary [La]}_{(\text{mg/g DM})}}{\text{Fecal [La]}_{(\text{mg/g DM})}} \times \frac{\text{Fecal [Nutrient]}_{(\text{g/g DM})}}{\text{Dietary [Nutrient]}_{(\text{g/g DM})}} \times 100 \quad [2]$$

### ***Sample Collection and Analysis***

Samples of feed ingredients and feed refusals were collected weekly. All samples were dried to constant weight at 55°C in a forced-air oven, and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ash concentration was determined after

combusting samples in a furnace (Thermolyne 30400, Barnstead International, Dubuque, IA) for 3 h at 500°C (AOAC 942.05). Crude protein concentration was calculated as percent N  $\times$  6.25 after combustion analysis (AOAC 990.03) using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ). Ash-free NDF and ADF concentrations were determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY). Sodium sulfite and  $\alpha$ -amylase (Ankom Technology) were included for NDF analysis (Ferreira and Mertens, 2007). ADF and lignin concentrations were determined sequentially. After determining ADF weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a DaisyII Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method of Hall (2009) with  $\alpha$ -amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspegillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland). Milk samples were collected on d 15 and 16 for analysis of milk fat, protein, lactose, and MUN concentrations with a CombiFoss FT+ Fourier transform infrared analyzer (Foss, Hillerød, Denmark) by United DHIA (Radford, VA).

### ***Statistical Analysis***

All variables were analyzed using the MIXED procedure of SAS (SAS version 9.3, SAS Institute Inc., Cary, NC). The statistical model included the effects of square (fixed; df = 5), treatment (fixed; df = 3), square by treatment interaction (fixed; df = 15), period (random; df = 3), and cow within square (random; df = 18), and the random residual error (df = 51). Orthogonal contrasts were used to test the main effects of xylanase supplementation (-XYL vs. +XYL) and forage type (CORN vs. SORG), and their interaction. Significant differences between main effects were declared at  $P < 0.05$ , and significant interactions were declared at  $P < 0.10$ .

## 5.4 RESULTS AND DISCUSSION

Corn silage had slightly higher concentrations of fat (3.7% vs. 3.2%), CP (7.1% vs. 6.8%), starch (31.2% vs. 29.2%), and lower concentrations of NDF (36.4% vs. 49.0%), ADF (22.3% vs. 32.7%), and ADL (2.1% vs. 5.7%) than sorghum silage. Due to the differences in composition between corn and sorghum silages, dietary CP, NDF, and starch concentrations ranged from 14.8 to 14.9%, 28.5 to 33.4%, and 28.9 to 30.7%, respectively. Grass hay used in this study contained 70.6% NDF, 43.5% ADF, 10.1% CP, and 4.7% lignin.

Contrary to our hypothesis, xylanase supplementation did not affect DMI ( $P < 0.10$ ), milk yield ( $P < 0.77$ ), milk fat percentage ( $P < 0.29$ ) and yield ( $P < 0.71$ ), milk protein percentage ( $P < 0.48$ ) and yield ( $P < 0.43$ ), lactose percentage ( $P < 0.84$ ) and yield ( $P < 0.51$ ), and MUN ( $P < 0.90$ ) (Table 2). The reasons for the lack of response in DMI and milk production are not clear. This study had shorter feeding periods compared with previous studies (Schingoethe et al., 1999; Rode et al., 1999; Arriola et al., 2011; Romero et al., 2016). Schingoethe et al. (1999) observed cows fed cellulase- and xylanase-treated forage after 100 d postpartum produced 9 to 15% more milk than cows receiving untreated forage. Romero et al. (2016) reported that cows fed xylanase-supplemented diets showed higher milk production than cows fed control diets after week 3 and persisted until week 7. However, Rode et al. (1999) showed milk yield remained consistently higher during 12-week experiment for cows fed diets supplemented with cellulase and xylanase.

Dry matter intake (28.8 vs. 25.5 kg/cow/day) and milk yield (51.6 vs. 48.9 kg/cow/day) were greater for cows fed CORN diets than for cows fed the SORG diets (Table 2). SORG diets contained higher NDF concentration than CORN diets (28.6% vs. 33.0%) and NDF digestibility was not affected by silage type ( $P < 0.12$ , Table 3). Neutral detergent fiber generally ferments more slowly than other dietary nutrients, therefore, it has greater filling effect over time than non-fibrous

feed components (Allen, 1996). Decreased DMI for cows fed SORG diets may be caused by increased filling effect caused by elevated NDF concentrations of SORG diets. Higher DMI and greater DM digestibility ( $P < 0.01$ ) for cows fed CORN resulted in higher milk production due to the positive relationship between milk yield and intake of digestible energy (Beauchemin and Holtshausen, 2010).

