MOLECULAR STRUCTURE FEATURES AND NUTRIENT AVAILABILITY AND UTILIZATION OF BARLEY SILAGE VARIETIES WITH VARYING DIGESTIBLE STRUCTURAL CARBOHYDRATE IN COMPARISON WITH A NEW SHORT-SEASON CORN SILAGE IN HIGH-PRODUCING DAIRY CATTLE

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By

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ABSTRACT

Barley silage is a main crop forage source that used by the dairy producer in Western Canada. There are many barley forage varieties that used for silage production. However, there is limited information in their nutritional characteristics and utilization in dairy cows, meanwhile new corn forages that developed to Western Canada that required less crop heat units to reach the maturity stage for silage production. The objectives of the Experiment 1 and 2 were: (1) to assess the magnitude of difference among barley silage varieties in comparison with short-season corn silage in terms of their chemical composition, energy values, protein and carbohydrates fractions, rumen degradation kinetics, and intestinal absorbed true protein supply to dairy cattle and (2) to define the interactive association between molecular structure of silages and carbohydrates or protein utilization in dairy cows. The two experiments were complete randomized design with four treatments: corn silage (P7213R), CDC Cowboy barley silage, CDC Copeland barley silage, and Xena barley silage. The barley silage varieties were selected based on varying rate of *in vitro* neutral detergent fiber digestibility (ivNDFD). Five cannulated lactating dairy cows were used for measuring *in situ* rumen degradation kinetics. Intestinal digestibility of rumen undegraded feed protein was estimated using three-step *in vitro* procedure. The protein and carbohydrates related-molecular structure spectral data was collected using attenuated total reflectance Fourier transform infrared (ATR-FT/IR) molecular vibrational spectroscopy. Corn silage showed the highest total digestible nutrient and energy content. Cowboy showed lower energy content and lower dry matter (DM) degradation in the rumen relative to other barley varieties. All studied silages exhibited the same level of metabolizable protein supply to dairy cows. Molecular structural analysis showed significant modifications in protein or carbohydrates related molecular spectral intensity. The protein structure α-helix to β-sheet ratio are correlated to total intestinally

absorbed protein supply. The spectral intensities of carbohydrates were highly correlated with the digestible carbohydrate content of silages.

The objective of the Experiment 3 was to evaluate the effect of barely silage varieties selected for varying rates of ivNDFD on DM intake (DMI), milk production, and total chewing activity of high-yield dairy cows in compared with short-season corn silage. Four mid-lactating multiparous Holstein cows (DIM = 101 ± 25; parities = 2.75 ± 0.83) were used in a 4 × 4 Latin square design. The CDC Cowboy with higher ivNDFD did not result in improvements in milk yield, feed efficiency, or total chewing activity compared with other barley silage varieties. Cows fed P7213R corn silage-based diet tended to have higher DMI (28.1 vs. 25.7; $P = 0.10$) and produce more milk (40.1 vs. 35.3 kg/d; $P = 0.01$) than those fed barley silage-based diets. This implies that the cows fed corn silage-based diet improved feed efficiency compared with those fed barley silage-based diets.

The objective of the Experiment 4 was to investigate the effects of barely silage with varying ivNDFD in comparison to short-season corn silage on rumen fermentation characteristics and microbial protein synthesis using a rumen simulation technique. The experiment was a randomized complete block design with four treatments that previously used in the dairy trial. The experiment consisted of 10 d of adaptation and 6 d of data collection. The main results of this study are: Cowboy barley silage did not affect rumen fermentation characteristics when compared with other barley silage varieties. On the other hand, the short-season corn silage had lower ruminal pH, a greater molar proportion of propionate, and lower acetate to propionate ratio relative to the average of all barley silage varieties. Nutrients digestibility of total mixed ration were not affected by the treatments. The corn silage had higher DM digestibility (DMD)

compared with the average all barley silage varieties. There was no significant effect of barely silage variety on the bacterial protein production, whereas the diet containing corn silage had exhibited higher bacterial protein production compared to barley silage.

To sum up, the results indicate that the new short-season corn silage had a higher energy content than barley silage. Feeding the new short-season corn silage would increase the milk yield, microbial protein synthesis, and feed efficiency in dairy cows relative to barley silage. The FT/IR could be used as a rapid potential tool to predict the ruminal degradation of fiber and the rumen degradation kinetics of CP by using molecular spectral bands intensities in structural carbohydrates and protein regions, respectively. The short-season corn silage could be used as an alternative to other conventional forages in Western Canada. Selecting barley silage varieties based on ivNDFD level is not a satisfactory approach to improve the milk production and DMI in dairy cows. Thus, for the next two experiments (5 and 6) it was intended to improve ivNDFD of barley silage or barley silage-based diet using an exogenous fibrolytic enzymes derived from *Trichoderma reesei* to enhance ivNDFD, and to correlate this increase with dairy cows' performance during mid-lactation or early-lactation, ultimately to see whether or not the effects on lactation performance in high producing dairy cows.

In the experiment 5, effects of fibrolytic enzymes on lactation performance, digestibility, and feeding behavior of dairy cows during mid-lactation were assessed. Dairy cows were fed barley silage-based diet pre-treated with a new fibrolytic enzymes derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK). Two studies were conducted to evaluate the effect of this product on barley silage and barley silage-based diet. Before starting the dairy trial, *in vitro* incubations were conducted to predict whether this product would have a

positive effect before proceeding to animal experiments. The dairy trial was performed using eight Holstein dairy cows. The cows were blocked by their parity and assigned randomly to one of 4 treatments: 0, 0.5, 0.75, and 1 mL of FETR / kg DM of diet in a replicated Latin square design. The application of FETR linearly ($P = 0.02$) increased *in vitro* DM digestibility and tended to improve ($P = 0.08$) ivNDFD in barley silage. The diet supplemented with an intermediate dosage level of FETR (0.75 ml FETR/ kg of TMR) had exhibited a higher milk fat (1.2 vs. 1.4 kg/cow/day) and fat-corrected milk (38.9 vs. 36.4 kg/d) compared to control. Increasing FETR levels resulted in a quadratic effect ($P < 0.05$) on feed efficiency. There was no effect ($P > 0.10$) of FETR level on feeding behavior. Based on the findings in this study, the optimal dosage of FETR was the 0.75 mL / kg DM of TMR. Adding this level of FETR to TMR, increased the digestibility of NDF, milk yield and milk fat yield in dairy cows.

In experiment 6, effects of pre-treating barley silage-based diet with a fibrolytic enzyme derived from *Trichoderma reese*i on lactation performance, omasal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile in Holstein dairy cows during early lactation were estimated. The application of FETR tended to decrease the DM intake compared to control (32.8 vs. 33.7; $P = 0.08$). There was no effect of FETR ($P > 0.10$) on rumen fermentation characteristics, ruminal pH profile, and omasal nutrient flow. There was a significant decrease ($P = 0.05$) in milk urea nitrogen as a consequence of adding FETR to the diet. In conclusion, dairy cows fed barley silage-based diet pre-treated with FETR had maintained milk yield with less amount of feed during early lactation. The positive effect of FETR may depend on diet composition, lactation stage and milk yield level.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AECP	Truly absorbable endogenous protein
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine
BCVFA	Branched-chain volatile fatty acids (isobutyrate + isovalerate)
bmr	Brown midrib
BS	Barley silage
CA1	Volatile fatty acids [Acetic + Propionic + (Butyric + Isobutyric)]
CA2	Lactic acid
CA3	Organic acid
CA4	Sugar (rapidly degradable carbohydrate fraction)
CB1	Starch (intermediately degradable carbohydrate fraction)
CB2	Soluble fiber (intermediately degradable carbohydrate fraction)
CB3	Digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction)
CC	Indigestible fiber (unavailable neutral detergent fiber)
CHO	Carbohydrate
CP	Crude protein
CNCPS	Cornell Net Carbohydrate and Protein System
DE_{3x}	Digestible energy at level (3x maintenance)
DM	Dry matter
DPB	Degraded protein balance
ECM	Energy-corrected milk
EDCP	Effective degradability crude protein
EDDM	Effective degradability dry matter
EDOM	Effective degradability organic matter
EDNDF	Effective degradability neutral detergent fiber
EE	Ether extract
FCM	Fat-corrected milk
FE	Fibrolytic enzyme
FMV	Feed milk value

FT/IR	Fourier transform infrared spectroscopy
IDP	Intestinal digestibility of protein
iNDF	Indigestible neutral detergent fiber
ivNDF	*In vitro* neutral detergent fiber digestibility
kd	Degradation rate of degradable fraction
kp	Passage rate
MCP	Microbial crude protein
ME	Metabolizable energy
ME_{P3x}	Metabolizable energy at a production level (3x maintenance)
MP	Metabolizable protein
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NEL_{P3x}	Net energy at a production level (3x maintenance).
NFC	Non-fiber carbohydrate
NRC	National Research Council
OM	Organic matter
PA1	Ammonia
PA2	Soluble true protein (rapidly degradable protein)
PB1	Insoluble true protein (moderately degradable protein)
PB2	Fiber-bound protein (slowly degradable protein)
PC	Indigestible protein
PCA	Principal components analysis
pdNDF	Potentially digestible neutral detergent fiber
peNDF	Physically effective neutral detergent fiber
RDCB3	Ruminal degradation of available neutral detergent fiber
RDP	Rumen degradable protein
RUDM	Rumen bypass or undegraded feed dry matter
RUNDF	Rumen bypass or undegraded feed neutral detergent fiber
RUOM	Rumen undegradable of organic matter
RUP	Rumen undegradable protein
SCP	Soluble crude protein
T_0	Lag time
TCL	Theoretical cut length
tdCP	Total truly digestible crude protein

tdFA	Total truly digestible fatty acid
tdNDF	Total truly digestible neutral detergent fiber
tdNFC	Total truly digestible non-fiber carbohydrate
TDN	Total digestible nutrients
TDN_{1x}	Total digestible nutrients at a maintenance level
TDP	Total ruminal and intestinal digestibility of protein
TMR	Total mixed ration
TRDC	Total degraded ruminal carbohydrates fraction
TRUC	Total escaped carbohydrates fraction
uNDF	undigested neutral detergent fiber
VFA	Volatile fatty acid

1. GENERAL INTRODUCTION

Single time point *in vitro* or *in situ* analysis is widely used as an alternative to *in vivo* methods for estimating ruminal fiber digestion of forages (Oba and Allen, 1999b; Oba and Allen, 2011). Oba and Allen (1999b) collected data from seven experiments with 13 comparisons and stated one-unit increase in 30-h *in vitro* neutral detergent fiber digestibility (ivNDFD) was correlated with a 0.17 kg increase in voluntary dry matter intake (DMI) and 0.25 kg increase in 4% fat corrected milk yield (FCM).

The correlation between feeding forages with high ivNDFD and milk production or DMI in dairy cows has been extensively studied for corn silage, and corn silage hybrids with enhanced ivNDFD (Oba and Allen, 1999b). However, there are few studies that have investigated the effect of feeding barley forages with enhanced ivNDFD on dairy cattle performance. In recent years, several new barley forages have been developed in Western Canada. However, there is little research associating ivNDFD for these varieties with dairy cow performance. Moreover, there are no reports on the molecular structure and metabolic characteristics of the protein in these varieties.

The corn grown in Western Canada is short-season corn and differs from conventional corn grown in warm climates in terms of its feeding value. These environmental differences lead to changes in the chemical and nutrient composition of silages. Abeysekara et al. (2013a, b) found short-season corn cultivars grown in Canadian prairie climatic conditions, i.e., P7213R, to have a nutrient composition similar to those grown in warm weather and could be used in cooler dry climates for the feeding of cattle. However, there are no studies on the effects of this new short season corn silages on the lactation performances of dairy cows.

A vibrational molecular spectroscopic method such as Fourier transform infrared (FT/IR) molecular spectroscopy has been developed in recent years as a rapid, direct, non-destructive and non-invasive bioanalytical technique (Yu et al., 2004b; Yu, 2011). This technique can be used to understand the quantity, composition, structure, and distribution of chemical constituents and functional groups in a tissue (feed and ingredients) within intact material. Intrinsic chemical structures were found to affect nutritive value, degradation characteristics, utilization, and the availability of feed (Yu and Nuez-Ortin, 2010). Knowledge of the internal structure of a feed ingredient is critical to understand nutritive quality, utilization, and availability. To our knowledge, there are no studies on the association of molecular structure features (functional groups) with metabolizable protein and carbohydrates and nutrient availability and utilization for the ensiled forages in high-producing dairy cattle.

Fibrolytic enzymes are commonly used in ruminant diets to improve forage fiber digestibility and production of lactating dairy cows. Enzyme supplementation directly to the feed has been found to improve the digestibility forage ivNDFD (Feng et al., 1996; McAllister et al., 1999; Kung et al., 2000; Yang et al., 2000). Nevertheless, results are inconsistent concerning the impacts of adding of these fibrolytic enzymes on dairy cow performance. Thus, the use of fibrolytic enzymes as feed additives has not yet been extensively embraced on commercial dairy farms. However, due to a continuous increase in feed costs, it is necessary to reconsider the use of fibrolytic enzymes as feed additives in ruminant diets as a strategy to improve feed efficiency and decrease the cost of milk production. More studies are required with an emphasis on enzyme-substrate specificity, enzyme activity, the method of supplementation, and the optimum dosage of enzymes.

2. LITERATURE REVIEW

2.1. The Main Forage Crops Grown for Silage Production in Canada

Many crop forages are grown for silage in Canada. Cereals, corn, peas and other field crops are widely used as silage. Whole crop cereal silages, such as barley, wheat, oat and triticale, are the most common silages in Western Canada. These silage species are well-adapted to the growing conditions in this region (Baron et al., 2000).

Barley silage is the most common cereal crop forage used by dairy producers in Western Canada. Barley has lower cost of production and water requirement compared to corn silage. The recommended seeding rate for silage production ranges between 125 and 150 kg/ha. The optimal seeding time is from early May to early June (Baron et al., 2000). To maximize the yield production, the fertilizer should be applied at seed time.

The barley forage should be harvested between mid-dough and hard-dough stage for silage production. Chemical composition and total digestible nutrients (TDN) for barley forage harvested at different stages of maturity is shown in Figure 2.1 (Nair, 2017). The percentage of DM is greatest at the late dough stage. The CP content is similar among the different maturity stages. The neutral detergent fiber (NDF) concentration decrease, while the starch and TDN increase, with advancing barley maturity.

Barley forage varieties have different optimal stages of harvesting. For example, Nair (2017) found that some barley forage varieties could produce high-quality forage (i.e., high total digestible nutrient, high NDFD and low iNDF) and high yield when harvested at hard-dough

stage, while other barley forage varieties could produce a poor forage (lower NDFD and higher iNDF content) if it reaches hard-dough stage (Nair, 2017).

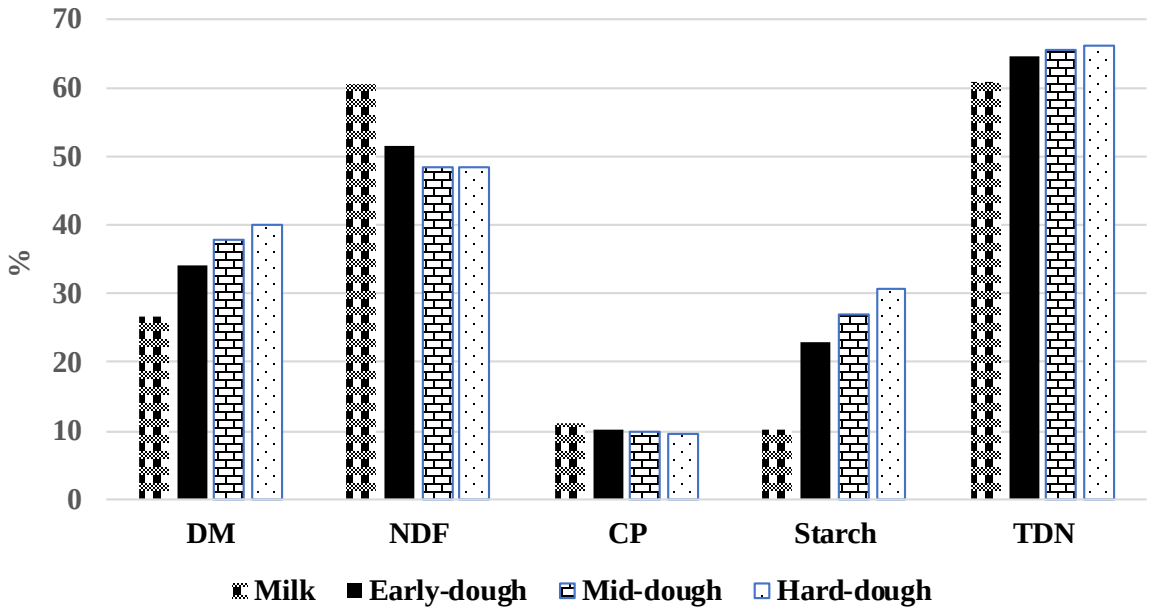

Figure 2.1. Nutrient content for barley forage at different stages of maturity (Nair, 2017)

The yield of barley silage is relatively lower compared to that of triticale silage when harvested at early-dough stage of maturity (Baron et al. 2000). Silage yield of barley and oat is equal at the early-dough stage (Baron et al. 2000). Whole crop barley silage is characterized by its high DM digestibility at all stages of maturity compared to that of oat and triticale (Kaulbars and King, 2004; Nadeau, 2007).

In recent years, many barley varieties were introduced in Western Canada; however, knowledge on their nutritional value and the association between their digestible fiber content and dairy cows' performance have not been extensively studied. Barley varieties can be used two

row (i.e., CDC Austenson, Conlon, CDC Copeland, and AC Metcalfe, CDC Cowboy and Xena) or six row (i.e., Falcon, Ranger, and Legacy).

Corn silage is the main crop forage in Eastern Canada. Corn forage is able to provide dairy cows with high-energy diet required for milk production (Mahanna, 2014). It is recommended to apply a seeding rate of at least 75,000 plants per hectare with 15 cm space between seeds and 75 cm row spacing (Kaulbars and King, 2004).

Recently, new corn forages have been successfully developed in Western Canada that can grow in areas with low Corn Heat Units (CHU; Baron et al. 2000). The CHU is an accumulation of heat through the growing season starting from mid-May and lasted until the first fall killing frost (-3-degree Celsius frost). The calculation of CHU assumes that growth of corn forage stops when the day temperatures are below 10 °C and night temperatures are below 4.4 °C. The Daily CHU are calculated using the following equation (Saskatchewan Ministry of Agriculture, 2016):

$$CHU = [1.8\ (Tmin - 4.4) + 3.3\ (Tmax - 10) - 0.084\ (Tmax - 10)^{2}] / 2. \qquad \text{Equation. 2.1}$$

It is recommended to harvest corn forage when the grain kernel development uniformly reaches the half milk line stage (Saskatchewan Ministry of Agriculture, 2016).

Selecting suitable corn hybrid for silage production is important to maximize forage yield and milk production. There are many factors to consider when selecting the best corn forage hybrid for silage production, such as grain to stalk ratio, whole plant yield, digestibility of DM per hectare, and the potential milk yield per hectare (Ballard et al., 2011). Other factors should be considered such as starch content and the forage ivNDFD (Mahanna, 2014).

The chop length and the mechanical processing of corn kernels are important during corn forage harvesting. The optimal theoretical cut length (TCL) for corn forage (30-35 %DM) is 19 mm, and the gap setting between the rollers should be 1 to 2 mm to ensure it cracks all the kernels (Ferraretto and Shaver, 2012). Mechanical processing of corn forage has been found to improve the total tract starch digestibility and milk production compared to unprocessed corn (Ferraretto and Shaver, 2012).

2.2. Importance of Forage Fiber in Dairy Rations

Silages are considered the most cost-effective feeding resource in ruminant nutrition. Grass and small-grain cereal silages are the most common sources of dietary energy, while leguminous silages are considered to be important sources of protein for ruminant livestock (Wilkinson et al., 2003). The quality of silage is an important determining factor in dairy cow performance; as the forage accounts for a large proportion of the diet supplying 35% up to 100% of DM (Nikkhah, 2013).

For high-producing dairy cows, high-quality silages with low fiber and higher fermentable carbohydrates are usually used to meet energy requirements (NRC, 2001). Nevertheless, insufficient physical effective of fiber reduces chewing activity, saliva production and rumen pH, and can cause rumen acidosis and laminitis (Yang and Beauchemin, 2007a). These can depress fibrolytic microbes and milk production (Beauchemin, 1991; NRC, 2001). National Research Council (NRC, 2001) stated that dairy rations should have a minimum of 25% NDF, 18.7% of which must come from forage for adequate rumen health (NRC, 2001). Although poor rumen fermentation and function can have negative impacts in dairy cattle rations deficient

in fiber; excessive levels of fiber over 44% may also have negative effects on intake and digestibility, due to rumen fill limitation (NRC, 2001).