Cows consuming CORN diets showed significantly reduced milk fat percentage (3.3% vs. 3.8%,  $P < 0.01$ ) and milk fat yield (1.68 kg/d vs. 1.81 kg/d,  $P < 0.01$ ), but similar 3.5% FCM ( $P < 0.25$ ) compared with cows fed SORG diets (Table 2). All diets contained about 12.6% (DM basis) wheat grain, which was the only grain source. Herrera-Saldana et al. (1990) showed starch disappearance rates were greater for wheat and barley and lower for corn and sorghum. Feeding highly fermentable starch can decrease ruminal pH by increasing lactic acid production (Silveira et al., 2007; Mohammed et al., 2010). Cows fed CORN diets had a greater intake of wheat grain than cows fed SORG (3.63 vs. 3.21 kg/d), and the reduction in fat-to-protein ratio in milk of cows fed CORN diets also suggest that rumen function was negatively affected by lowered pH caused by higher intake of wheat grain (Slater et al., 2000) and resulted in decreased milk fat percentage and yield. Moreover, the major long chain fatty acid in corn or sorghum silage is linoleic acid (C18:2) (Baldin et al., 2017). Linoleic acid was 9.2% higher in corn silage (46.2 vs. 37.0%) compared to sorghum silage (Table 4). Increased linoleic acid in CORN diets may increase the risk for biohydrogenation-induced milk fat depression because it has been reported to be the parent compound for most of the bioactive *trans* fatty acids that inhibited milk fat synthesis (Baldin et al., 2017).

Neither milk protein percentage ( $P < 0.60$ ) nor milk lactose percentage ( $P < 0.51$ ) was affected by silage type. Milk protein yield ( $P < 0.01$ ) and milk lactose yield ( $P < 0.01$ ) were

increased for cows fed CORN diets due to the greater milk yields for cows fed CORN. Cows consuming CORN diets had an increase in total-tract CP digestibility (78.0 vs. 72.4%,  $P < 0.01$ ) than cows fed SORG diets. Cows fed CORN diets had the higher intake of starch which increases the energy from ruminal fermentation and duodenum starch flow. Increased starch digestion in the small intestine may increase milk protein production because increased starch digestion in the small intestine may spare amino acid utilization in the gut and liver (Reynolds et al., 1994).

The interaction between main effects was significant for total tract digestibility of DM and CP, and total tract digestibility of DM and CP was lower for CORN+XYL. Also, cows fed CORN diets with xylanase supplementation showed lower total tract digestibility of NDF, hemicellulose, and ADF. The reasons for this lower fiber digestibility are not clear since cows fed either CORN+XYL or CORN-XYL had similar intake of NDF and no previous study reported the negative effects of supplementation xylanase (Schingoethe et al., 1999; Rode et al., 1999; Kung et al., 2000; Arriola et al., 2011; Peters et al., 2015; Romero et al., 2016; Arriola et al., 2017). Cows fed CORN+XYL had the lowest milk fat percentage and milk fat yields, which may imply that these cows had lower ruminal pH than cows in other treatments. At lower ruminal pH, hydrogen ions displace cations bound to the fiber, which reduces the number of available attachment sites for rumen bacteria and may decrease fiber digestibility by reducing enzymatic digestion by ruminal bacteria (Huhtanen and Khalili, 1992). Total tract digestibility of starch was lower for SORG ( $P < 0.01$ ). Cows fed SORG diets had the higher intake of NDF and peNDF which may have resulted in increased rumination. Increased rumination increases rumen movement and increases the escape rate of small feed particles from the rumen (Allen, 1996; Harvatine and Allen, 2006).