The NRC recommendations regarding the total NDF and forage NDF contents of dairy rations are presented in Table 2.1 (NRC, 2001). In general, the minimum NDF contents that are recommended for dairy ration will depend on the dietary contents of non-fiber carbohydrate (NFC), a physical effective fiber, and the source of the fiber. It is well established that the fiber from forage sources stimulates more salivation and cud-chewing activity than non-forage fiber sources (Yang and Beauchemin, 2007a). Consequently, the major factor for evaluating the efficiency of dietary NDF capability is the coarse particles of NDF in forages. It has become very important to prevent acute and subacute rumen acidosis and maintain milk fat level; hence evaluating the physical effective NDF (peNDF) of diets is important for maintaining the rumen pH and fiber digestion.

Table 2.1. Recommended minimum NDF concentration based on the proportion of NDF coming from forage sources (NRC, 2001).

Minimum NDF from forage (% of DM)	Minimum NDF from forage (%NDF)	Minimum NDF in TMR[1] (% of DM)
19	75	25
18	66	27
17	58	29
16	51	31

[1]TMR = total mixed ration

The amount of peNDF in the diet is dependent on the chop length of forages, dietary NDF, and forage-to-concentrate ratio (Mertens, 1997). The peNDF intake can stimulate the chewing activity and can minimize the incidence of ruminal acidosis (Teimouri Yansari et al., 2004). Many studies have examined the effects of peNDF on lactation performance (Heinrichs and Kononoff, 2002; Krause et al., 2003; Huhtanen et al., 2007a; Yang and Beauchemin, 2007a, 2009). The peNDF of feed could be calculated from the NDF content multiplied by a physical effectiveness factor (pef). The pef ranges between 0 (not effective at stimulating chewing) and 1 (100% effective at stimulating chewing). Numerous feed models such as Cornell Net Carbohydrate and Protein System (CNCPS) presently use peNDF as an important input for the model to predict lactational performance. The forage and TMR particle size distribution recommendations are presented in Table 2.2 (Heinrichs and Kononoff, 2002).

Table 2.2. Forage and total mixed ration particle size distribution using Penn state particle separator as reported by Heinrichs and Kononoff (2002).

Sieve size	Feed type		
	Corn silage	Haylage	TMR[1]
>19.0 mm	3-8	10-20	2-8
19.0–8.0 mm	45-65	45-75	30-50
8.0–4.0 mm	20-30	30-40	10-20
<4.0 mm (bottom ban)	<10	<10	30-40

[1]TMR= total mixed ration

2.3. Evaluation of Fiber Digestibility in Dairy Cows

Understanding the mechanism of fiber digestion is very important to accurately estimate the digestible energy of fiber and to improve animal performance. Fiber is digested primarily in the rumen and its degradability affected by the chemical nature of the fiber and by the passage rate and digestion rate of fiber within the digestive tract of the animal. The potentially digestible NDF (pdNDF) content and the digestion rate (Kd) vary greatly between and within different silage types (Waldo et al., 1972).

Several models have been developed to describe the process of digestion in the rumen. Most of these models have been developed by fractional schemes to correlate the disappearance or gas production curves with rumen digestibility of feed components. This assumes that the feed component includes at least two portions: a potentially degradable fraction and an undegradable fraction. The potentially degradable portion will be degraded at a fractional rate (per hour), after a discrete lag time (h). The undegradable fraction is calculated from the longer time of incubation as proposed by Waldo et al. (1972). By using this model, Allen and Mertens (1988) developed mathematical equations to define fiber digestibility and rumen fill. For fiber digestibility, the following equations were developed:

$$\text{Digestible fiber pool} = (\text{pdNDF (fiber intake per unit of time)}) / (\text{Kd} + \text{Kp}) \qquad \text{Equation. 2.2}$$

$$\text{Indigestible fiber pool} = (\text{iNDF (fiber intake per unit of time)}) / (\text{Kp}) \qquad \text{Equation 2.3}$$

Finally, the rumen fill is estimated as the sum of the digestible and indigestible fiber pools in the rumen. Equation (2.2) shows that rumen digestibility is directly related to (pdNDF) and (Kd), and inversely proportional (Kd + Kp; the rate of total fiber digestibility). Thus, as the ruminal retention time increases (1/Kp), the extent of ruminal digestibility increases (Huhtanen et al., 2007b). The fiber weight in the rumen is dependent on fiber intake per unit of time, and digestible (pdNDF), and indigestible (iNDF) components, as well as digestion rates (Kd) and passage (Kp). Jung and Allen (1995) ranked the factors that influence ruminal fiber fill, and the most important element was considered to be the fiber content, followed by Kp, iNDF, and finally the lowest factor was the Kd. The forage fiber digestibility is calculated according to the following equation (Allen and Mertens, 1988) :

$$\text{Digestibility} = \text{pdNDF} * [\text{Kd} / (\text{Kd} + \text{Kp})] \qquad \text{Equation. 2.4}$$

Based on this equation, the forage fiber digestibility is related to 1) the ratio of degradation rate of fiber in the rumen to disappearance rate of fiber in the rumen (Kd + Kp); 2) the digestible fiber fraction (pdNDF).

The digestion kinetics of fiber can be measured *in vivo* using marker or rumen evacuation technique, where cannulated animals are used for measuring the digestible and indigestible fiber pools that flow from the rumen (Weiss, 1998). In the rumen evacuation technique, the total digesta of rumen contents can be determined by removing ruminal content of each animal at different time point. It has been proved that the use of other biological methods (i.e. *in vitro* or *in situ* techniques) could give better characterization to the degradation kinetics of the fibrous fraction of forages. Over the last 50 years, the *in vitro* system has not been widely used for analysis of forages due to its difficulty to perform in farms. This situation has changed in recent

years with the use of a shorter digestion time (30 or 48 h) along with improvements in spectral analysis using near-infrared spectroscopies. In these cases, laboratories were facilitated to assess the digestion of forages without the need to obtain rumen fluid. Some mathematical equations have been developed, which can use single time points like 24 or 30 h ivNDFD along with fixed lag time and lignin content in the forages to calculate the Kd rates (Van Amburgh et al., 2004).

In recent times, feeding studies have found that the iNDF after long incubation time (240 h *in vitro* or 288 h *in situ*) was highly correlated with DMI and can be used to predict pdNDF (Van Amburgh et al., 2015). Furthermore, there were sufficient data on iNDF analyses being created by commercial laboratories. Thus, the iNDF was applied as a new approach rather than using lignin × 2.4 to calculate pdNDF (CB3) and iNDF (CC) of the updated CNCPS 6.5 (Van Amburgh et al., 2015).

It has been found that a model, which could accurately predict NDF digestibility, should partition NDF into iNDF and pdNDF, fractionate feed particles by their retention and passage in the rumen, and use a predicted Kd by an *in vitro* system (Huhtanen et al., 2007b). Based on this approach, Combs developed a new method for predicting fiber digestibility; where shorter incubation time (24, 30, and 48 h) along with undigested NDF (uNDF-240 h) were used to predict Kd of CB3 fraction (KdCB3; Combs, 2013). The KdCB3 rates derived from *in vitro* analysis are used as inputs to calculate the ruminal fiber digestibility of CB3 fraction (RDCB3) according to this equation; RDCB3 = CB3 × KdCB3/ (KdCB3 + Kp). Finally, the *in vitro* total-tract NDFD (ivttNDFD) is calculated assuming that the intestinal digestibility of available NDF (CB3) escaping rumen digestion is 10%.

The *in vivo* total-tract NDF digestibility was found to be highly correlated with the ivttNDFD (Lopes et al., 2015). The regression equation to describe the relationship was described as follows: *in vivo* total-tract NDFD (%) = −3.62 + 1.11 × ivttNDFD (%) with $R^2 = 0.70$, RMS = 4.27, *P*-value < 0.01; *n* = 21 diets. The differences between two methods (ivttNDFD and *in vivo* total-tract NDFD) were not significant, and mean values varied by only 1%, showing promise for this approach (Lopes et al., 2015).

The use of high-resolution spectroscopic techniques (e.g., high-field nuclear magnetic resonance, mid-infrared, Raman spectroscopy, and pyrolysis mass spectrometry) is finding increased application in feed evaluation (Yu et al., 2004b; Yang et al., 2013). The molecular vibrational spectroscopy such as Fourier transform infrared (FT/IR) spectroscopy has been developed as a rapid, direct, nondestructive and noninvasive bioanalytical technique (Yu et al., 2004b). Thereby, this technique paves the way to better understand the quantity, composition, structure, and distribution of chemical constituents and functional groups in a tissue (feed and ingredients) (Yu, 2012; Huang et al., 2017).

Intrinsic molecular structures were found to affect the nutritive value, degradation characteristics, utilization, and availability of feed (Nuez-Ortín and Yu, 2010; Yu and Nuez-Ortín, 2010). Several studies have reported that FT/IR would accurately predict concentrations of lignin, ferulic, and coumaric acids in fresh forage samples (Abeysekara et al., 2013b; Xin et al., 2013; Yang et al., 2013; Prates et al., 2018). However, there is limited information on the association between molecular structure of ensiled forages and their ruminal degradation profile.

2.4. Fiber Digestion and Utilization in Ruminants

2.4.1. Plant Cell-Wall Carbohydrates

Forages are diverse in their characteristics, and this variation results in differences in quality of animal feed. Plant cell-wall carbohydrates are the most important components in forages that influence silage quality. Ruminants can digest and degrade plant cell-wall polysaccharides. The plant cell-wall chemistry and anatomical structure will determine the digestion characteristics of cell types (Grabber et al., 1997). The fiber fraction for the main silages is presented in Table 2.3.

Table 2.3. Fiber fraction for NDF concentrations based on the proportion of NDF derived from forage sources (Varga and Ishler, 2017)

Silage	Legume silage	Grass silage	Corn silage	Winter cereals
DM	37	31	33	29
NDF, %DM[1]	47	62	45	52
ADF, %DM[2]	39	41	26	31
Hemicellulose, %DM[3]	8.9	21	19	21
Lignin, %DM	7.7	6.4	2.8	4.3

[1]NDF = neutral detergent fiber
[2]ADF = acid detergent fiber
[3]Hemicellulose = ADF- NDF

The main groups of plant cell-wall carbohydrates are hemicellulose and cellulose. Cellulose is water-insoluble β-glucan composed of a linear molecule of D-anhydroglucopyranose residues linked by a β-(1→4) bond (Figure 2.3). In contrary to cellulose, hemicellulose has

various groups of polymers that are characterized by heterogeneous composition. Xylan is the main component of hemicellulose and compromises about 30–35% of the cell-wall material of annual plants. The main chain of xylan is composed of 1,4-*β*-linked D-xylopyranose units (Bhat and Hazlewood, 2001).

The collaborative activity of the cellulolytic and noncellulolytic microorganisms in the rumen is critical in fiber digestion (Flint and Forsberg, 1995). Rumen cell-wall degradation is initiated by the attachment of rumen microbes to feed particles and the bacterial species specialized to start this attachment/colonization process are the cellulolytic species *Ruminococcus albus*, *R. flavefaciens*, and *Fibrobacter succinogenes*.

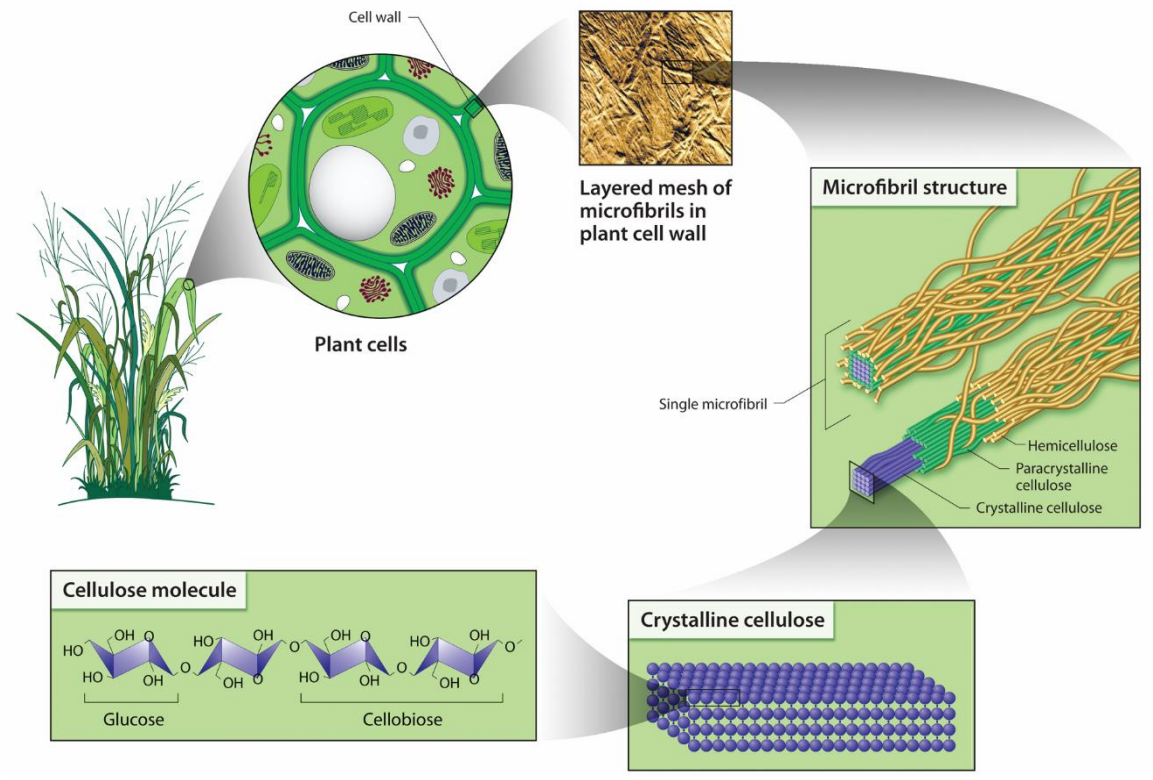

Figure. 2.2. Cellulose structure (courtesy of the US Department of Energy Genome Program, available at http://genomics.energy.gov)

Rumen fungi and protozoa also colonize and degrade plant fragments to differing degrees (Akin, 1986). The fermentation of structural carbohydrates by cellulolytic consortium results in the progressive process where volatile fatty acids (VFAs) are liberated at a lower rate than starch fermentation of non-structural carbohydrate. The fermentation of structural carbohydrates is associated with increases in the proportions of acetic and butyric acid (Johnson et al., 1996).

Following absorption, a large proportion of acetate is not changed by hepatic metabolism and may be augmented by endogenous acetate production in the liver. The posthepatic supply of acetate to peripheral tissues constitutes a major part of the total energy available to the animal and may be either oxidized to produce adenosine triphosphate (ATP) or used as a substrate in the production of long-chain fatty acids (Beever et al., 2000). While ruminally derived butyrate is quantitatively metabolized to β-OH-butyrate for epithelial cells absorption through the rumen epithelium, in posthepatic tissues it has a similar metabolic fate to that of acetate (Beever et al., 2000).

2.4.2. Lignin and Phenolic Acids

Lignin is an indigestible polymer in plants that plays an important role in the structural integrity of plant tissue. Although lignin comprises little of the total structural carbohydrate system in plants, it has been recognized to exert a negative effect on cell-wall polysaccharide digestibility by coating the plant cell-wall polysaccharides from enzymatic hydrolysis (Paloheimo et al., 2010). Lignin arises from an enzyme-initiated dehydrogenative polymerization of three originators: *p*-coumaryl alcohols, coniferyl, and sinapyl. The phenylpropanoid metabolism and shikimic acid pathway lead to the synthesis of lignin intermediates like *p*-coumaric acid, ferulic acid, and diferulic acid (Humphreys and Chapple, 2002), which are

converted into coniferyl, sinapyl, and *p*-coumaryl alcohols and ultimately to guaiacyl, syringyl, or *p*-hydroxyphenyl lignin, respectively. With the maturation of forage cell wall, the guaiacyl-type lignin changes to lignin-rich syringyl units, and the digestibility of mature cell walls decreases. Taboada et al. (2010) found that guaiacyl and syringyl contents have negative correlations with organic matter and DM digestibility in ruminants fed silage. They concluded that guaiacyl and syringyl could be used as predictors for forage digestibility rather than total lignin content in silage (Taboada et al., 2010).

The brown midrib (bm3) mutation in annual C4 grasses such as corn and sorghum results in both a reduction in lignin concentration and a shift in lignin composition to a more degradable-guaiacyl-rich polymer (Jung and Allen, 1995). Jung and Deetz (1993) have suggested that the improved digestibility of cell walls in bm3 mutants is a result of both the reduced lignin concentration and the reduction in syringyl lignin content.

Cross-linking of lignin to cell-wall polysaccharides has been reported as an additional mechanism for limiting forage fiber digestibility in ruminants (Figure. 2.3; Ralph et al., 1994; Jung and Allen, 1995). In grasses, ferulate and *p*-coumarate molecules are esterified to arabinoxylans in one side, and *p*-coumarates are linked to lignin on the other side through ester or covalent bonding (Ralph et al., 1994). As forages mature and lignin concentrations increase, ferulates that were esterified to arabinoxylan become etherified to lignin forming cross-linkage between lignin and the cell-wall polysaccharides (Iiyama et al., 1990).

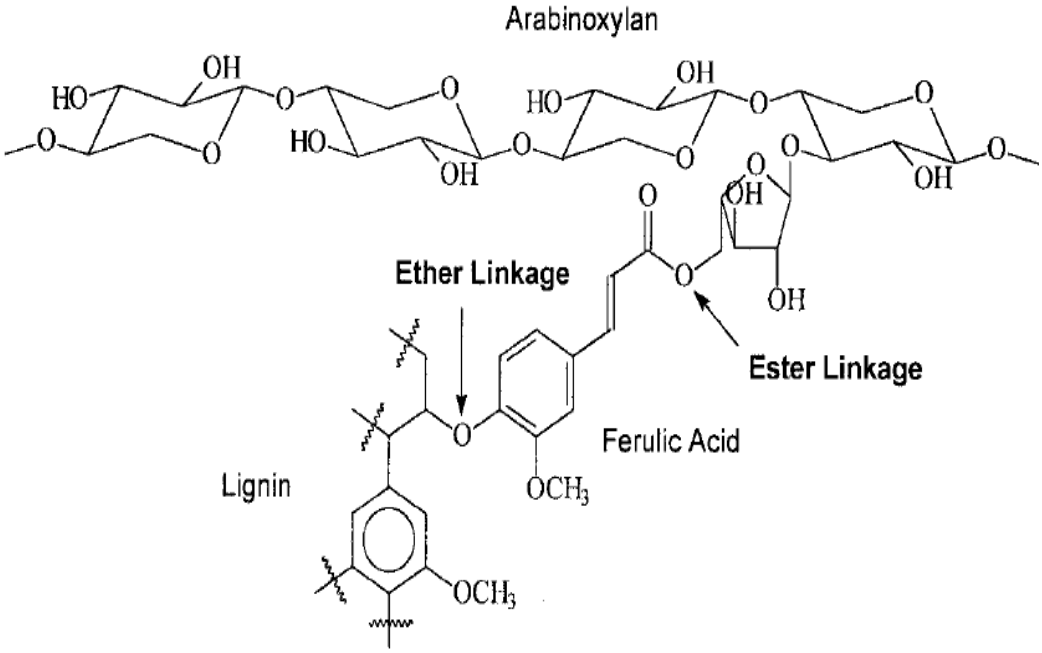

Figure.2.3. Illustration of the cross-linking structure in grasses where ferulate esters of arabinoxylan form bridges to lignin. Modified from (Ralph et al., 1994; Jung and Allen, 1995).

The degree of lignin/arabinoxylan cross-linkage by ferulates affects the digestibility of plant cell-wall polysaccharides as this linkages prevents the access of microbial enzymes to polysaccharides (Grabber et al., 1997). The presence of phenolic esters negatively affects ruminal cell-wall degradability (Casler et al., 2008). However, the reduction in degradability caused by esterified ferulic acid only limits the degradation rate of polysaccharide, rather than its extent, because ruminal fungi and ruminal bacteria are able to possess an esterase activity (Borneman et al., 1993; Casler et al., 2008).