Feed cost has been reported as one of the most important input cost control measures for dairy farms because it is the largest cost of farm total expenses (Hadrich and Johnson, 2015). The

economic outcome of choosing alternative silage types for replacing corn silage is critical information for dairy farmers to control feed costs. The reliance only on corn silage, grown continuously, may decrease corn silage yields and increase feed cost due to pest, diseases, and drought (Harper et al., 2017). Sorghum silage has shown potential as alternative forage resource for lactating dairy cows because it can perform better than corn silage on soils with low water-holding capacity (Harper et al., 2017). In this study, the SORG diets would have resulted in higher milk prices than CORN diets due to the greater milk fat concentrations (Table 5). Under the assumption that sorghum silage is cheaper than corn silage, feeding SORG diets may have resulted in greater income over feed costs than CORN diets (Table 5).

## **5.5 CONCLUSIONS**

Contrary to our hypothesis, supplementing xylanase did not affect production performance and nutrient digestibility of cows fed corn or sorghum silage-based diets. However, cows fed sorghum silage-based diets resulted in better economic outcomes due to greater milk fat percentage and yield. Thus, future studies should investigate the long-term effects of supplementing xylanase on production performance and nutrient digestibility of cows fed diets containing sorghum silage as the forage source.

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**Table 5.1** Ingredient and chemical composition of diets (% DM basis).

Ingredients	Diet <sup>1</sup>			
	CORN		SORG	
	-XYL	+XYL	-XYL	+XYL
Corn silage <sup>2</sup>	33.5	33.4	-	-
Sorghum Silage <sup>3</sup>	-	-	33.5	33.4
Grass Hay <sup>4</sup>	8.4	8.4	8.4	8.4
Corn Grain	7.1	7.1	7.1	7.1
Wheat Grain	12.6	12.6	12.6	12.6
Soybean meal	13.0	13.0	13.0	13.0
Dry Distilled Grains	4.2	4.2	4.2	4.2
Wheat Middlings	16.8	16.7	16.8	16.7
Calcium salts of fatty acids <sup>5</sup>	1.0	1.0	1.0	1.0
Sodium Bicarbonate	1.1	1.1	1.1	1.1
Salt	0.5	0.5	0.5	0.5
Magnesium Oxide	0.2	0.2	0.2	0.2
Xylanase <sup>6</sup>	-	0.2	-	0.2
Trace mineral premix <sup>7</sup>	0.4	0.4	0.4	0.4
Vitamin ADE <sup>8</sup>	0.04	0.04	0.04	0.04
Vitamin E <sup>9</sup>	0.003	0.003	0.003	0.003
Rumensin 90 <sup>10</sup>	0.008	0.008	0.008	0.008
Nutrients				
OM	92.2	92.4	91.8	92.0
CP	14.9	14.9	14.8	14.8
NDF	29.1	28.5	33.4	32.7
Forage NDF	12.2	12.0	14.0	13.7
Starch	29.5	30.7	28.9	30.0
NFC	43.2	44.0	38.6	39.5

<sup>1</sup> **CORN**: corn silage-based diet; **SORG**: sorghum silage-based diet; **-XYL**: control; **+XYL**: xylanase supplemented.

<sup>2</sup> Corn silage composition: 34.3% DM, 3.7% fat, 7.1% CP, 36.4% NDF, 22.3% ADF, 2.1% lignin, and 31.2% starch.

<sup>3</sup> Sorghum silage composition: 32.9% DM, 3.2% fat, 6.8% CP, 49.0% NDF, 32.7% ADF, 5.7% lignin, and 29.2% starch.

<sup>4</sup> Grass hay composition: 89.0% DM, 10.1% CP, 70.6% NDF, 43.5% ADF, 4.7% lignin, and 2.1% starch.

<sup>5</sup> Calcium salts of fatty acids (Virtus Nutrition, LLC, Corcoran, CA).

<sup>6</sup> Ronozyme WX (DSM Nutritional Products, Parsippany, NJ).

<sup>7</sup> Contained 22.25% calcium; 7.50% magnesium; 2.75% potassium; 3.90% sulfur; 1.50% manganese; 1.50% zinc; 9,500 ppm iron; 2,500 ppm copper; 200 ppm iodine; 200 ppm cobalt; 66 ppm selenium; 227,273 IU/kg Vitamin A; 136,364 IU/kg Vitamin D3; 636 IU/kg Vitamin E.

<sup>8</sup> Contained 3,500 IU/kg Vitamin A; 950 IU/kg Vitamin D3; 2,000 IU/g Vitamin E.

<sup>9</sup> Contained 500 IU/g of premix.

<sup>10</sup> Contained 200 mg of monensin per gram of product (Elanco Animal Health, Indianapolis, IN).