2.5. Enhancing Fiber Digestibility and Utilization of Silage

Ruminal digestibility of forage NDF can range from less than 25% to over 75% for different forage types (NRC, 2001). Most research with brown midrib mutant corn silage found that lactating dairy cows will consume more DM and produce more milk when fed corn silages that have greater ivNDFD (Dado and Allen, 1995; Oba and Allen, 1999b, 2000). Oba and Allen (1999b) found a relationship between ivNDFD and animal performance, they reported that one-unit increase in forage ivNDFD was associated with increases of 0.17 kg d^{-1} of dry matter intake, 0.23 kg d^{-1} of milk yield, and 0.25 kg d^{-1} of 4% fat-corrected milk. Using high-quality silage in dairy cattle rations could reduce physical rumen fill, allow cattle to consume more feed, and produce more milk (Dado and Allen, 1995). Many factors influence the quality of silage. These factors include silage species, silage varieties, cutting height, growing conditions, silage additives, adding fibrolytic enzymes, and stage of harvest.

2.5.1. Silages Species

The most practical approach for increasing ivNDFD is based on increasing the amount of pdNDF in forages. Grass silages often have a greater proportion of pdNDF to iNDF and are higher in ivNDFD than legume silages, but the rate of digestion of legume pdNDF is frequently faster and could increase the total NDF digested *in vivo* (Dado and Allen, 1995; Oba and Allen, 1999b, 2000). Chemical and structural features have been identified, that may reduce fiber digestion. Of these, lignin is the most often reported (Jung and Deetz, 1993). Lignin constrains ruminal fiber digestion, which acts as a physical barrier for microbial digestion. The involvement of cross-linking of lignin to polysaccharides by ferulate linkages as an additional factor that

inhibits the digestion of grass fiber has been identified (Jung and Allen, 1995). However, a similar lignin cross-linking to fiber polysaccharides in legumes has not yet been determined.

There is an important role of plant anatomy in fiber digestibility (Akin, 1986). The vascular tissue, sclerenchyma, and stem epidermis are degraded at a slower rate in the rumen where they contain a higher amount of indigestible or highly lignified components. Leaf blades of C4 grasses are typically less digestible than those of C3 grasses due to the existence of mesophyll cells. In C3 species, stem tissue cells such as parenchyma bundle sheath, mesophyll, phloem, and epidermal cells, are degraded, but these tissues are partially or slowly degraded in C4 species. In an earlier study Akin and Burdick (1975) found that C4 grasses are less digestible than C3 species due to the existence of vascular tissue and parenchyma bundle sheath cells in larger amounts than in C3 grasses.

The total-tract digestibility of whole-crop cereal silage, legumes, or corn silage is often lower than that of grass silage. However, the lower digestibility is mostly alleviated by higher feed intake such that energy intake is maintained (Sinclair et al., 2003). Many studies have shown that partial replacement of grass silage with whole-crop cereals may not have a negative impact on milk production in dairy cows (Ahvenjärvi et al., 2006). However, the effects of feeding barley silage on DMI have been inconsistent, probably attributable to differences in the quality of the forages across studies. For example, Ahvenjärvi et al. (2006) noted a reduction in fiber digestibility when grass silage was replaced with whole-crop barley silage. This reduction in NDF digestibility was related to a lower pdNDF pool size in the rumen and higher iNDF content of barley silage compared with that of grass silage (Sinclair et al., 2003).

Whole-crop cereal species also vary in their quality and digestibility; for example, barley and oat silages when harvested at the same maturity stage (milk to soft dough stage) have been found to enhance the feed intake and average daily gain in heifers when compared with triticale silage (Nadeau, 2007). Dairy cows that are fed on barley silage had a higher feed intake than cows fed oat silage when crop were harvested at the maturity stage (early to a mid-dough stage of maturity). Such difference in feed intake is a consequence of variation in chemical composition and ear: stalk ratio of whole-crop cereals. Barley contains starch than oats and triticale because of the higher ear: stalk ratio in barley. Since most fibers exist in plant stalk, barley contains lower fiber than oats and triticale when harvested at the same stage of maturity. The higher starch resulted in a lower fiber content in barley silage; hence, barley can enhance the OM digestion compared with oats or triticale silages when fed to dairy cows (Nadeau, 2007).

2.5.2. Selecting Varieties with Enhanced Fiber Digestibility

The brown midrib mutation mutants (bm3) of corn were discovered for the first time at the University of Minnesota in 1924; the bm3 genes have been found in sorghum, Sudan grass, millet, and corn. The brown midrib corn forage (bmr) has about 25% less lignin and lower cross-linkages with lignin. Corn silage with the brown midrib mutation has a lower iNDF (34% less lignin) and had 19% higher ivNDFD than conventional corn silage (Eastridge, 1999; Lim et al., 2015).

Several studies confirmed the positive effect of feeding bmr corn on DMI and productivity of dairy cattle (Weiss and Wyatt, 2006; Stone et al., 2012), but responses have not been consistent in all experiments (Gehman et al., 2008). Table 2.4 summarizes some studies that

have evaluated the effect of selecting corn forage hybrids with enhanced ivNDFD on lactation performance in dairy cows.

In a meta-analysis by Ferraretto and Shaver (2015), it was found the milk yield was higher (+1.5 kg) for dairy cows fed bmr corn silage than cows fed conventional corn silage. Feeding dairy cows with TMR composed of bmr corn silage has increased milk protein yield due to improving the microbial protein synthesis compared to conventional corn silage. However, they reported that feed efficiency was not affected, and starch digestibility was decreased when feeding bmr corn silage.

Selecting corn forage varieties based on their ivNDFD has been embraced in the United States as an approach for variety selection programs for silage production and supports its usage in the diet formulation for high-producing dairy cows. However, there is limited information on the effect of selecting barley silage varieties with high ivNDFD on lactation performance. Oba and Swift (2014) found that feeding barley silage with enhanced ivNDFD (+4 percentage units of ivNDFD) improved feed efficiency in dairy cows. These authors found that higher ivNDFD resulted in greater availability of dietary energy without affecting DMI.

2.5.3. Agronomic Practices to Enhance Fiber Digestibility

2.5.3.1. Seeding Time

Environmental temperature has a significant impact on forage digestibility. Forages grown under a high environmental temperature had higher lignin content (Fahey and Hussein, 1999). Altering the time of seeding can advance the stage of maturity when plants are exposed to greater ambient temperature, moisture availability, and photoperiod intensity. Chow et al.

(2008) found that exposure of forages to a lower environmental temperature during heading stage increased ivNDFD.

2.5.3.2. Fertilization

It is well-established that the nitrogen fertilization can increase the protein content and forage yield and decrease fiber content of silage. Campos et al. (2013) reported a reduction in hemicellulose content and arabinose proportion of the fiber fraction in Milenio grass by nitrogen fertilization. They also found that fertilization increased fiber digestibility due to increase in (arabinose + glucose) to xylose ratio (Campos et al., 2013).

Table 2.4. Effects of silage varieties with enhanced 30-h ivNDFD on DM intake and milk yield

Reference	CS hybrid[1]	30h-ivNDFD (%)[2]	DM intake (kg/d)	Milk yield (kg/d)	4% FCM[3] (kg/d)
(Akins and Shaver, 2014)	Dual-purpose	41.9	23.9	38.8	42.0
	bmr	54.9	24.4	40.7	42.5
(Castro et al., 2010)	Conventional	51.3	24.7	40.6	35.2
	bmr	60.3	26.4	41.0	36.8
(Ebling and Kung, 2004)	Conventional	39.9	23.4	41.4	36.2
	bmr	54.0	25.9	44.3	37.3
(Gehman et al., 2008)	Dual-purpose	49.1	20.1	36.4	34.1
	bmr	61.0	21.1	39.5	37.4
(Holt et al., 2013)	Conventional	62.2	21.7	42.3	45.6
	bmr	71.4	21.7	43.1	45.0
(Oba and Allen, 1999a)	Conventional	39.4	23.5	38.9	35.7
	bmr	49.1	25.6	41.7	38.2
(Oba and Allen, 2000)	Conventional	46.5	22.8	33.5	31.8
	bmr	55.9	23.6	36.9	32.9
(Taylor and Allen, 2005)	Conventional	54.0	23.6	39.8	36.9
	bmr	66.6	25.5	42.5	39.2
(Thomas et al., 2001)	Dual-purpose	49.2	28.6	45.1	44.4
	leafy	53.9	27.7	46.6	45.8
(Weiss and Wyatt, 2002)	Dual-purpose	35.4	23.9	33.3	33.3
	high fiber	40.1	23.7	34.0	33.3
(Weiss and Wyatt, 2006)	Dual-purpose	58.3	24.8	34.9	35.1
	bmr	65.2	24.5	36.4	34.4

[1]CS = corn silage; bmr = brown midrib corn silage

[2] ivNDFD = *in vitro* NDF digestibility

[3]FCM = fat-corrected milk

2.5.3.3. Stage of Maturity

Fiber digestibility depends largely on plant maturity. The effect of harvest maturity of whole-crop annual forages is more variable concerning fiber content. Rosser et al. (2013) reported a reduction in NDF content by advancing the maturity of barley and oat forage from head elongation to ripe, with a reduction in NDF content from 13.8 to 9.6%. The NDF concentration of whole-crop barley was not changed during the milk and soft dough stages, but it increased somewhat between the soft and hard dough stages, while this change was not observed in whole-crop oat forage (Bolsen and Berger, 1976). Bolsen and Berger (1976) reported a reduction in total-tract DM digestibility of barley silage at milk stage, compared to advanced mature stage due to the increasing grain content. Rustas et al. (2011) found no change in DM and NDF digestibility of wheat forage ensiled at milk and dough stages.

With advancing maturity of grass silage, digestibility dramatically drops because the tensile strength of stems increases to support the weight of the plant, and the leaf-to-stem ratio declines (Huhtanen et al., 2007a; Yang and Beauchemin, 2007b). In grass silage, OM digestibility dropped from 79% in early growth to 73% in late growth, and NDF digestibility decreased from 73% in early growth to 66% when the plant maturity reached late growth stage. In legumes, NDF digestibility is less than that in grasses or small grains during the early vegetative stage of growth but drops slower with advancing maturity.

In corn silage, the stage of maturity affects the fiber fraction (NDF, ADF and ADL). The fibrous content has been observed to decline with increasing maturity in whole-corn plants, but no significant change in lignin concentration from early dent to black layer (Owens, 2005). Coors et al. (1997) suggested the observed drop in fiber concentration with increasing maturity

to the dilution effect with increasing percentage of grain as the corn plant matures. Fiber concentration of corn stover increases as maturity increases (Xu et al., 1995).

2.5.3.4. Cutting Height

Increasing the cut height, which results in leaving a larger proportion of less digestible stalk in the field, may increase the feeding value of silage for lactating dairy cows. It has been reported that corn silage digestibility was enhanced at cutting heights of 45–50 cm at the expense of DM yield. Curran (2000) reported a reduction in hemicellulose, cellulose, and lignin and greater effective degradability of silage cut at 50 versus 10 cm. Neylon and Kung (2003) examined the effects of corn plant-cutting height and maturity on silage nutrient value. Plants were cut at 12.7 and 45.7 cm and harvested between one-third and two-third milk line and then again at black layer. As anticipated, NDF was less in silages cut higher, and ADF content decreased significantly. At higher maturity, the lignin contents were not influenced by increased cutting height. The cutting height only influenced ivNDFD, with the higher cut being more digestible. By increasing the cutting height of corn silage, the nutritive value was increased by decreasing NDF, ADF, and lignin concentrations and increasing the starch concentration. They also found that as corn plants were cut higher, there was a tendency for increased milk production and increased feed efficiency in dairy cows. Kung et al. (2008) also observed a decrease in fiber fraction concentrations and an increase in starch, and crude protein concentrations as cutting height was increased. These observations are all logical, because when cutting height is increased, more lignified and less digestible stems are left in the field, while increasing the concentration of more digestible leaves and kernels.

2.5.4. Silage Inoculants

Silage inoculants can be added to the freshly harvested forages to obtain a high-quality silage. The first studies on adding inoculants for improving the quality of silage used inoculants that contain homolactic bacteria (LAB), such as *Lactobacillus plantarum*, which accelerates the reduction in silage pH. This rapid drop in pH inhibits the growth of yeasts, spoilage bacteria, and fungi, meanwhile maintaining the sugars in the silage without decomposition (Baah et al., 2011). If this occurs, the yeast utilizes the lactic acid for its growth causing an increase in silage pH. At this stage, yeast and mold can quickly take advantage of sugars for their growth and reduce the density of nutrients in silage. Due to losses in silage-nutrient content, the studies on developing the inoculant production came up with the second-generation silage inoculants generated from *Propionibacteria* spp. and *Lactobacillus buchneri* (Baah et al., 2011; Addah et al., 2014). Overall, studies have shown that *Lactobacillus buchneri* inoculants are more effective in improving aerobic stability of barley silage than *Propionibacteria* inoculant. *Lactobacillus buchneri* is a heterolactic bacteria, which is able to ferment lactic acid to acetic acid; the acetic acid in turn has an inhibitory effect on the growth of yeast and subsequently prolong the silage shelf-life and reduce deterioration of silage nutrients (Reich and Kung, 2010). It was proposed that *Lactobacillus buchneri* inoculation would reduce feed intake in ruminant livestock as a result of acetic acid production. However, no effect of inoculant on feed intake has been reported when *Lactobacillus buchneri*-treated silage has been fed (Driehuis et al., 1999; Ranjit et al., 2002; Taylor et al., 2002; Kendall et al., 2009).

The first and second generation of inoculants focused only on improving silage stability without addressing the improvement of nutrient availability by animals. The main reason for the

limited effect in the first or second generation was that the inoculants did not produce enzymes that digest the plant cell walls. The third-generation silage inoculants were introduced more recently, through feeding silage inoculated with lactic acid bacteria with ferulic acid esterase activity. Previous studies by Yu et al. (2005a, b). have shown *Aspergillus ferulic* acid esterase and *Trichoderma xylanase* act synergistically to release ferulic acid from feruloyl-polysaccharides in complex plant cell walls. Their activities open the rest of the polysaccharides for more hydrolytic attack and facilitates the accessibility of the main polysaccharide chain to cellulase, thereby increasing the release of reducing sugars (Yu et al., 2005a, b). Nsereko et al. (2008) performed a screening study on 1000 esterase-producing *Lactobacillus* bacteria and found that half of this number would be able to produce ferulic acid esterase, and a more detailed study on eight of the bacteria. When compared to untreated perennial ryegrass, all inoculated samples had 9–11% greater ivNDFD. Moreover, they found that the inoculation of four corn silage hybrids with a combination of *L. buchneri* and *L. paracasei tolerans* enhanced ivNDFD by 7% (Nsereko et al., 2008).

Several studies have confirmed that esterase enzymes can complement the effect of cellulase and hemicellulase enzymes on plant cell walls, thereby increasing DM and NDF digestibility (Krueger et al., 2008). Conversely, some studies have reported no effect from adding ferulic acid esterase-producing inoculant on fiber digestibility of silage (Lynch et al., 2015). Kang et al. (2009) reported an enhancement in NDF digestibility when corn hybrids were treated with a third-generation inoculant. The author suggested these effects are attributed to the properties of the forage to which they are applied. Other studies have reported improvements in digestibility and performance of steers fed barley silage treated with a third-generation inoculant (Addah et al., 2011, 2012).

2.5.5. Using Enzymes to Enhance Fiber Utilization

There is increasing interest in using exogenous enzymes as a cost-effective method for improving animal productivity. The main enzyme products marketed for livestock are derived mainly from only four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Streptococcus faecium*) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei*, and *Saccharomyces cerevisiae*) species. Other fungal species, including *Humicola insolens* and *Thermomyces lanuginosus*, are marketed to a lesser extent (Muirhead, 1996). Several studies have confirmed that adding enzymes to rations of dairy cows can increase DMI and fiber digestibility in dairy cows (Adesogan et al., 2007).

Exogenous feed enzymes with fibrolytic activities have been reported to enhance fiber digestion in the rumen (Yang et al., 1999; Kung et al., 2002). Most commercial products investigated in dairy cows have had cellulases and xylanases activates, with proteases and amylases being tested in a minor number of studies. Table 2.5 shows studies performed in dairy cows fed TMR supplemented with enzymes characterized by cellulase and/or xylanase activities. Beauchemin et al. (2003a) reported that DMI would increase by 1.0 ± 1.3 kg/d and milk yield by 1.1 ± 1.5 kg/d with the addition of exogenous fibrolytic enzymes to dairy cow diets. It is evident from the dispersion of data from the mean of the responses to the addition of enzymes fibrolytic to ruminant diets were fluctuating in milk yield response. Therefore, the use of fibrolytic enzyme products in the dairy has not been adapted widely in the commercial operations. However, due to the concern about using antibiotics in animal feed and continuous increase in the cost of milk production, use of feed enzymes has been reconsidered in ruminant diets as a strategy to improve dairy cow performance.

Table 2.5. Effect of commercial fibrolytic enzymes on dairy cattle performance

Source of enzymes	Forage level %	Source of forage[1]	DM intake	Milk	Digestibility	References
Xylanase and cellulase	39%	24% CS + 15% AH	no effect	no effect	DM, OM, NDF, ADF, and CP	Rode et al., 1999
Cellulase and xylanase	55%	10% BS + 43% AS	no effect	increased	increased: OM, and NDF	Yang et al., 1999
β-glucanase, xylanase, and endocellulase	45%	22.5% BS + 22.5 %AG	increased	no effect	increased: DM, and decreased NDF	Beauchemin et al., 2000
Cellulase, hemicellulase, and xylanase	50%	45% CS + 5% AH	no effect	increased	not reported	Kung et al., 2000
Xylanase	38%	24% CS + 14% AH	no effect	increased	increased: DM	Yang et al., 2000
Xylanase and cellulose	55%	37% BS + 18% AH	no effect	no effect	increased: DM, OM, NDF, ADF, and CP	Bowman et al., 2002
Cellulase	45 to 61%	CS + AS	no effect	no effect	no effect	Knowlton et al., 2002
Xylanase and endoglucanase	43 to 57%	CS+ AH + GS	no effect	no effect	not reported	Vicini et al., 2003
Cellulose and xylanase	50%	26% CS + 17% AS + 7% AH	no effect	no effect	not reported	Reddish and Kung, 2007
Amylase and xylanase	40%	25% AH + 15% GH	no effect	no effect	increased: DM, OM, and CP	Hristov et al., 2008
Cellulase and xylanase	50%	30% CS + 20% GS	no effect	no effect	no effect	Peters et al., 2010
Xylanase, exoglucanase, and endoglucanase	52 to 67%	49% CS +13.5% AH	no effect	no effect	increased: DM, OM, NDF, ADF, and CP	Arriola et al., 2011
Xylanase and endoglucanase	52%	20% BS + 21%AS+ 11%AH	decreased	no effect	not reported	Holtshausen et al., 2011
Cellulase and xylanase	60%	40% CS + 20% GS	no effect	Increased: ECM.	no effect	Peters et al., 2015

[1] CS = corn silage; AH = alfalfa hay; BS = barley silage; AS = alfalfa silage; AG = alfalfa haylage; GS = grass silage.

It is well-established that applying the exogenous enzymes before feeding is more effective when applied as a liquid form than as a powder (Rode et al., 1999; Yang et al., 2000). Meanwhile, spraying enzymes onto the wet feed, such as silage, is more effective than on dry feed, such as hay and grain, where the wet feed is easier for enzymes to decompose the complex carbohydrates from polymers. This hydrolysis may enhance and simplify the microbial attachment, and hence reduce the lag time required for microbial colonization (Bowman et al., 2002). Enzymes that bind to feed seem more active, perhaps due to better resistance to proteolytic inhibition in the rumen. Rumen ecosystem was found to have a minor effect on exogenous enzymes because of glycosylation (Morgavi et al., 2001). It has also been found that nonglycosylated enzymes could sustain in the rumen and resist the proteolytic activity by ruminal microbiota, but this will depend on microbial sources of enzymes (Fontes et al., 1995).

In high-producing dairy cattle, stage of lactation has an important effect on the efficiency of enzyme additives. For instance, Schingoethe et al. (1999) found that cows in early lactation responded to enzyme supplementation, but they detected no effect of enzymes on the cows in mid-lactation. Differences in the response of early- and mid-lactation cows to enzyme supplementation were also reported in other studies (Zheng et al., 2000; Knowlton et al., 2002).

Feed specific enzyme activity should be considered when assessing the effect of enzyme on forage. Beauchemin et al. (1995) examined the enzyme-forage specificity on three forages (alfalfa hay, timothy hay, and barley silage). The authors noted the enzyme was more effective in improving steers' weight gain for the pretreated alfalfa hay and timothy hay but that was not effective for barley silage pretreated with enzyme.

2.6. Literature Review Summary, Overall Research Objectives and Hypothesis

2.6.1. Summary

High ivNDFD of corn forage has been found to enhance DMI and milk yield in dairy cows. Several varieties of barley forage have been widely developed for silage production in Western Canada. However, there is limited information regarding the association between ivNDFD level of these varieties and DMI or milk production in dairy cows. Consequently, more studies are necessary to reveal the association between ivNDFD level of these varieties and milk production for these developed barley silages.

In recent years, several corn forage varieties have been introduced in Western Canada that require 2000 or more CHU to reach the optimal harvest stage of maturity for silage production. However, there are no studies on their effect on lactation performance, the metabolic characteristics of protein in the gastrointestinal tract, and total truly metabolizable protein supply to dairy cattle in comparison with other conventional silages such as barley silage.

The FT/IR molecular spectroscopy has been developed to detect and quantify the molecular structural features feeds biopolymers. However, studies on protein and carbohydrate internal structures in terms of functional groups of ensiled forages are scarce. Therefore, studies are required to study the correlation between ensiled forage molecular structural characteristics and their digestion and utilization in the digestive tracts of dairy cows.

2.6.2. Research Hypotheses

- Barley silage variety with high 30h-ivNDFD will result in high DMI and milk production relative to varieties with low 30h-ivNDFD.
- New short-season corn silage will improve the dairy cows' performance and could be successfully used as an alternative to barley silage in Western Canada.
- The molecular structure changes in forage will affect energy and protein availability in dairy cows.
- Enhancing fiber digestion with exogenous fibrolytic enzymes supplementation will improve milk production efficiency in dairy.

2.6.3. Research Objectives

- To assess the differences among barley silage varieties regarding; lactation performance, ruminal degradability, ruminal fermentation and microbial protein synthesis in dairy cows.
- To evaluate the differences of feeding with barley silage and short-season corn silage upon the production performance of dairy cows.
- To quantify the association between the spectral profiles of carbohydrate and protein of barley and corn silages in relation to their chemical composition and ruminal degradability in dairy cows.
- To measure the effect of supplementing a novel fibrolytic enzyme on dairy cows' performance.

3. Physicochemical Characteristics and Molecular Structures for Digestible Carbohydrates of Silages

3.1. Abstract

The main objectives of this study were (1) to assess the magnitude of differences among barley silage varieties (BS) selected for varying rates of *in vitro* neutral detergent fiber (NDF) digestibility (ivNDFD; Cowboy BS with higher ivNDFD, Copeland BS with intermediate ivNDFD, and Xena BS with lower ivNDFD) with regard to their molecular structure related to CHO region, CHO chemical fractions, and rumen degradability in dairy cows in comparison with a new corn silage hybrid (Pioneer 7213R) and (2) to quantify the strength and pattern of association between the molecular structures and digestibility of carbohydrates. The carbohydrate-related molecular structure spectral data was measured using advanced vibrational molecular spectroscopy (FT/IR). In comparison to BS, corn silage showed a significantly ($P < 0.05$) higher level of starch and energy content and higher degradation of dry matter (DM). Cowboy BS had lower feeding value (higher indigestible fiber content and lower starch content) and lower DM degradation in the rumen compared to other BS varieties ($P < 0.05$). The spectral intensities of carbohydrates were significantly correlated with digestible carbohydrate content of the silages. In conclusion, the univariate approach with only one-factor consideration (ivNDFD) might not be a satisfactory method for evaluating and ranking BS quality. FT/IR molecular spectroscopy can be used to evaluate silage quality rapidly, particularly the digestible fiber content.

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3.2. Introduction

It is important to provide high-producing dairy cattle with adequate amount of good quality forages to support high levels of milk production, optimum rumen function, metabolic health, and efficiency of fermentative digestion (NRC, 2001). However, in the regions of northern latitude, it is a challenge to produce forage crops due to the restricted growing season that limits the growth range of cropping alternatives. Whole-crop barley (*Hordeum vulgare* L.) silage (BS) is the main forage component of dairy and beef rations in Western Canada, because the crop is well adapted for production in Western Canadian growing season (Wallsten and Hatfield, 2016).

Next to starch, the cell-wall carbohydrates are the most important components of BS. An efficient microbial digestion of cell-wall carbohydrates in the rumen is critical for optimizing the utilization of BS in ruminant feeding (Hofmann, 1989). A meta-analysis of data from 7 experiments with 13 comparisons shows that a ten-unit increase in *in vitro* neutral detergent fiber digestibility (ivNDFD) is associated with a 1.7 kg/d increase in dry matter intake (DMI) and a 2.5 kg/d increase in 4% fat-corrected milk yield (Oba and Allen, 1999b).

The positive effects of feeding forages with enhanced ivNDFD on DMI and milk production have been extensively studied for corn silage, resulting in the development of corn silage hybrids with enhanced ivNDFD (Oba and Allen, 1999b). However, limited studies have investigated the variation in ivNDFD of BS, and no specific study has investigated the effect of feeding BS with enhanced ivNDFD on dairy cattle performance. Recently, many new barley forage varieties have been developed in Western Canada; however, no systematic research has

been conducted on the molecular structure and physicochemical characteristics of these newly developed barley forage varieties.

The corn grown in the Canadian prairies is different from the corn varieties grown in warmer climates such as the United States (Mahanna, 2010). These differences are attributed to some limitations such as lower growth temperature and shorter growing season. Successful growth and grain filling of corn in Canadian prairies depends on the availability of crop heat unit, and corn is considered more suitable to areas receiving a minimum of 2000 to 2100 crop heat units (McCartney et al., 2009). Recently, new corn hybrids, requiring lower crop heat unit for maturation, were introduced in Western Canada (Abeysekara et al., 2013b). To establish these hybrids as a forage crop and optimize their utilization in animal rations, their nutritional value needs to be compared against other conventional forage crops such as BS.

A vibrational infrared biomolecular spectroscopic method, such as Fourier transform infrared vibrational molecular spectroscopy (ATR-FT/IR), has been developed as a rapid, direct, nondestructive, and noninvasive bioanalytical technique of analysis of feed physicochemical characteristics (Yu et al., 2004b; Damiran and Yu, 2011). Intrinsic chemical structures affect nutritive value, ruminal degradation characteristics, and postruminal digestibility and utilization of feed (Yu and Nuez-Ortín, 2010; Abeysekara et al., 2013a). Therefore, quantifying the carbohydrates' (CHO) inherent molecular makeup may be important for understanding the variation in CHO fractions, and digestibility in different types of forages. The current study was conducted to assess the magnitude of differences among BS varieties in comparison with a new corn silage variety as regards to (1) CHO molecular makeup (2) CHO chemical fractions and rumen degradation kinetics of dry matter (DM) and NDF in dairy cows, and (3) to quantify the

association between the molecular structural features of CHO, and chemical profile and digestibility of CHO.

3.3. Materials and Methods

All experimental procedures used in this study were approved by the University of Saskatchewan Animal Care Committee. All cows were housed at the Rayner Dairy Research and Teaching Facility farm, University of Saskatchewan, Saskatoon, Canada, and cared for according to guidelines of the Canadian Council on Animal Care (2009).

3.3.1. Silage Sampling

The barley varieties used in the present study were selected based on the screening study of Nair et al. (2016) on seven varieties of BS using ivNDFD as an approach for ranking the silage. The seven varieties (CDC Cowboy, Falcon, Metcalfe, CDC Copeland, Conlon, Xena, and Legacy) harvested as silage at the mid-dough stage of maturity were collected within two years (2012 and 2013) from dairy and beef operators in the Canadian provinces (Saskatchewan and Alberta). These barley varieties show a significant difference in ivNDFD that ranged from 28% to 37%. Three varieties (CDC Cowboy, CDC Copeland and Xena) with varying ivNDFD were selected for this study. The ivNDFD of CDC Cowboy (Ardell Seeds, Vanscoy, SK), CDC Copeland (Wylie Farms Ltd., Biggar, SK), and Xena (Crop Production Services, Bow Island, AB) were 37%, 31% and 29%, respectively.

One corn forage variety was selected in the current study (P7213R) based on screening study done at the University of Saskatchewan (Abeysekara et al., 2013a; b). The results of this

screening study showed that P7213R has higher nutritive value and a higher NDF degradation rate compared to other forages.

All three barley forages (CDC Cowboy BS, CDC Copeland BS, Xena BS) and corn forage (P7213R) were seeded at the University of Saskatchewan research farm (latitude: 52.2 ° N, longitude: 106.6° W and altitude 491m). All forages were grown on a nonirrigated land at the University of Saskatchewan. Seeding, harvest and ensiling management were as described before (Refat et al., 2018).

Two representative samples from each silage variety were taken at two different dates (expected chemical and nutrient changes within increasing ensiling time), such that each silage was represented by two replicates. Each sample was used for (1) chemical profile analysis (2) undigested NDF (uNDF-288) analysis (3) rumen degradation using *in situ* technique and (4) molecular structure study. For the chemical, uNDF-288, and *in situ* study, the silage samples were oven dried at 55°C for 48 h. The dried samples were ground through a 1 mm screen for chemical and uNDF analyses or through a 3-mm screen for the *in situ* study (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK).

3.3.2. Carbohydrate Fractions

The Cornell Net Carbohydrate and Protein System (CNCPS 6.5) was used to partition CHO into volatile fatty acids (CA1), lactic acid (CA2), other organic acid (CA3), sugars (CA4), starch (CB1), soluble fibre (CB2), NDF (CB3), and indigestible/unavailable fiber (CC) (Higgs et al., 2015). The CC fraction in the CNCPS model was calculated according to the equation of Higgs et al. (2015) : CC = NDF× uNDF%, where uNDF is the undigested NDF after a 288-h *in*

situ incubation (Higgs et al., 2015). The CB3 fraction was calculated as: CB3 = NDF– CC (Higgs et al., 2015).

3.3.3. Energy Values

Energy content of total digestible CP (tdCP), fatty acids (tdFA), NDF (tdNDF), NFC (tdNFC), total digestible nutrients at 1× maintenance ($TDN_{1\times}$), digestible energy (DE) at the production level of intake ($DE_{3\times}$), metabolizable energy (ME) at the production level of intake ($ME_{3\times}$), and net energy for lactation at the production level of intake ($NEL_{3\times}$) were estimated for the tested feeds using a summative approach from NRC (Weiss et al., 1992; NRC, 2001).

3.3.4. In Situ Rumen Degradation Kinetics of DM and NDF

Six lactating Holstein dairy cows fitted with rumen cannula (inner diameter of 10 cm; Bar Diamond, Parma, ID, USA) were used for *in situ* incubation to estimate rumen degradation kinetics. The cows were housed individually in a tie-stall with rubber bedding during the trial. The cows were fed a total mixed ration twice daily at 0800 and 1600. The total mixed ration feed (kg DM feed/d) was formulated with 6.3 kg of BS, 3.4 kg of corn silage, 4.5 kg of chopped alfalfa hay, and 13.8 kg of barley-based concentrate to meet the NRC requirement for lactating dairy cows producing 42 kg of milk (NRC, 2001).

The rumen degradation kinetics of DM and NDF were determined using *in situ* technique as described in detail by Yu et al. (2003). Around seven grams of ground samples (3 mm) were weighed and put in nylon bags (10 cm × 20 cm; Nitex 03-41/31 monofilament open mash fabric, Screentec Corp., Mississauga, ON) with the pore size of 40μm. The rumen incubations were performed based on the ‘‘gradual addition/all out’’ schedule and samples were incubated in the

rumen for 0, 6, 12, 24, 48, and 72 h. At the end of incubation, bags were removed from the rumen and then hand washed along with 0 h bags in detergent-free cold water to rinse off ruminal content, then dried at 55°C for 48 h. The dry residues were pooled based on treatments, animals, and incubation time for chemical analysis (n = 2). The pooled samples were ground through a 1 mm screen using a Retsch ZM 200 rotor mill and then prepared for residual chemical analysis.

Rumen degradation parameters of each component were estimated according to the modified first order kinetics model (Ørskov and McDonald, 1979; Robinson et al., 1986). The results were calculated using the non-linear (NLIN) procedure of SAS and iterative least-squares regression (Gauss Newton method). The model used for measuring rumen degradation kinetics of DM was as follows:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)}$$

where R(t) is the residue at the time of incubation (%), S is the soluble fraction (%), U is the undegradable fraction (%), T0 is lag time (h), and Kd is degradation rate (%/h). Then rumen degradable DM (EDDM) was calculated according to the NRC-2001 using the nonlinear parameters predicted by the above equation (S, U, and Kd) as

$$EDDM = S + D \times (Kd / Kd + Kp)$$

The rumen undegradable DM (RUDM)) was calculated as

$$RUDM = U + D \times (Kp / Kd + Kp)$$

where, Kp is the estimated passage rate from the rumen (%/h). All effective degradability data presented in this study were calculated assuming a rate of passage of 4%/h (Van Vuuren et al., 1993).

The descendent model (the residue decreases as time increases) used for measuring rumen NDF kinetics was:

$$R(t) = U + (100 - U) \times e^{-Kd \times (t - T0)}$$

where R(t) is the residue at the time of incubation (%), U is the undegradable fraction (%), T0 is lag time (h), and Kd is degradation rate (%/h). Then rumen undegradable NDF (RUNDF) was calculated according to the NRC-2001:

$$RUNDF = U + D \times (Kp / Kp + Kd)$$

3.3.5. Study on Carbohydrate Molecular Structures Using ATR-FT/IR Spectroscopy

Fourier transform infrared spectroscopy was used for analysis of the carbohydrate related-molecular structure spectral features. The evaluations were carried out at the molecular spectroscopy laboratory at Department of Animal and Poultry Science, University of Saskatchewan. Dried, fine ground silage samples (0.50 mm) were used to collect spectra related to the molecular structure of carbohydrates. The spectra were generated in the mid-IR region ($4000 - 800\ cm^{-1}$) using JASCO Spectra manager II software (JASCO Corporation, Tokyo, Japan) with a spectral resolution of $4\ cm^{-1}$ and 128 coadded scans. The FT/IR molecular spectrometer was equipped with a ceramic IR light source and a deuterated $_{L}$-alanine doped triglycine sulfate detector (JASCO Corporation, Tokyo, Japan), and fitted with a MIRacle™

attenuated total reflectance (ATR) accessory module as well as a ZnSe crystal and pressure clamp (PIKE Technologies, Madison, WI, USA).

3.3.5.1. Univariate Molecular Spectral Analysis of Carbohydrates Spectral Data

The spectral bands related to CHO structure related functional groups were identified according to published literature (Li et al., 2015, 2016). The carbohydrate related molecular spectral bands include the following: (1) LIG_A (peak area region and baseline, ca. 1525−1487 cm^{-1} mainly associated with ligneous compounds, within which one major peak ca.1512 cm^{-1} (2) STC_A (peak area region and baseline: ca. 1487−1189 cm^{-1}) mainly associated with hemi- and cellulosic compounds and within which three major peaks ca. 1416, 1372, and 1320 cm^{-1}; (3) CEC_A (peak area region and baseline: ca. 1292−1189 cm^{-1}) mainly associated with cellulosic compounds and within this region one major peak ca. 1242 cm^{-1}; (4) TC_A (peak region and baseline: ca. 1189−909 cm^{-1}) mainly associated with total CHO and within which three peaks ca. 1152, 1100, and 1030 cm^{-1}; (5) NSTC_A (peaks area region and baseline: ca. 909−880 cm^{-1}) mainly associated with nonstructural CHO and within this region one major peak ca. 898 cm^{-1}.

3.3.5.2. Multivariate Molecular Spectral Analysis of Carbohydrates Spectral Data

Two widely used methods were employed to perform multivariate spectral analysis on the overall spectral data on ligneous compounds (ca. 1525−1487 cm^{-1}), cellulosic compounds (ca. 1292−1189 cm^{-1}), structural CHO (ca. 1487- 1189 cm^{-1}), total CHO (ca. 1189−909 cm^{-1}) and nonstructural CHO (ca. 909−880 cm^{-1}) functional groups using Statistical 8.0 (StatSoft Inc., Tulsa, OK, USA). The Agglomerative Hierarchical Cluster Analysis (CLA), uses Ward's Algorithm method without parametrization for clustering of closely correlated data and presents results as dendrograms (Yu, 2005). The Principal Component Analysis (PCA) transforms all

interrelated variances into new uncorrelated variances called principal components (PCs) (Yu, 2005). The results of PCA are presented as a scatter plot between the first two main PCs (PC1 vs. PC2), based on loading scores. The first PC would account for >95% of remaining observed variance.

3.3.6. Chemical Analysis

Dry matter (method 930.15), ash (method 942.05), and crude fat (method 2003.05) were analyzed according to AOAC (2000). For estimation of CP (N × 6.25), N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI). The methods described in Van Soest et al. (1991) and AOAC **(**method 2002.04**)** were used to determine the contents of NDF. This detergent solution was combined with heat-stable α-amylase before neutral detergent extraction. Acid detergent fiber (ADF; method 973.18) and acid detergent lignin (ADL; method 973.18) were analyzed according to AOAC (Van Soest et al., 1991). These NDF, ADF and ADL methods were adapted for an ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, USA). Ethanol soluble carbohydrate and starch were determined using the methods described by Hall et al. (1999). The uNDF-288 content of each feed sample was determined following *in situ* incubations for 288 h in the rumen using two lactating dairy cows, using two consecutive runs as replicate (Ahvenjärvi et al., 2000). Around 3 g of ground sample (1 mm) was weighed in triplicate into 5 × 10 cm size custom-made *in situ* bags (6 µm pore size, part no. 07 -6/5, Sefar America Inc., Depew, NY) and were randomly assigned to the cows. After removal from the rumen, the bags were rinsed with cold water and oven dried at 55ºC for 48 h. Subsequently, the residues were analyzed for NDF content.

3.3.7. Statistical Analysis

Statistical analyses were performed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). Chemical profile was analyzed using completely randomized design. The model used for analyzing this design was as follows: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is an observation of the dependent variable; μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, e_{ij} is the random error associated with the observation ij.

In situ degradability data (DM and NDF) were analyzed using a randomized complete block design. The model used for this design was as follow: $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y_{ijk} is an observation of the dependent variable ij; μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, B_j is a block effect with *in situ* animals, as a random effect, and e_{ijk} is the random error associated with the observation ij.

The ATR-FT/IR spectroscopic data were analyzed using a completely randomized design model with subsampling (spectra reading; n= 5 scans): $Y_{ij} = \mu + T_i + S(T_i) + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij (chemical functional groups such as LIG_A); μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, S is the subsample (5 scan) which nested within each treatment, e_{ij} is the random error associated with the observation ij. The significance was declared at $P < 0.05$, and trends at $P \leq 0.10$. Differences among the treatments were evaluated using Tukey's multiple comparison test.

Correlations between the structural spectral data (n = 8) of silage and *in situ* rumen degradation, CHO chemical profile and CHO fraction partitioned by the updated CNCPS model were analyzed using the CORR procedure of SAS software (SAS Institute, Cary, NC) with a parametric correlation method (Pearson) or nonparametric correlation method (Spearman),

depending on data normality. Normality tests were performed using the UNIVARIATE procedure of SAS with Normal and PLOT options.

Multiple regression analysis (with model variable selection) was performed to select the best spectral parameters that explain the variation in chemical profile, CHO subfraction and degradability using the PROC REG procedure of SAS with a reversed stepwise option. The following model was used for the multiple regression: model Y= spectral parameter 1 + spectral parameter 2 + spectral parameter 3 + spectral parameter 4 + ... + error. The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". All variables left in the final prediction models were significant at the 0.05 level. Residual analysis was performed using the Univariate procedure of SAS with Normal and Plot options.

3.4. Results

3.4.1. Chemical and Nutrient Profile

All three barley forages had similar ($P > 0.10$) starch, NDF, and ADF, with mean values of 12%, 46%, and 35% DM, respectively (Table 3.1). Starch content of corn silage was higher ($P < 0.05$; 26.4% vs 8.7–15.3% DM), while those of NDF (38.6 vs 43.1–49.2% DM) and ADF (26.3 vs 34.0–36.0% DM) were lower ($P < 0.05$) in corn silage when compared with all BS. Lignin content was higher ($P < 0.05$) in Copeland (4.9% DM) and Cowboy (5.2% DM) BS, intermediate in Xena BS (4.5% DM), and lower ($P < 0.05$) in corn silage (2.9% DM). Indigestible fiber fraction expressed as %NDF was higher ($P = 0.05$) in Cowboy BS (46.2%), intermediate in Copeland BS (42.8%), and lower ($P = 0.05$) in Xena BS (34.7%) and corn silage (34.7% NDF).