**Table 5.2** Effects of supplementation of xylanase enzyme on production performance of cows consuming diets containing corn or sorghum silage as the forage source.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>		
	CORN		SORG			Silage	XYL	S×X
	-XYL	+XYL	-XYL	+XYL				
DMI, kg/d	29.3	28.3	26.2	24.7	1.08	0.01	0.10	0.75
Milk yield, kg/d	51.3	51.9	48.9	48.9	2.01	0.01	0.76	0.70
Milk fat, %	3.31	3.28	3.91	3.76	0.14	0.01	0.29	0.48
Milk fat yield, kg/d	1.68	1.67	1.89	1.82	0.11	0.01	0.41	0.53
Milk protein, %	2.88	2.93	2.88	2.89	0.07	0.60	0.48	0.55
Milk protein yield, kg/d	1.47	1.50	1.39	1.39	0.04	0.01	0.62	0.49
Milk lactose, %	4.75	4.80	4.82	4.79	0.07	0.51	0.84	0.32
Milk lactose yield, kg/d	2.43	2.48	2.35	2.34	0.11	0.01	0.84	0.51
SCC Score	1.99	1.95	1.94	2.02	0.14	0.87	0.76	0.25
Fat to protein ratio	1.15	1.12	1.36	1.31	0.06	0.01	0.17	0.75
3.5% FCM, kg/d	49.4	49.6	51.7	50.7	2.37	0.08	0.57	0.51

<sup>1</sup> **CORN**: corn silage-based diet; **SORG**: sorghum silage-based diet; **-XYL**: control; **+XYL**: xylanase supplemented.

<sup>2</sup> **Silage**: effect of silage type; **XYL**: effect of xylanase supplementation; **S×X**: effect of interaction.



**Table 5.3** Apparent total tract nutrient digestibility of dairy cows fed diets containing corn or sorghum silage as the forage source with or without xylanase supplementation.

	Diet <sup>1</sup>				SEM	<i>P</i> value <sup>2</sup>		
	CORN		SORG			Silage	XYL	S×X
	-XYL	+XYL	-XYL	+XYL				
DM, %	78.4	76.1	73.8	73.6	0.85	0.01	0.01	0.05
CP, %	79.3	76.7	72.6	72.2	0.77	0.01	0.02	0.08
NDF, %	57.0	51.0	56.3	54.8	1.75	0.18	0.01	0.05
Starch, %	99.3	99.1	96.3	96.8	0.62	0.01	0.36	0.10

<sup>1</sup> **CORN**: corn silage-based diet; **SORG**: sorghum silage-based diet; **-XYL**: control; **+XYL**: xylanase supplemented.

<sup>2</sup> **Silage**: effect of silage type; **XYL**: effect of xylanase supplementation; **S×X**: effect of interaction.

**Table 5.4** Fatty acid profile (g/100 g fat) of corn or sorghum silage used as the forage source.

	Silage	
	Corn	Sorghum
C14:0	0.27	0.50
C14:1	0.02	0.01
C16:0	14.61	17.40
C16:1	0.14	0.38
C18:0	2.34	2.30
C18:1 (cis 9)	23.49	27.06
C18:1 (cis 11)	0.65	1.15
C18:2 (cis 9, cis 12)	46.24	36.98
C18:3 (cis 9, cis12, cis15)	6.83	4.92
C20:0	0.58	1.48
C20:1 (cis11)	0.23	0.20
C20:2	0.04	0.03
C20:4	0.04	0.03
C20:3	0.05	0.06
C20:5	0.17	0.20
C22:0	0.49	1.06
C22:1	0.02	0.02
C22:6	0.01	0.05
C24:0	0.67	2.01
C24:1 (cis15)	0.01	0.02
Others	3.10	4.14

**Table 5.5** The economic outcomes of using corn or sorghum silage as the forage source.

	Diet <sup>1</sup>	
	CORN	SORG
Milk price <sup>2</sup> , \$/cwt	12.9	14.1
Income, \$/cow/day	14.6	15.2
Feeding cost <sup>3</sup> , \$/cow/day	8.1	7.4
Income over feed cost, \$/cow/day	6.5	7.8
Income over feed cost, \$/cwt	5.7	7.3

<sup>1</sup> **CORN**: corn silage-based diet; **SORG**: sorghum silage-based diet.

<sup>2</sup> Fat price: \$2.3334/lb.; Class III skim milk price: \$5.38/cwt.