Table 3.1. Chemical profiles and indigestible NDF contents for corn and barley silages

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Basic chemical profile						
DM, %	33.8*a*	27.5*b*	31.0*ab*	30.5*ab*	0.60	0.01
Ash, %DM	5.7*b*	8.4*a*	7.4*ab*	8.2*a*	0.39	0.03
Ether extract, %DM	2.7	2.9	2.8	2.6	0.10	0.32
Carbohydrates and fiber chemical profile, %DM [2]						
NFC	44.0*a*	27.8*c*	35.4*b*	32.9*bc*	1.30	0.01
NSC	27.5*a*	11.1*c*	15.8*bc*	17.2*b*	0.99	0.01
Starch	26.4*a*	8.7*b*	13.3*b*	15.3*b*	1.35	<0.01
Sugar	1.1	2.5	2.6	2.0	0.47	0.25
NDF	38.6*b*	49.2*a*	43.1*ab*	45.6*ab*	1.33	0.02
ADF	26.3*b*	36.0*a*	34.0*a*	34.2*a*	1.26	0.02
Hemicellulose	12.3*ab*	13.3*a*	9.2*b*	11.5*ab*	0.58	0.02
Cellulose	23.4*b*	30.7*a*	29.1*ab*	29.7*ab*	1.13	0.03
Lignin and indigestible NDF contents[3]						
ADL, %DM	2.9*b*	5.2*a*	4.9*a*	4.5*a*	0.15	<0.01
ADL, %NDF	7.4*c*	10.6*a*	11.3*a*	9.8*b*	0.21	<0.01
uNDF, %DM	13.4*c*	22.7*a*	18.5*b*	15.8*bc*	0.68	<0.01
uNDF, %NDF	34.7*b*	46.2*a*	42.8*ab*	34.7	2.33	0.05

[1]SEM= standard error of mean; means with different letters in the same row differ ($P < 0.05$).
[2] NFC non-fiber carbohydrate = 100 – (NDF– NDICP) – EE – CP – Ash; NSC = Nonstructural carbohydrates; NSC = starch + ethanol soluble sugar; NDF = neutral detergent fiber; ADF = acid detergent fiber; hemicellulose = NDF – ADF; Cellulose = ADF – ADL.
[3]ADL= acid detergent lignin; uNDF = undigested NDF (288 h in situ incubation).

3.4.2. The CNCPS Carbohydrate Fractions

Table 3.2 shows the carbohydrate subfractions of BS compared with corn silage. There were no differences ($P > 0.10$) in the contents (% CHO) of CB2 (soluble fiber) and CB3 (available NDF) among the tested silages (Table 3.2). However, corn silage had higher ($P < 0.05$) content of CB1 (31.9 vs 11.2–19.5% CHO) and lower content of CC (16.8 vs 20.8–30.1%

CHO) as compared to silages from the three barley varieties. Moreover, significant differences were detected among barley silages for CC with Xena having lower (20% CHO), CDC Copland intermediate (22% CHO), and CDC Cowboy higher (30% CHO) CC contents.

3.4.3. Digestible Nutrients and Energy Values

Cowboy BS tended to have a higher tdNDF ($P = 0.06$; Table 3.2) than Copeland BS and corn silage (26% vs 22% DM). The tdNFC was higher ($P < 0.05$) in corn silage as compared to the three BS varieties (44.2% vs 28.6–36% DM). Corn silage had the greatest $TDN_{1\times}$ (71.0% DM) and Cowboy BS had the lowest level (62% DM), whereas Copeland and Xena BS had intermediate $TDN_{1\times}$ values (averaged 64% DM).

The energy estimated $DE_{3\times}$, $ME_{p3\times}$, and $NEL_{p3\times}$ contents were higher in corn silage (2.8, 2.4, and 1.5 Mcal/kg of DM, respectively), intermediate in Copeland BS (2.6, 2.2, and 1.4 Mcal/kg of DM, respectively), and lower in Xena and Cowboy (2.5, 2.1, and 1.3 Mcal/kg of DM, respectively) BS (Table 3.2).

Table 3.2. Carbohydrates fraction and energy values: Comparison of barley varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
The CNCPS carbohydrate fractions, %CHO[2]						
CB1	31.9*a*	11.2*b*	16.9*b*	19.5*b*	1.82	<0.01
CB2	9.9	6.9	13.1	10.1	1.63	0.20
CB3	29.9	33.8	32.5	37.3	1.97	0.21
CC	16.8*c*	30.1*a*	22.4*b*	20.8*bc*	0.70	0.01
Truly digestible nutrients and total digestible nutrient, %DM[3]						
tdNDF	22.1	25.6	22.0	24.3	0.74	0.06
tdCP	8.0*b*	10.5*a*	9.9*ab*	9.3*ab*	0.41	0.05
tdNFC	44.2*a*	28.6*c*	36.0*b*	33.5*bc*	1.16	0.01
tdFA	1.7	1.9	1.8	1.6	0.10	0.32
TDN_{1x}	71.0*a*	61.9*c*	65.0*b*	63.7*bc*	0.40	<0.01
Predicted energy values, Mcal/kg DM (NRC-2001 dairy)[4]						
DE_{1x}	3.1*a*	2.7*c*	2.9*b*	2.8*bc*	0.02	<0.01
DE_{p3x}	2.8*a*	2.5*d*	2.6*b*	2.6*c*	0.02	<0.01
ME_{p3x}	2.4*a*	2.1*c*	2.2*b*	2.1*c*	0.02	<0.01
NEL_{p3x}	1.5*a*	1.3*c*	1.4*b*	1.3*bc*	0.01	<0.01

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]CNCPS = Cornell Net Carbohydrate and Protein System; CB1 = starches; CB2 = soluble fibers; CB3 = available NDF; CC = unavailable cell walls.
[3]tdNFC = truly digestible non-fiber carbohydrates; tdCP = total digestible crude protein; tdNDF = total digestible neutral detergent fiber; tdFA = total digestible fatty acid; TDN_{1x} = total digestible nutrients at maintenance level (1x).
[4]DE_{1x} = digestible energy; DEp_{3x} = digestible energy at a production level (3x maintenance); MEp_{3x} = metabolizable energy at a production level (3x maintenance); $NELp_{3x}$ = net energy at a production level (3x maintenance).

3.4.4. Rumen Degradation Kinetics of Nutrients in Barley and Corn Silages

The results showed there were no significant differences in degradation rates of DM and lag time (T0) among all silages ($P > 0.10$; Table 3.3). The S-fraction of DM was significantly different among all silages ($P < 0.05$), being higher in Xena BS (34%), intermediate in Copeland BS (29%), and lower in Cowboy BS (22.8%) and corn silage (23%). On the other hand, corn silage had the highest value potentially rumen degradable D-fraction (51.7% DM), and lower undegradable U-fraction (25.4%). There were no significant differences among BS varieties in D-fraction of DM, ranging from 38.9% to 41.1% DM. However, the U fraction varied significantly among BS, ranging from 37% DM (Cowboy BS) to 27% DM (Xena BS). Among the silages, Xena BS had greater ($P < 0.05$) EDDM (50.2% DM) and Cowboy BS had smaller (43.1% DM), while corn silage (48.0% DM) and Copeland BS (45.2% DM) had intermediate EDDM.

There were no significant differences in degradation rates of NDF between all silages (Kd, 3.2%/h; $P > 0.10$; Table 3.3). The lag time was significantly ($P < 0.05$) longer in corn silage (9.3 h), intermediate in Xena BS (6 h), and shorter in Copeland (2.8 h) and Cowboy (3.9 h) BS. The undegradable fraction of NDF was significantly lower in Xena BS (26%), intermediate in Copeland BS (36%), and higher in Cowboy BS (44%). The lower ($P < 0.05$) effective degradation of NDF was observed in Copeland BS (22.7% NDF) and greater in Xena BS (35.9% NDF).

Table 3.3. In situ rumen degradation kinetics of DM and NDF: Comparison of barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
In situ rumen DM degradation[2]						
Kd, %/h	4.22	4.41	3.23	2.91	0.882	0.44
T0, h	1.7	1.4	2.1	4.6	1.05	0.20
S, %	23.0*c*	22.8*c*	29.2*b*	33.8*a*	0.36	<0.01
D, %	51.7*a*	39.9*ab*	41.1*ab*	38.9*b*	3.58	0.05
U, %	25.4*b*	37.4*a*	29.8*ab*	27.3*ab*	3.48	0.01
EDDM, %	48.0*ab*	43.1*b*	45.2*ab*	50.2*a*	1.25	<0.01
RUDM, %	52.0*ab*	56.9*a*	54.8*ab*	49.8*b*	1.25	<0.01
In situ rumen NDF degradation[3]						
Kd, %/h	3.73	4.22	2.41	2.72	0.782	0.18
T0, h	9.3*a*	3.9*b*	2.8*b*	6.2*ab*	0.92	0.01
D, %	67.4*ab*	55.9*b*	63.6*ab*	74.5*a*	4.98	0.03
U, %	32.6*ab*	44.1*a*	36.4*ab*	25.5*b*	4.98	0.03
RUNDF, %	67.8*ab*	71.2*ab*	77.4*a*	64.1*b*	2.22	0.01
EDNDF, %	32.2*ab*	28.8*ab*	22.7*b*	35.9*a*	2.18	0.01

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]Kd = the rate of degradation of D fraction (%/h); T0 = lag time; S = washable fraction; U = undegradable degradable fractions; D = degradable fractions; RUDM = rumen bypass or undegraded feed DM; EDDM = effective degradability of DM.
[3]RUNDF = rumen bypass or undegraded feed neutral detergent fiber; EDNDF = effective degradability of neutral detergent fiber

Table 3.4. Inherent molecular structural characteristics revealed by Fourier transform/infrared-attenuated total reflectance (ATR-FT/IR) vibrational molecular spectroscopy: Comparison of barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Ligneous compounds, absorbance unit (AU)[2]						
LIG_A	0.79	0.74	0.33	0.67	0.164	0.52
H_ 1512	0.032	0.032	0.024	0.034	0.0064	0.73
Structural CHO related spectral profile, AU[3]						
STC_A	22.14	20.15	17.48	18.39	1.113	0.13
H_1416	0.102*b*	0.108*ab*	0.100*b*	0.113*a*	0.0032	<0.01
H_1372	0.111*a*	0.106*ab*	0.094b	0.101*ab*	0.0031	0.01
H_1320	0.087	0.082	0.069	0.068	0.0059	0.22
Cellulosic compounds related spectral profile, AU[4]						
CEC_A	4.29	3.73	2.96	3.04	0.507	0.33
H_1242	0.085	0.072	0.059	0.061	0.0084	0.27
Total CHO related spectral profiles, AU[5]						
TC_A	81.80	82.55	81.70	79.50	2.313	0.87
H_1152	0.170	0.151	0.152	0.151	0.0077	0.36
H_1100	0.298	0.334	0.311	0.301	0.0111	0.25
H_1030	0.673	0.683	0.677	0.641	0.0193	0.50
Non-structural CHO related spectral profile, AU[6]						
NSTC_A	0.20*b*	0.29*a*	0.33*a*	0.27*a*	0.018	0.01
H_897	0.017*b*	0.022*a*	0.024*a*	0.022*a*	0.0012	0.01
Spectral ratio profile						
STC_A/TC_A	0.281	0.245	0.214	0.239	0.0153	0.14
CEC_A/TC_A	0.054	0.045	0.036	0.039	0.0061	0.30
CEC_A/STC_A	0.194	0.188	0.168	0.166	0.0079	0.60
LIG_A/STC_A	0.035	0.037	0.018	0.037	0.0112	0.60
LIG_A/TC_A	0.010	0.009	0.004	0.009	0.0031	0.56

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]LIG_A (peaks area region and baseline: ca. 1525- 1487 cm^{-1}) mainly associated with ligneous compound, within this region one major peak ca. 1512 cm^{-1}
[3]STC_A (peaks area region and baseline: ca. 1487- 1189 cm^{-1}) mainly associated with hemi- cellulosic compounds and within this region three major peaks ca. 1416, 1372, 1320 cm^{-1}
[4]CEC_A (peaks area region and baseline: ca. 1292-1189 cm^{-1}) mainly associated with cellulosic compounds and within this region one major peak ca. 1242 cm^{-1};
[5]TC_A (peaks region and baseline: ca. 1189-909 cm^{-1}) mainly associated with total CHO and within this region three peak heights ca. 1152, 1100, and 1030 cm^{-1}.
[6]NSTC_A (peaks area region and baseline: ca. 909- 880 cm^{-1}) mainly associated with non-structural CHO and within this region one major peak ca. 898 cm^{-1}.

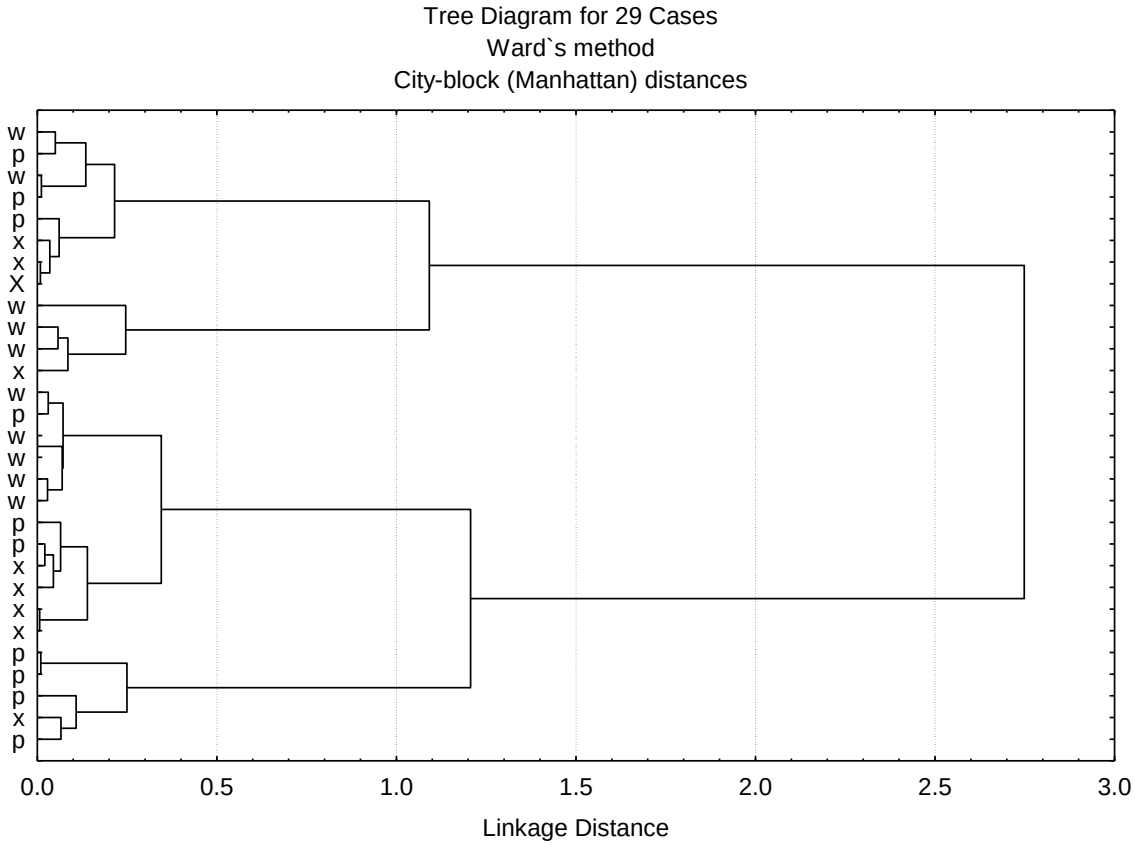
3.4.5. Carbohydrate Molecular Spectroscopic Features of Barley and Corn Silage with Different Digestible Carbohydrates Content

Peak height and area values of ligneous compounds in all forages were not significantly different ($P > 0.10$; Table 3.4). Also, all forages had similar ($P > 0.10$) structural carbohydrates peak area (STC_A; averaged 20 absorbance unit; AU). There were three spectral peaks within the STC_A ca. 1416, 1372, and 1320 cm^{-1}. Xena BS had higher ($P < 0.05$) peak height at 1416 cm^{-1} than corn silage and Copeland BS, while corn silage had higher ($P < 0.05$) peak height at 1372 cm^{-1} than Copeland BS. The CEC peak area and height, and total CHO peak area intensities were not different ($P > 0.10$) among barley and corn silages. Also, the absorbed intensities of CHO peaks at 1152, 1100, and 1030 cm^{-1} were not different among the silages ($P > 0.10$). The non-structural CHO peak area (NSTC_A), peak height H_898, and NSTC spectral ratios were significantly lower ($P < 0.05$) in corn silage compared with all BS varieties.

3.4.6. Multivariate Analysis of Carbohydrate Molecular Spectral Profiles Related to Barley and Corn Silages

Results of multivariate molecular spectral analysis related to fingerprint regions: (1) ligneous compounds fingerprint region, ca. 1525–1487cm^{-1}; (2) structural carbohydrate fingerprint region, ca. 1487–1189 cm^{-1}; (3) total carbohydrates fingerprint region, ca. 1189–909 cm^{-1}; and (4) non-structural carbohydrates fingerprint region, ca. 909–880 cm^{-1} are displayed in Figure 3.1 to 3.4. Results from CLA indicated no cluster classes of these regions could be discriminated from each other. For the PCA analysis, the first principal component explained 96.5%, 97.5%, 98.3, and 99.8% of the variation, respectively.

(a)


Tree Diagram for 29 Cases
Ward`s method
City-block (Manhattan) distances
w
p
w
p
p
x
x
X
w
w
w
x
w
p
w
w
w
w
p
p
x
x
x
x
p
p
p
x
p
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Linkage Distance

(b)

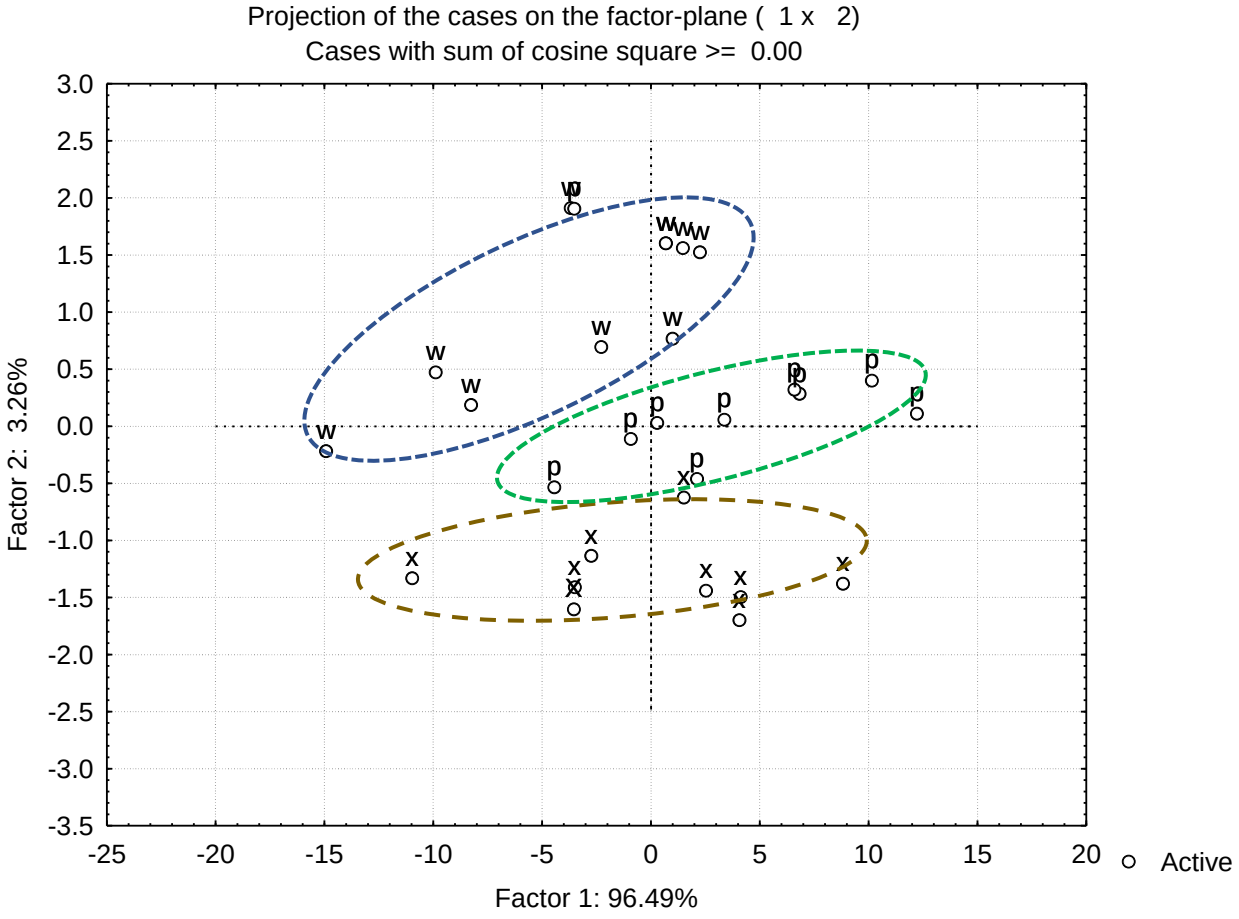
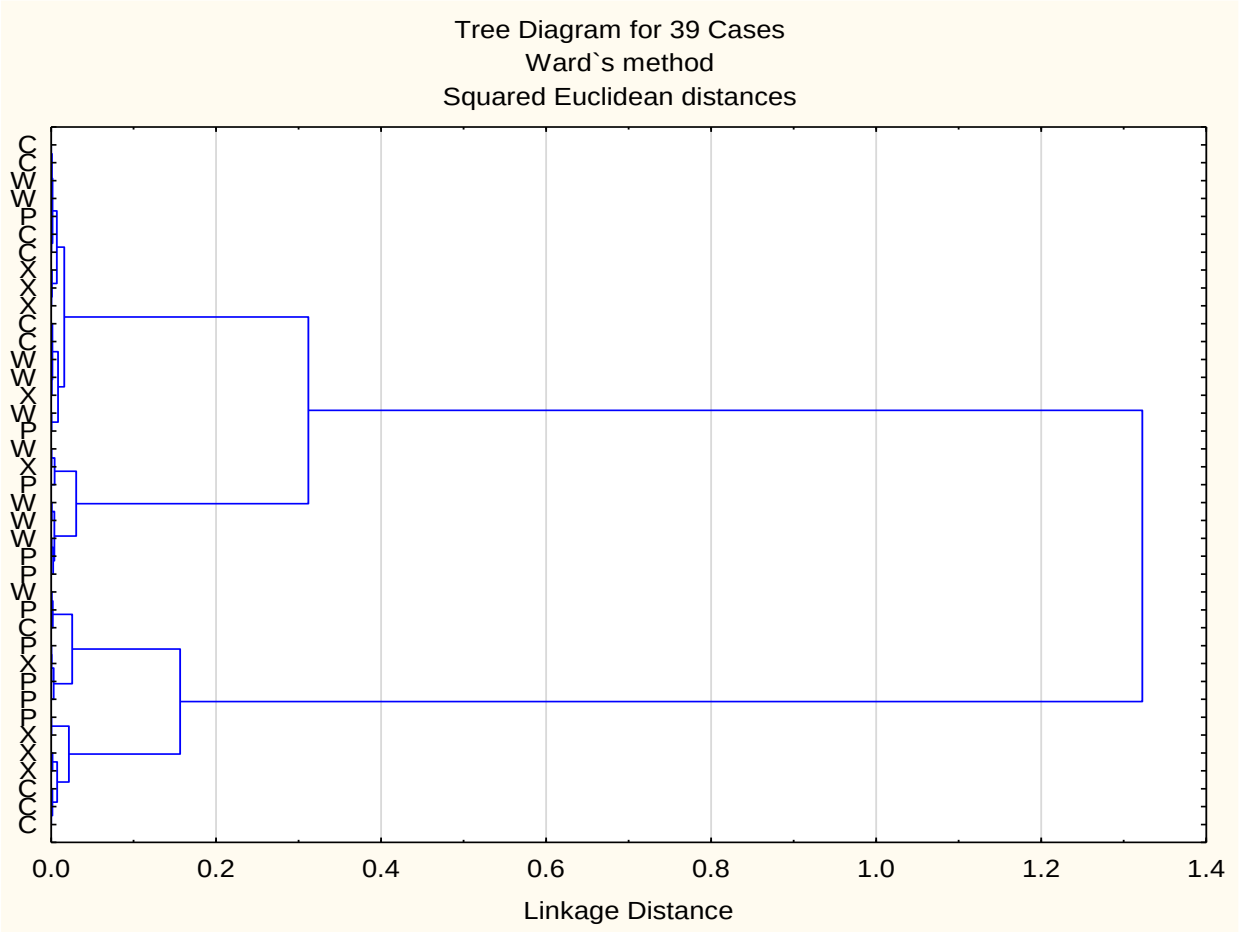


Figure 3.1. Multivariate spectral analyses for different barley silages: (a) Cluster analysis. Select spectral region: Ligneous region (ca. 1525- 1487 cm^{-1}). Cluster method: Ward's algorithm; (b) Principal component analysis. Scatter plots of the first principal components (PC1) vs the second principal component (PC2).

(a)


Tree Diagram for 39 Cases
Ward`s method
Squared Euclidean distances
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Linkage Distance

(b)

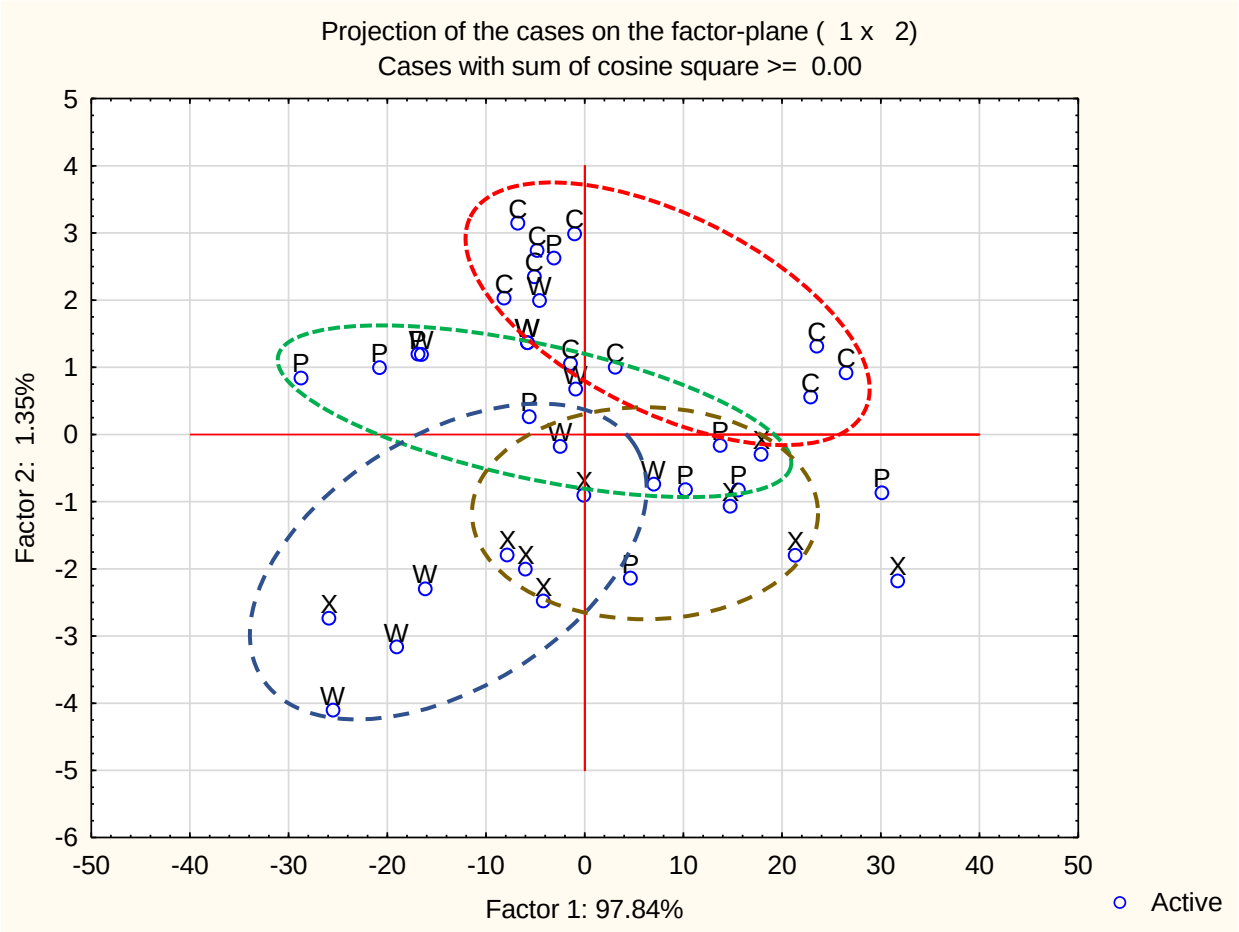
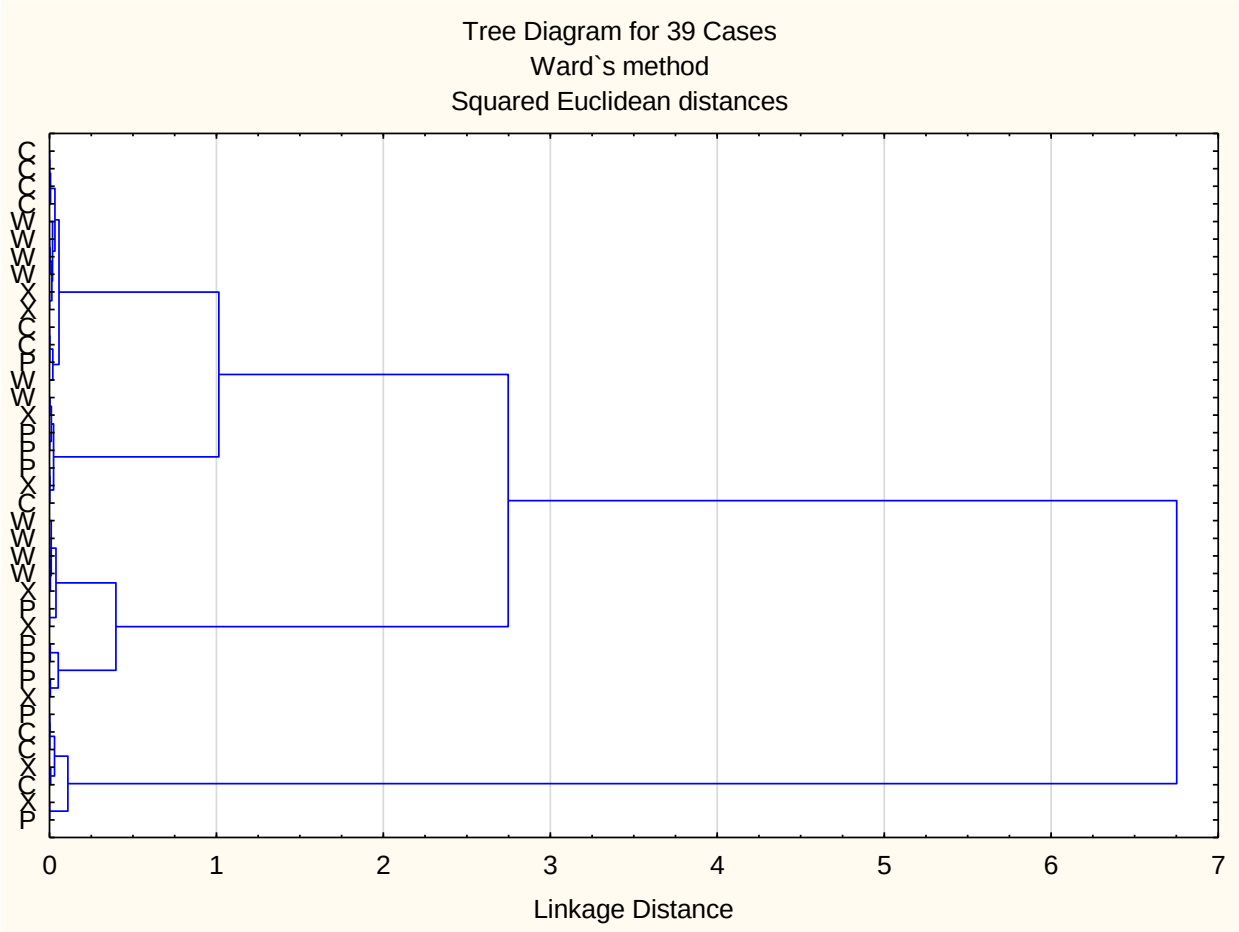


Figure 3.2. Multivariate spectral analyses of different forages: comparison between corn and barley silages: (a) Cluster analysis. Select spectral region: structural CHO region (ca. 1487- 1189 cm^{-1}). Cluster method: Ward's algorithm; (b) Principal component analysis. Scatter plots of the first principal components (PC1) vs the second principal component (PC2).

(a)


Tree Diagram for 39 Cases
Ward`s method
Squared Euclidean distances
0
1
2
3
4
5
6
7
Linkage Distance

(b)

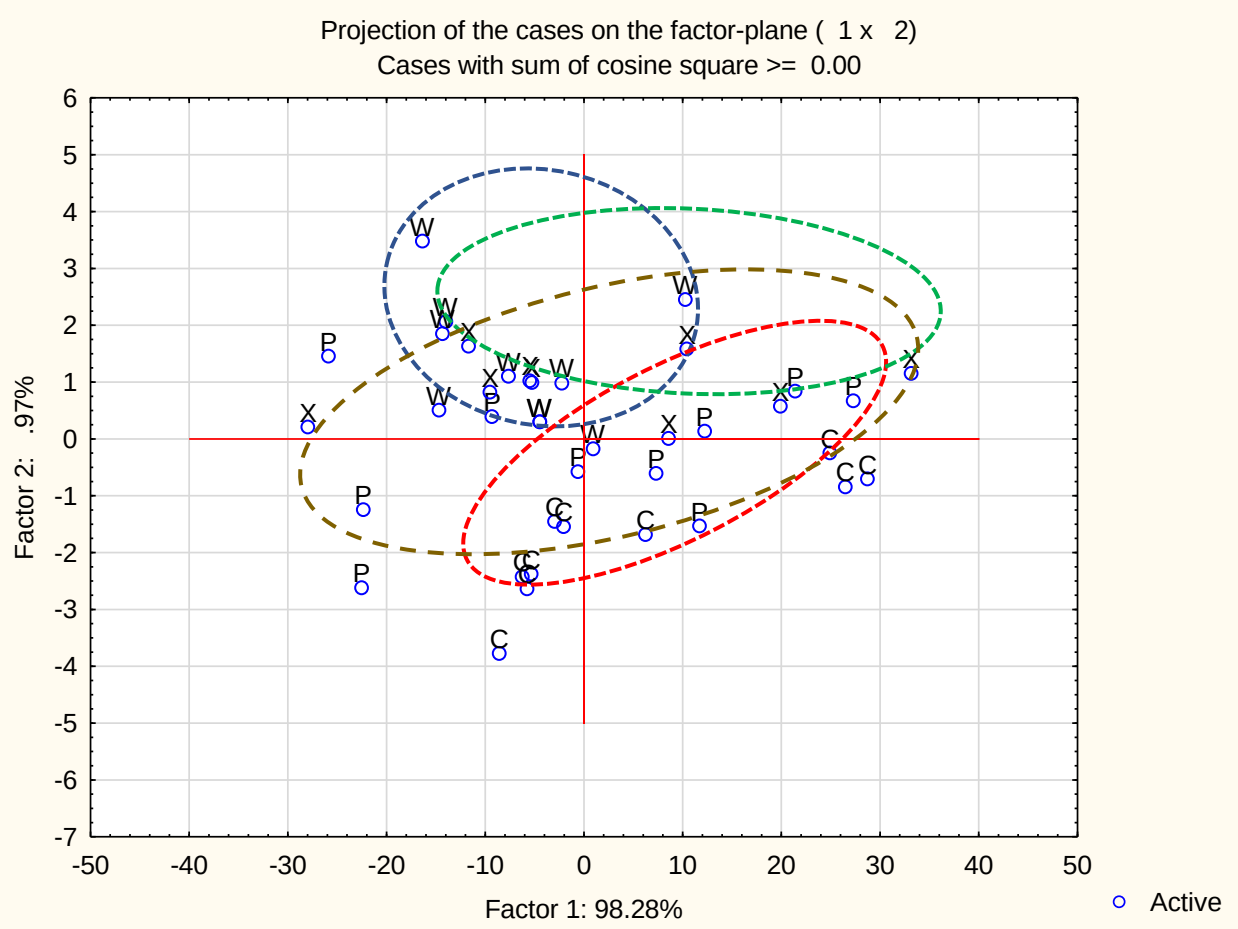
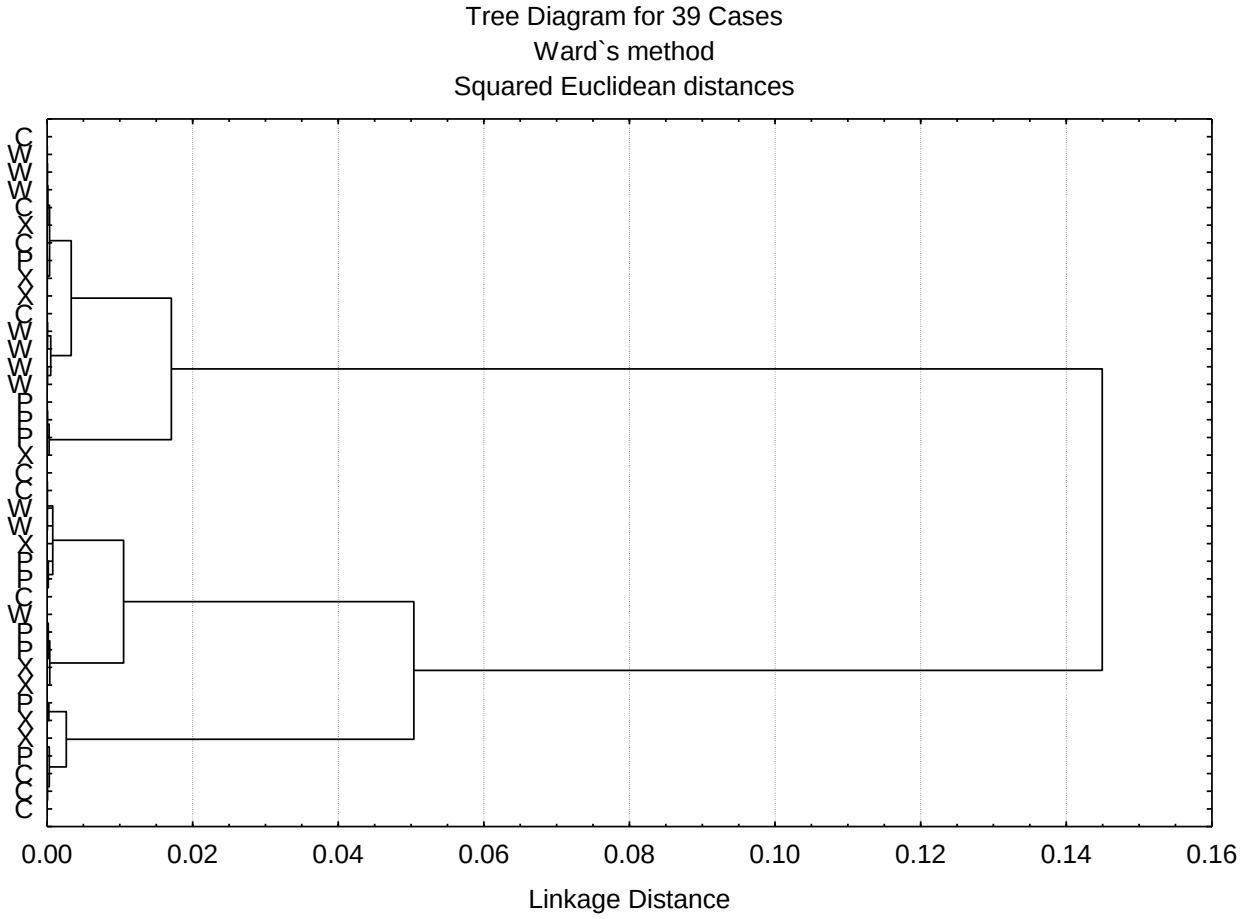


Figure 3.3. Multivariate spectral analyses of different forages: comparison between corn and barley silages: (a) Cluster analysis. Select spectral region: total CHO region (ca. 1189-909 cm^{-1}). Cluster method: Ward's algorithm; (b) Principal component analysis. Scatter plots of the first principal components (PC1) vs the second principal component (PC2).