<sup>3</sup> Concentrate price: \$322/ton; corn silage price: \$35/ton; sorghum silage price: \$32/ton; grass hay price: \$62/ton.

## CHAPTER 6 CONCLUSIONS

Cereal grains are commonly included in dairy rations as the energy source for lactating dairy cows. Corn grain is a predominant energy source used to meet the high energy requirements of lactating dairy cows during early- or mid- lactation. When drought stress occurs, one immediate effect is an increase in corn prices (USDA, 2012a,b) and a reduction in net farm income due to increased feed costs. For example, corn price increased from \$6.37 per bushel (\$250/Mg) in June 2012 (USDA, 2012a) to \$7.63 per bushel (\$300/Mg) in August 2012 (USDA, 2012b), when drought stress was most extreme. Increased feed prices translate into greater feed costs in dairy farming systems, which may reduce income over feed costs and profitability. When corn prices increase, alternative feeding strategies, such as replacing corn grain with barley grain is necessary to be evaluated. Barley grain is not widely used for feeding lactating dairy cows because the degradability of barley starch is higher than corn starch. Feeding rapid fermentable starch may lead to subacute ruminal acidosis. Also, barley grain contains lower starch and higher fiber concentration than corn grain.

Hulless barley has a loose husk surrounding the kernel of grain that can be easily removed during harvesting and processing. Due to less husk, hulless barley has been reported to contain 8 to 14% greater digestible energy, higher starch, and lower fiber concentration than hulled barley. Due to higher starch and lower fiber concentration than hulled barley, hulless barley has a high potential for replacing corn grain as the energy source in dairy rations. In study 1, hulless barley grain used as the grain source in diets containing 30% starch and 30% NDF to replace corn grain. The inclusion of hulless barley as an energy source in diets for lactating dairy cows resulted in similar production performance and nutrient digestibility as for corn grain. This research determined that hulless barley grain can be an alternative grain source for replacing corn grain and

increasing the concentration of NDF is not necessary when feeding hulless barley to lactating dairy cows.

In study 2, we evaluated the production performance and nutrient digestibility of lactating dairy cows by feeding hulled or hulless barley grain-based diets with different forage to concentrate ratios. we hypothesized that rumen function is altered when cows are fed low-forage diets containing barley grains and that an altered rumen function would be reflected in lower lactation performance, a reduction of de novo fatty acids in milk fatty acid profile, or a combination of both. According to results, we concluded that feeding hulled barley or hulless barley grain-based diets with different forage to concentrate ratios resulted in similar production performance and nutrient digestibility in lactating dairy cows. Even with low forage diets (40% forage and 13% forage NDF), feeding both barley grains did not affect milk fat synthesis and cow performance. Hulled and hulless barley are the good energy sources for sustaining high milk production and maintaining ruminal health of lactating dairy cows. Feeding diets with 17% forage NDF may results in higher milk yield than that of feeding diets with 13% forage NDF.

According to study 2, dairy cows require sufficient NDF and forage NDF in their diet to maintain rumen function and maximize milk production. Adequate fiber will increase rumination time and will simulate more production of saliva which can buffer the rumen fluid (Allen, 1997). However, increased fiber concentration is negatively related to DMI and milk production due to the increased rumen fill (Allen, 1996). The effect of fill caused by forage NDF content on DMI may gradually diminish as digestibility increases (Allen, 1996). Feeding exogenous fibrolytic enzyme is a feasible strategy to improve production performance by increasing fiber digestibility by dairy cows. In study 3, we evaluated the effects of supplementing xylanase enzyme on production performance and nutrient digestibility of lactating dairy cows consuming corn or sorghum silage-

based diets. We concluded that supplementation of xylanase for 19 d did not affect cow performance and feeding diets containing 12.6% wheat grain (dry matter basis) and 33% NDF resulted in a reduction in milk fat percentage and yield. future studies should investigate the long-term effects of supplementing xylanase on production performance and nutrient digestibility of cows fed diets containing sorghum silage as the forage source.

For future studies, more studies are required to investigate protein matrix around endosperm of cereal grains. The different ruminal starch degradability of cereal grains is due to protein matrix that covers endosperm. Effect of protein matrix on the starch digestion by lactating dairy cows should be evaluated. Also, identification and quantification of protein-hydrolyzing ruminal bacterial should be further invested in order to understand better the digestion of grain starch.