(a)


Tree Diagram for 39 Cases
Ward`s method
Squared Euclidean distances
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
Linkage Distance

(b)

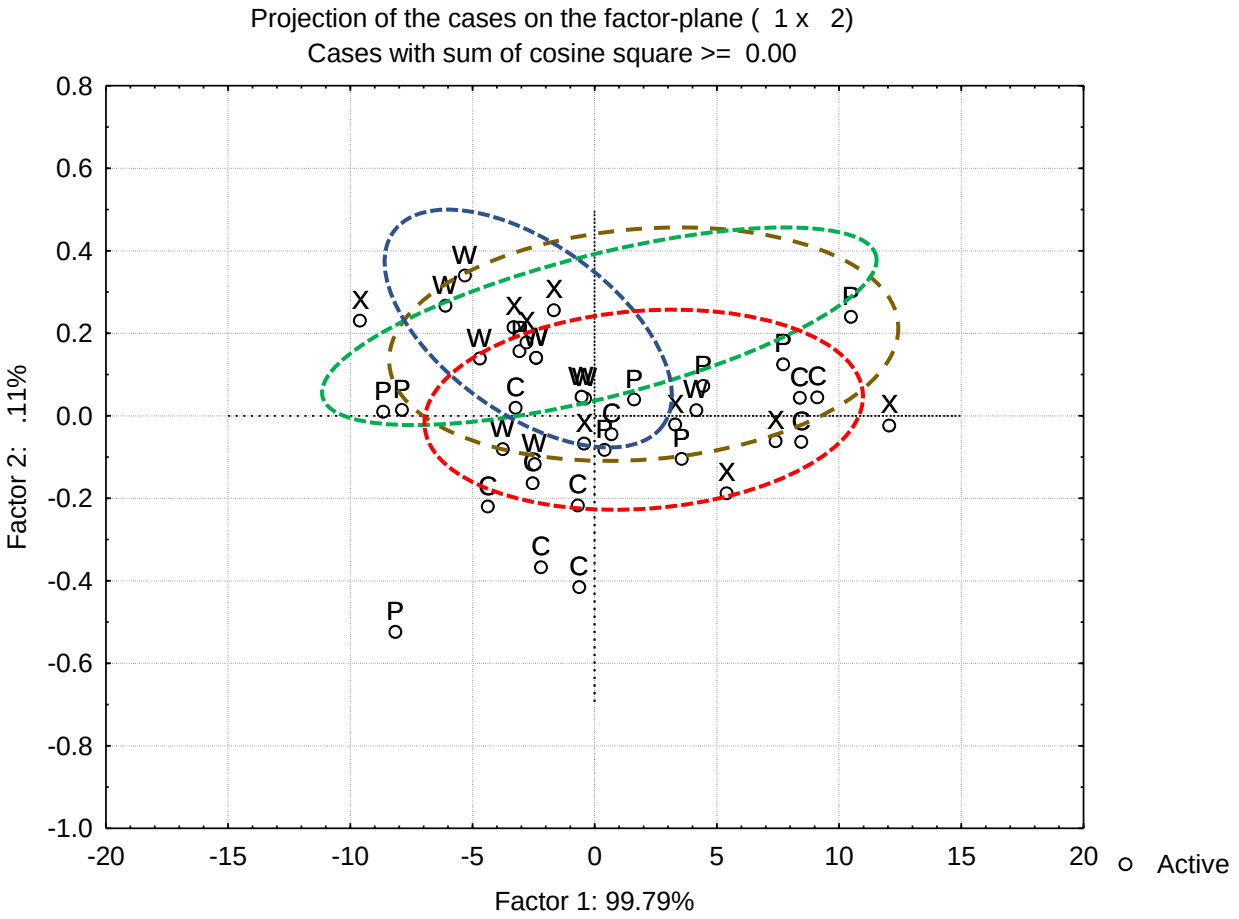


Figure 3.4. Multivariate spectral analyses of different forages: comparison between corn and barley silages: (a) Cluster analysis. Select spectral region: non- structural CHO region (ca. 1909-880 cm^{-1}). Cluster method: Ward's algorithm; (b) Principal component analysis. Scatter plots of the first principal components (PC1) vs the second principal component (PC2).

The principal components (PCs) for all forages (corn and barley silages) overlapped in the STC, TC, and NSTC regions. For the ligneous region, three separated classes related to each BS were distinguished (Figure 3.1b).

3.4.7. Carbohydrate Molecular Structural Profiles in Relation to Digestible Carbohydrate Contents

Spectral intensity of NSTC_A had a negative correlation with starch ($r = -0.76$, $P = 0.03$; Table 3.5), and NSC ($r = -0.76$, $P = 0.03$) content and a positive correlation with ADL ($r = 0.81$, $P = 0.01$) content. Spectra area intensity of STC had a negative correlation with ADF content ($r = -0.72$, $P = 0.05$). There were no significant correlations ($P > 0.05$) between carbohydrate molecular structural profiles and uNDF-288. For CNCPS carbohydrates fractions, only NSTC_A was positively correlated with CC fraction ($r = 0.86$, $P = 0.01$) and negatively correlated with CB1 fraction ($r = -0.74$, $P = 0.01$). For energy values, TDN_{1x} ($r = -0.75$, $P = 0.03$) and NE_{Lp3x} ($r = -0.72$, $P = 0.04$) were negatively correlated with amide NSTC_A. Ligneous compounds spectral intensity was positively related to rate ($r = 0.82$, $P = 0.01$) and extent ($r = 0.79$, $P = 0.02$) of NDF degradation in the rumen.

Table 3.6 presents multiple regression equations and their power to estimate CHO chemical profile, CNCPS CHO fractions, energy values and *in situ* NDF degradation parameters. The equations show that NDF content (% DM) can be predicted ($R^2 = 0.79$) from the peaks height at ca. 1030 and 1100 cm^{-1} of total CHO. Moreover, the peak height of total CHO at ca. 1100 cm^{-1} can predict uNDF-288 with reasonably good accuracy ($R^2 = 0.56$). The equations show that CHO spectral intensities can predict CNCPS fraction CB1 ($R^2 = 0.96$), CB3 ($R^2 = 0.80$) and CC ($R^2 = 0.92$). The peak area ratio of LIG_A/STC_A and peak area intensities

LIG_A, NSTC_A, STC_A, H-1100 were the best parameters to predict CNCPS fractions in all studied forages (Table 3.6). Moreover, the contents of TDN_{1x} (R^2 = 0.56) and NE_{Lp3x} (R^2 = 0.52) can be predicted from NSTC_A. The peak area intensities, LIG_A and LIG_A/STC_A ratio were the best estimator of the rate (R^2 = 0.66) and extent (R^2 = 0.68) of NDF degradation.

3.5. Discussion

The results of the current study showed that starch concentration was significantly higher in corn silage compared to all BS varieties. There were also significant differences among BS varieties in starch concentration, where Cowboy had a lower content compared to Xena BS. Moreover, BS contained 19% more NDF and 32% more ADF compared with corn silage. In agreement with our findings, a previous study reported higher starch and a lower NDF concentrations in corn silage as compared with BS (Benchaar et al., 2014). Higher concentration of starch and lower concentration of NDF in corn silage would result in higher passage rates of feed and higher propionate proportion associated with increased DMI in dairy cows (Oba and Allen, 2000). The lignin (ADL %NDF) and uNDF (%NDF) contents were lower in Xena BS and corn silage, compared to Cowboy BS. The uNDF-288 has been reported to provide a more accurate estimation of the concentration of digestible CHO, hence predicting energy supply and DMI compared with the traditional method lignin/NDF × 2.4 (Krizsan et al., 2013; Lopes et al., 2015).

Table 3.5. Correlation between carbohydrate molecular structural spectral features and carbohydrate nutrient profiles in combined feed of barley and corn silages

Items		LIG_A[1]	STC_A	CEC_A	TC_A	NSTC_A
Structural carbohydrate and non-structural carbohydrates						
Starch % DM	[2]r	0.13	0.52	0.45	-0.15	-0.76
	P value	0.76	0.19	0.26	0.72	0.03
NSC % DM	R	0.15	0.51	0.45	-0.17	-0.76
	P value	0.72	0.19	0.26	0.68	0.03
NDF % DM	R	0.00	-0.50	-0.42	0.23	0.59
	P value	1.00	0.21	0.30	0.59	0.12
ADF % DM	R	-0.22	-0.72	-0.65	0.20	0.82
	P value	0.60	0.05	0.08	0.63	0.01
ADL % DM	R	-0.25	0.48	0.41	-0.41	0.81
	P value	0.54	0.23	0.31	0.31	0.01
uNDF, % NDF	R	-0.21	-0.21	-0.13	0.49	0.64
	P value	0.61	0.62	0.75	0.22	0.09
CNCPS carbohydrates subfractions, %CHO						
CB1	R	0.10	0.50	0.44	-0.14	-0.74
	P value	0.81	0.20	0.28	0.75	0.04
CB3	R	0.06	-0.33	-0.25	0.24	0.41
	P value	0.89	0.42	0.54	0.57	0.31
CC	R	-0.28	-0.64	-0.57	0.29	0.86
	P value	0.51	0.09	0.14	0.49	0.01
Estimated energy profiles						
TDN_{1x}, %DM	R	0.18	0.60	0.53	-0.23	-0.75
	P value	0.67	0.12	0.18	0.59	0.03
MEp_{3x}, Mcal/kg	R	0.13	0.60	0.54	-0.19	-0.71
	P value	0.76	0.11	0.17	0.65	0.05
NEL_{p3x}, Mcal/kg	R	0.13	0.62	0.56	-0.18	-0.72
	P value	0.77	0.10	0.15	0.68	0.04
In situ rumen degradation kinetics of NDF						
Kd, %/h	R	0.82	0.42	0.36	-0.52	-0.50
	P value	0.01	0.31	0.38	0.18	0.21
EDNDF, %	R	0.79	0.12	0.06	-0.71	-0.64
	P value	0.02	0.78	0.90	0.05	0.09

[1]Lig_A (peaks area region and baseline: ca. 1525- 1487 cm^{-1}) mainly associated with ligneous compound; STC_A (peaks area region and baseline: ca. 1487- 1189 cm^{-1}) mainly associated with hemi- cellulosic compounds; CEC_A (peaks area region and baseline: ca. 1292-1189 cm^{-1}) mainly associated with cellulosic compounds; TC_A (peaks region and baseline: ca. 1189-909 cm^{-1}) mainly associated with total CHO; NSTC_A (peaks area region and baseline: ca. 909- 880 cm^{-1}) mainly associated with non-structural CHO.
[2]r: correlation coefficient.

Table 3.6. Multiple regression analysis to choose the most important CHO spectral parameters to predict CHO nutrient supply

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Prediction eq. (test model: $Y = a + b_1 \times_1 + b_2 \times_2 + ..$)	Model R^2	RSD*	*P* value
Carbohydrates chemical profile					
NDF, % DM	H_1030, H_1100	Y = 50.7 – 112.0 × H_1030 + 220.2 × H_1100	0.79	2.36	0.02
ADL, % DM	LIG_A, NSTC_A	Y = -3.4 + 2.1 × LIG_A + 23.6 × NSTC_A	0.95	0.26	< 0.01
uNDF, %NDF	H_1100	Y = -24.1 +133.2 × H_1100	0.56	2.69	0.03
CNCPS CHO fractions, %CHO					
CB1	LIG_A, NSTC_A, CEC_A/STC_A	Y = 129.7 - 29.5 × LIG_A-249.5 × NSTC_A – 138.5 × CEC_STC	0.96	2.01	< 0.01
CB3	H_1100, LIG_A/STC_A	Y = -21.3 + 187.2 × H_1100 + 166.5 × LIG_A/STC_A	0.80	2.21	0.02
CC	LIG_A, NSTC_A	Y = -11.9 + 6.48× LIG_A + 77.1 ×NSTC_A	0.92	1.05	< 0.01
Predicted energy values by NRC (2001)					
TDN_{1x}, %DM	NSTC_A	Y = 79.5 - 51.7× NSTC_A	0.56	6.91	0.03
NEL_{p3x}, Mcal/kg DM	NSTC_A	Y = 1.8 - 1.3× NSTC_A	0.52	0.005	0.04
In situ rumen degradation kinetics of NDF					
Kd, %/h	LIG_A	Y = 0.98+ 3.40× LIG_A	0.66	0.780	0.01
EDNDF, %	LIG _A/STC_A	Y = 18.8 +335.0 × LIG _A/STC_A	0.68	3.62	0.01

*RSD = residual standard deviation.

The high uNDF-288 content of Cowboy BS reflects that the Cowboy BS would have an adverse effect on DMI and milk production particularly in high producing dairy cows. All the predicted energy values (DE, ME, and NE_l) were highest in corn silage, intermediate in Copeland and Xena BS, and lowest in Cowboy BS. The lower predicted energy values in Cowboy BS is attributed to low starch content and hence low tdNFC compared to other forages (Weiss et al., 1992).

All barley silages were similar in CB3 content, while the CC fraction was higher in Cowboy BS. Corn silage had the greatest degradability of CB1 and total carbohydrates. In contrast, a previous study showed that Cowboy BS was higher in ivNDFD (37% DM), compared with Xena (28%) and Copeland (31.1%) BS (Nair et al., 2016). This discrepancy in digestible CHO content is probably a result of genotype by environment interaction.

Results from *in situ* DM degradation showed that all forages had similar Kd values for the slowly degraded DM fraction. Corn silage had the highest value of slowly degraded DM fraction. Since soluble carbohydrates are totally degraded in the rumen and starch has high ruminal digestion, the high digestible DM fraction of corn silage could be related to high content of starch (Johnson et al., 1999). The effectively degraded DM fraction was higher in Xena BS than in Cowboy BS. The higher EDDM in Xena is the result of the degradation of soluble DM, and part of the NDF fraction.

The potentially digestible NDF fraction comprised large fractions of the NDF pool in all studied forages ranging from 56% to 74%. Xena BS had greater potentially degradable NDF fraction compared with Cowboy BS. The extent of rumen degradation of NDF was lower in Copeland BS than Xena BS. This can partially be explained by the difference in potentially

digestible NDF and undegradable fractions between Copland and Xena BS. Next to genetic differences, the extent of rumen degradation has been reported to be affected by variables like the soil type, climate conditions, growing conditions, and stage of maturity (Varga et al., 1983; Cherney et al., 1993; Yu et al., 2004a). In the previous study, Xena BS has been reported to be lower in ivNDFD compared to Cowboy BS (Nair et al., 2016). The difference between the two studies results may be attributed to effect of growing condition, which can alter the chemical composition, particularly ivNDFD. Increases in the *in situ* NDF degradability of the Xena BS could have the potential to substantially improve the productivity of dairy cows fed diets containing relatively high contents of barley silage without negatively influencing feed intake due to greater extent of rumen degradation of NDF. However, more research is required to determine the effects of feeding these barley silage varieties on lactation performance of dairy cows.

There were five characteristic spectral regions which were mainly associated with the carbohydrate and ligneous conformation analyzed in the present study. The results of carbohydrate inherent molecular structural characteristics showed that Copeland BS had relatively low intensities of STC peak heights ca. 1416 cm^{-1} and ca. 1372 cm^{-1}, while corn silage and Xena BS had high peak heights of STC. However, the area intensities of STC_A and CEC_A absorbed intensities were similar for all silages. The STC and CEC absorbed intensities are mainly related to hemicellulosic and cellulosic compounds (Xin et al., 2013). The differences in spectral parameters located within STC_A, could explain the variations in the digestive behavior between forages. The non-structural CHO peak area (NSTC_A), peak height H_898, and NSTC spectral ratios were significantly lower in corn silage, compared with all BS varieties. A previous study done by Yu (2012) found that the peak area and height within the NSTC region

were the best indicators for predicting the rapidly degradable CHO fraction (CB1) and undegradable CHO fraction (CC) of bioethanol coproducts. The differences between barley and corn silages in NSTC spectral intensities reflect the competence of FT/IR technique to detect the starch and simple sugar contents in silages.

Principal component analysis is a highly useful tool for assessing the differences and similarities in molecular structures between samples, for example PCA analysis can explore relationships in the structural makeup data of feed samples (Allison et al., 2009). The PCA analysis showed the molecular structure of different barely forages were different in their ligneous content, because their spectral data formed separated classes (Figure. 3.1b). These findings are consistent with the biological measurements of indigestible NDF values (uNDF-288h). All forages were not distinguishable in spectral characteristics for other regions, such as structural carbohydrates, non-structural carbohydrates, and total carbohydrates regions. This is due to mixed-cluster groups in CLA and overlapping ellipses in PCA (Figure 3.2−3.4). There were differences in the results of the univariate statistical analysis and multivariate analysis. These differences were also detected in research by Huang et al. (2017). It should be noted that peak heights and areas give incomplete information about molecular structural characteristics in a specific spectral region and cannot give complete information about the whole spectral region (Yu, 2005). The multivariate analysis is based on statistical variable reduction, which reflects the whole spectral information within the specific regions, i.e., the region representing total carbohydrates and non-structural carbohydrates (Yu, 2005). The multivariate analysis in the current study was based on the original spectrum. Although some forages had different heights, areas, and some spectral ratios, these changes could not be picked up by the multivariate analyses based on the original spectrum.

Some associations have been found between CHO and spectral parameters associated with CHO. For example, starch content was negatively correlated with NSTC_A, while STC_A was negatively correlated with ADF content. These findings are in agreement with previous studies, where they reported a negative correlation between structural CHO area and ADF content in corn stover, *Brassica carinata* seed and transgenic alfalfa forage samples (Xin et al., 2013; Li et al., 2016). The NSTC_A related spectral profile was more correlated with energy profiles of forages, indicating that NSTC region played an important role in defining the nutrient utilization of forages in dairy cattle.

The CHO fractions partitioned by the CNCPS model had similar tendencies. For instance, the NSTC_A was negatively correlated with CB1 fraction, and negatively correlated with CC fraction (calculated for *in situ* incubation of feed for 288 h). The STC_A tended to correlate with CC fraction.

The *in situ* NDF rumen degradation kinetics also had correlations with CHO molecular spectral parameters in the present study. The rate and extent of NDF rumen degradation were positively correlated with LIG_A. To date, several studies on correlation analysis between molecular structural features and feeding value and nutrients digestibility have been published (Zhang and Yu, 2012; Li et al., 2015; Xin et al., 2017). Similar trends were found for the relationship of CHO molecular features to nutrient profiles in different morphological sections of corn stover (n = 18), and transgenic alfalfa forage (n = 6) (Li et al., 2015; Xin et al., 2017). However, few correlations have been found in *Brassica carinata* seed and canola seed (n = 6) (Xin et al., 2013). These discrepancies between studies could be attributed to feed type differences in the sample pool, which influence correlation coefficients in correlation analysis.

3.6. Conclusion

The results indicate that the univariate approach with only one factor consideration (ivNDFD) might not be a satisfactory method for evaluating and ranking barley silage quality. The new short-season corn silage had greater energy content relative to barley silage varieties due to its higher digestible fiber content compared with barley silage. The carbohydrate spectral profiles (functional groups) are highly related to the nutritive values of barley and corn silages.

4. Molecular Structure of Protein in Relation to Nutrient Utilization and Availability of Barley and Corn Silages in Dairy Cows

4.1. Abstract

This study was conducted to examine the differences between barley silage and a new short-season corn silage in terms of their rumen degradation kinetics of crude protein (CP), intestinal digestibility of CP and the estimated metabolizable protein supply (MP) in the small intestine to dairy cattle; and to identify the pattern of interactive association between the molecular structures of barley and corn silage in relation to metabolic characteristics of protein in dairy cows. Three BS varieties (Cowboy BS, Copeland BS, and Xena BS) and one variety of a new short-season corn silage (P7213R) were selected for this study. The protein-related molecular structure spectral information was collected using an advanced vibrational molecular spectroscopy (Fourier transform infrared; FT/IR). The resulted showed that Xena BS had a lower ($P < 0.05$) rumen undegradable CP (g/kg DM) compared to other BS varieties. In comparison to BS, corn silage exhibited a lower rumen degradable CP and a lower degraded protein balance ($P < 0.05$). However, the truly absorbed bypass CP and MP of the new short-season corn silage was similar to BS varieties. All BS varieties had similar MP content ($P > 0.10$). The results indicated that the protein molecular structural bands were significantly ($P < 0.05$) correlated with CP fractions and truly absorbed rumen-undegradable protein in the small intestine. In conclusion, there was no effect of BS variety on the estimated protein supply in dairy cattle. The new short-season corn silage variety had less degradable protein content in the rumen compared to barley silage. The FT/IR molecular spectroscopy can be used to evaluate silage quality and protein utilization in dairy cows.

4.2. Introduction

Ensiling is an important method of preserving forages, particularly in the areas where the climate is not favourable for making hay (Wallsten and Martinsson, 2009). Maximizing the utilization and profitability of silages in the dairy industry could be obtained by using high-quality forages that have high forage yields. Corn silage and alfalfa silage are widely used as forage sources in the world, however, small grain cereals (e.g., triticale, barley, oats) are usually used as forage source in Northern climates (Chapman et al., 2005; Wallsten and Martinsson, 2009; Gill et al., 2013). Whole crop barley silage (BS) is a cool-season crop, which considers the main forage source in Western Canada (Gill et al., 2013). Generally, the energy value of BS is lower than the energy value of corn silage, but the protein concentration is greater than in corn (Gill et al., 2013; Brunette et al., 2014). Nevertheless, there are many variables that also affect nutrient yield and digestibility of BS such as variety, environmental conditions during the growing season, and stage maturity at harvesting (Gill et al., 2013; Nair et al., 2016). More recently, several relatively new barley forage varieties have been developed in Western Canada, however, there is no study that investigated the differences among these varieties with regard to their protein metabolic characteristics in dairy cows.

The corn grown in the Northern latitude region is different from the corn varieties grown in warmer climates (Mahanna, 2010). These differences are attributed to some limitation during growing season. Recently, new hybrids were introduced in Western Canada. These new hybrids requires a lower crop heat units (Abeysekara et al., 2013b). To establish these hybrids as a forage crops, they must be compared against other conventional forage crops such as BS.

A spectroscopic method, such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT/IR), has been developed as a rapid, direct, non-destructive, and non-invasive bioanalytical technique for feed evaluation (Yu, 2012). Several studies found that intrinsic chemical structures influence degradation characteristics, utilization, and availability of protein in several feed resources (Yu and Nuez-Ortín, 2010; Yu, 2012). Therefore, measuring the protein inherent molecular makeup may be important for understanding the variation in protein fractions, and digestibility for different types of forages or among forages varieties. The current study was conducted: (1) to assess the magnitude of differences among barley silage (BS) varieties in comparison with a new short season-corn silage variety in regards to their protein profile, rumen degradation kinetics of OM and protein, intestinal digestibility of rumen bypass CP (RUP), and metabolizable protein (MP) supply to dairy cattle, and (2) to identify the pattern of interactive association between the molecular structure of protein and digestible protein content in silages.

4.3. Materials and Methods

4.3.1. Silage Sampling

Three barley varieties were selected for this study. All three varieties were of the two-row type: CDC Cowboy (Ardell Seeds, Vanscoy, SK), CDC Copeland (Wylie Farms Ltd., Biggar, SK) and Xena (Crop Production Services, Bow Island, AB). A newer corn forage variety was selected in the current study (P7213R) based on its higher nutritive values compared to other corn forages as reported by (Abeysekara et al., 2013a; b). All three barley forages (CDC Cowboy BS, CDC Copeland BS, Xena BS) and corn forage (P7213R) were seeded at the University of Saskatchewan research farm (latitude: 52.2 ° N, longitude: 106.6° W and altitude 491m). All

forages were grown on a non-irrigated land at the University of Saskatchewan. Seeding, harvest and ensiling management were as described before by Refat et al. (2018).

For the current study, two representative samples from each silage variety were taken at two different dates such that each silage was represented by two replicates. Each sample was used for (1) protein profile analysis (2) rumen degradation using *in situ* technique and (3) molecular structure study. For the chemical, and *in situ* study, the silage samples were oven dried at 55°C for 48 h. The dried samples were ground through a 1-mm screen for chemical analyses or through a 3-mm for the situ study (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK).

4.3.2. Protein Fractions

In this study, the Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5 was used to partition crude protein (CP) (Higgs et al., 2015). In the CNCPS v6.5, CP is partitioned based on their ruminal degradation characteristics: ammonia (PA1), non-ammonia soluble true protein (PA2), moderately degradable true protein (PB1), slowly degradable true protein, bound in NDF (PB2), and completely undegradable CP (PC).

4.3.3. Rumen Degradation and Intestinal Digestibility

Six lactating Holstein cows fitted with rumen cannula (inner diameter of 10 cm; Bar Diamond, Parma, ID, USA) were used for *in situ* incubation to study the rumen degradation kinetics of OM and CP as described in detail in Yu et al. (2003). The cows were housed individually in a tie-stall with rubber bedding during the trial. The rumen incubations were performed based on the 'gradual addition/all out' schedule and samples were incubated in the

rumen for 0, 6, 12, 24, 48 and 72h. At the end of incubation, bags were removed from the rumen and then hand washed along with 0 h bags in detergent-free cold water to rinse off ruminal content, then dried at 55°C for 48 hours. The dry residues were pooled based on treatments, animals and incubation time for chemical analysis. The pooled samples were ground through a 1-mm screen using a Retsch ZM 200 rotor mill and then prepared for residual chemical analysis.

Rumen degradation parameters of each component were estimated according to the modified first order kinetics model (Ørskov and McDonald, 1979; Robinson et al., 1986). The results were calculated using the non-linear (NLIN) procedure of SAS and iterative least-squares regression (Gauss Newton method). The model used for measuring rumen degradation kinetics of OM and CP was as follow:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)},$$

where R(t) is the residue at h of incubation (%), S is the soluble fraction (%), U is the undegradable fraction (%), T0 is lag time (h), and Kd is degradation rate (%/h). Then rumen degradable of OM (EDOM) and CP (EDCP) were calculated according to the NRC-2001 using the nonlinear parameters predictable by the above equation (S, U, and Kd) as:

$$\text{EDOM / EDCP (g/kg DM)} = S + (D \times Kd) / (Kd + Kp)$$

The rumen undegradable OM (RUDM) and CP (RUP) were calculated as:

$$\text{RUOM / RUP (g/kg DM)} = U + D \times (Kp / Kd + Kp)$$

where, Kp is the estimated passage rate from the rumen (%/h). All effective degradability data presented in this study were calculated assuming a rate of passage of 4%/h (Van Vuuren et al., 1993).

The 3-step *in vitro* method of Calsamiglia and Stern (1995) was applied to analyze intestinal digestibility of RUP (IDP) of rumen residues of 12 h rumen incubation.

4.3.4. Predicted Truly Absorbed Protein Supply to Dairy Cows and Feed Milk Value

The nutrient metabolizable protein (MP) supply to dairy cows was estimated according to NRC (2001) model:

$$\text{MP (g/kg DM)} = \text{ARUP} + \text{AMCP} + \text{AECP}$$

where, AMCP is the truly absorbable rumen-synthesized (MCP) in the small intestine , ARUP is the truly absorbable bypass CP in the small intestine, and AECP is the truly absorbable endogenous protein (ECP) reaching the small intestine (NRC, 2001). The detailed descriptions of the equations are given by Peng et al. (2014).

The predicted feed milk values (FMV) is used for estimating the efficiency of MP of feed to be converted to milk (NRC, 2001; Theodoridou and Yu, 2013a). The efficiency of MP to be converted to milk is 0.67, and 1 kg of milk contains approximately 33 g of milk protein. (NRC, 2001). Consequently, FMV can be estimated according to the following equation:

$$\text{FMV} = \frac{\text{MP (g/kg DM)}}{33} \times 0.67$$

4.3.5. Univariate Molecular Spectral Analysis of Protein Spectral Data

Fourier transform infrared spectroscopy molecular spectroscopy was used for analysis the protein related-molecular structure spectral features. The dried, ground silage samples (0.50 mm) were used to collect spectra related to the molecular structure of protein. The Spectra were

generated from the mid-IR region (4000-800 cm^{-1}) using JASCO Spectramanager II software (JASCO Corporation, Tokyo, Japan) with a spectral resolution of 4 cm^{-1} and 128 co-added scans.

The Univariate spectral analysis was performed using OMINIC 7.3 Software (Thermo-Nicolet, Madison, WI, USA). For molecular structure, every sample was spectroscopically scan for five times. The molecular spectral data of samples were collected and corrected for the background spectrum using FT/IR molecular spectroscopy (Jasco 4200, Jasco International Co. Ltd., Tokyo, Japan). The regions related to the molecular structure of protein in includes the protein amide I and II in the infrared regions of approximately ca. 1706–1487 cm^{-1} (Abeysekara et al., 2013a, b). The amide I peak was further resolved into several multi-component peaks in which α-helices (ca. 1652 cm^{-1}) and *β*-sheets (ca. 1631 cm^{-1}) was identified.

4.3.6. Chemical Analysis

For estimation of CP (N × 6.25), N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI) (AOAC, 2000). The total soluble CP (SCP) was analyzed by incubating the sample with a bicarbonate–phosphate buffer and filtering it through Whatman 54 filter paper (Roe et al., 1990). The acid detergent fiber insoluble protein (ADICP) and neutral detergent fiber insoluble protein (NDICP) values were also measured as described by Licitra et al. (1996). Ammonia N concentrations were measured according to the method described by Rhine et al. (1998).

4.3.7. Statistical Analysis

Statistical analyses were performed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). Chemical profile was analyzed using completely randomized design. The

model used for analyzing this design was as follow: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is an observation of the dependent variable; μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, e_{ij} are the random error associated with the observation ij.

In situ degradability of OM and CP data were analyzed using a randomized complete block design. The model used for this design was as follow: $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y_{ijk} is an observation of the dependent variable ij; μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, B_j is a block effect with *in situ* animals, as a random effect, and e_{ijk} is the random error associated with the observation ij.

The ATR-FT/IR spectroscopic data were analyzed using a completely randomized design model with subsampling (spectra reading; n= 5 scans): $Y_{ij} = \mu + T_i + S(T_i) + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij (chemical functional groups such as; μ is the population mean for the variable; T_i is the treatment effect, as a fixed effect, S is the subsample (5 scans) which nested within each treatment, e_{ij} are the random error associated with the observation ij. The significance was declared at $P < 0.05$, and trends at $P \leq 0.10$. Differences among the treatments were evaluated using Tukey's multiple comparison test or Tukey.

Correlations between the structural spectral data in silage and *in situ* rumen degradation, predicted predicted truly absorbed protein supply to dairy cows, and feed milk value were analyzed using the CORR procedure of SAS software (SAS Institute, 2003) with a parametric correlation method (Pearson) or nonparametric correlation method (Spearman) which depends on data normality. Normality tests are performed using the UNIVARIATE procedure of SAS with Normal and PLOT options.

4.4. Results and Discussion

4.4.1. Nutrient Profiles and Protein Fractions Partitioning by the Updated CNCPS Model

The protein profiles of barley and corn silages are presented in Table 4.1. There were no significant differences among barley silage varieties in CP (averaged 11.2 %DM). On the other hand, CP content differed significantly between barley silage and corn silage, where the Cowboy BS had a higher CP 11.7 (%DM) compared to corn silage 9.1 (%DM). The CP of barley and corn silage used in the present study was relatively similar to data reported in previous studies (Mustafa et al., 2000; Chow et al., 2008; Chaves et al., 2012).

In present study, Cowboy BS had higher SCP ($P = 0.03$) than corn silage (65.4 vs. 51.6 %CP). Ensiled forages have higher soluble crude protein values compared with fresh forages and it is considered as the main CP fraction in silage (Licitra et al., 1996). The SCP in corn silage and BS in this study were similar to those obtained in previous studies (Benchaar et al., 2014; Brunette et al., 2014).

The neutral detergent insoluble CP (NDICP) is the protein fraction retains available to dairy cows but is relatively resistant to ruminal degradation, while the acid detergent insoluble CP (ADICP) is the protein fraction associated with the ADF, and is highly resistant to rumen degradation (Licitra et al., 1996). The results of the current study showed all silages had comparable content of NDICP and ADICP.

Table 4.1. Crude protein, and degradable and undegradable protein sub-fractions: Comparison different barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Protein subtractions[2]						
CP, %DM	9.1*b*	11.7*a*	11.2*ab*	10.7*ab*	0.42	0.04
SCP, %DM	4.7*b*	7.7*a*	7.2*ab*	6.4*ab*	0.45	0.03
SCP, %CP	51.9	65.4	64.4	59.5	2.55	0.06
NDICP, %DM	1.1	1.4	1.3	1.4	0.15	0.57
NDICP, %CP	12.0	11.6	11.7	12.4	1.09	0.94
ADICP, %DM	0.90	1.10	1.15	1.25	0.11	0.27
ADICP, %CP	10.0	9.3	10.1	11.8	1.04	0.44
CNCPS protein subtractions, %CP[3]						
PA1	8.6	13.9	12.6	13.8	1.21	0.09
PA2	43.3	51.5	51.8	45.7	3.31	0.32
PB1	36.1	23.1	24.0	28.1	2.74	0.08
PB2	2.1	2.3	1.6	0.6	0.80	0.56
PC	10.0	9.3	10.1	11.8	1.04	0.44

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]CP = crude protein; SCP = soluble protein; ADICP = acid detergent insoluble crude protein; NDICP = neutral detergent insoluble crude protein.
[3]CNCPS = The Cornell Net Carbohydrate and Protein System (CNCPS); PA1= ammonia; PA2= soluble non-ammonia CP; PB1= moderately degradable protein; PB2 = slowly degradable protein bound in NDF; PC= indigestible protein.

Table 4.1 shows the protein fractions portioned by CNCPS model for the studied silages. The results showed that all barley silage varieties had similar rapidly degradable, moderately and slowly degradable protein sub-fractions, with mean %CP values of 13.4 (PA1), 49.7 (PA2), 25 (PB1), 1.5 (PB2), respectively. Corn silage tended to have a higher moderately degradable protein sub-fractions PB1 (36 %CP) compared with barley silage varieties (25 %CP; $P = 0.08$). The PB1 is moderately degraded in the rumen (3 to 20 %/h; Higgs et al., 2015). The higher PB1 in corn silage would, in turn, contribute to increasing the digestible CP content in the rumen. The PB2 slowly degrades in the rumen (4–9 %/h), and a large fraction of PB2 bypasses the rumen and digests in the small intestine (Higgs et al., 2015). The PB2 fraction was similar among all forages (1.6 %CP; $P > 0.10$). The PC fraction in the feed includes the indigestible part of the protein, which cannot be used effectively by the ruminants (Van Amburgh et al., 2015). Our findings did not show any significant differences between corn and barley and among barley varieties in PC fraction (average 10.3 %CP; $P > 0.10$).

4.4.2. Univariate Molecular Spectral Analysis of Protein Functional Groups

The protein molecular structure parameters for the four studied forages are presented in Table 4.2. In the univariate analysis, total amide area differed significantly among the four studied forages, where the corn silage exhibited lower ($P < 0.05$) total amide area (10 AU) compared to Cowboy and Xena BS (15 AU). These differences in the amide area is in consistent with the “wet chemistry” analysis (Table 4.1). The results showed that amide I and amide II heights differ significantly among the corn and barley forages, where corn silage exhibited lower heights compared to barely silages (Table 4.2, $P < 0.05$). There were also differences among barley silages in protein molecular structure, where Xena BS exhibited higher amide area ($P <$

0.05) when compared to Copeland BS. However, there was no significant difference among BS varieties in the Amide I to amide II ratio. These findings are in agreement with previous studies that found an association between amide I and II height and area with CP content in different feedstuff (Khan et al., 2014; Peng et al., 2014).

Because the feed protein amide I component peaks overlay each other, the secondary derivative was used to identify amide I component peak frequencies (Yu et al., 2004b). The secondary protein molecular structure in terms of the α-helix and β-sheet peaks for the studied forages are presented in Table 4.2. The intensity of the β-sheet peak height was similar for BS varieties (averaged 0.110 AU, $P > 0.10$). The α-helix was significantly higher ($P < 0.05$) in Xena BS compared to Cowboy BS. The corn forage exhibited lower values ($P < 0.05$) of α-helix, and β sheet spectral intestines. It has been reported the ratios of amide I/II and α-helix/β-sheet ratios reveal information about the protein's molecular makeup in feed ingredients such as barley grains, flaxseed, canola meal, and carinata meal (Khan et al., 2014; Peng et al., 2014). Thus, the differences in the amide I/II or α-helix/β-sheet ratio between studied forages could influence their solubility and access to microbes and proteolytic enzymes (Khan et al., 2014).

Table 4.2. Differences in protein molecular structure spectral profiles: Comparison different barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Protein (primary structure spectral features), absorbance unit (AU)[2]						
Amide area,	10.31*c*	14.67*ab*	12.27*bc*	15.64*a*	0.6847	<0.01
Amide I peak height,	0.084*c*	0.102*ab*	0.094*bc*	0.114*a*	0.0041	<0.01
Amide II peak height	0.027*b*	0.041*b*	0.040*b*	0.077*a*	0.0070	<0.01
Ratio of amide peak heights (I to II)	3.02*a*	2.53*ab*	2.58*ab*	1.72*b*	0.261	0.01
Protein (secondary structure spectral features), AU[3]						
β-Sheet	0.087*b*	0.113*a*	0.095*ab*	0.110*a*	0.0057	< 0.01
α-Helix	0.046*c*	0.063*bc*	0.073*b*	0.096*a*	0.0056	<0.01
Ratio of α-helix to *β*-sheet	0.53*b*	0.55*b*	0.77*a*	0.87*a*	0.001	0.01

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]Baseline for protein spectral peak, (ca. 1706–1487 cm^{-1}); Amide I and Amide II peaks ca. 1636 cm^{-1}; and 1542 cm^{-1}, respectively.
[3]*β*-Sheet (ca. 1631 cm^{-1}); α-Helix (ca. 1652 cm^{-1}).

4.4.3. Ruminal Degradation of OM

The *in situ* rumen degradation kinetics of OM for barely and corn silages are shown in Table 4.3. There were no significant differences in degradation rates among silages (Kd, 3.8%/h). The soluble fraction of OM was significantly ($P < 0.05$) different among all silages, being higher in Xena (31%), intermediate in Copeland BS (26%) and lower in Cowboy BS and corn silage (20%). The slowly degradable OM fraction was significantly higher in corn silage compared (54%) to Xena BS (41%). All studied forages showed differences in the undegradable OM fraction, where the Cowboy BS had the greatest amount of undigested OM (38%), while corn silage had the lowest content of the rumen-undegradable OM (25%). The effective degradability of OM differed significantly among the studied forages ($P < 0.05$), where the new short-season corn silage and Xena BS had the greatest degradability (48%), while Cowboy BS had the lowest EDOM (41%). These results would indicate a lower degradation of Cowboy BS compared to other forages, which may exert an adverse effect on animal performance by limiting the feed intake in dairy cows (Nousiainen et al., 2009).

4.4.4. Ruminal Degradation of CP

The *in situ* ruminal degradation of CP for barley and corn silages are presented in Table 4.3. The rate of degradation of the D fraction tended be slower in Xena BS and corn silage compared to the other studied forages ($P = 0.10$). The degradation rate of CP in corn silage in the current study is relatively similar to that reported before by Abeysekara et al. (2013b). There were no differences among BS in the soluble CP content (average 49%). However, BS had a greater amount ($P < 0.05$) of soluble CP (47%) compared to corn silage (38%).

Table 4.3. *In situ* rumen degradation kinetics of OM and CP: Comparison different barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
In situ rumen OM degradation[2]						
Kd, %/h	4.22	4.43	3.44	3.01	0.844	0.50
T0, h	1.8	1.3	2.1	4.7	1.07	0.47
S, %	21.2*c*	18.8*c*	26.1*b*	31.2*a*	0.62	<0.01
D, %	53.8*a*	43.0*ab*	42.6*ab*	40.5*b*	3.48	0.01
U, %	25.1*b*	38.2*a*	31.3*ab*	28.4*ab*	3.36	0.04
RUOM, %	52.8*b*	59.4*a*	56.4*ab*	51.4*b*	1.42	0.01
EDOM, %	47.2*a*	40.6*b*	43.6*ab*	48.6*a*	1.42	0.01
Ruminal degradation of protein[3]						
Kd, %/h	1.91	7.01	8.93	2.31	2.401	0.10
T0, h	2.6	0.0	1.2	1.0	1.51	0.68
S, %	37.9*b*	46.0*a*	47.5*a*	52.9*a*	1.91	<0.01
D, %	54.8*a*	18.5*b*	15.3*b*	22.6*b*	6.00	<0.01
U, %	7.4*b*	35.5*a*	37.2*a*	24.5*ab*	6.33	0.01
RUP, %	48.3*a*	42.6*ab*	43.4*ab*	41.1*b*	1.47	0.02
RUP, g/kg DM	51.0*b*	60.1*a*	60.5*a*	52.2*b*	1.34	0.01
EDCP, %	51.7*b*	57.4*ab*	56.6*ab*	58.9*a*	1.47	0.02
EDCP, g/kg DM	54.7*b*	81.0*a*	78.9*a*	75.9*a*	4.17	<0.01

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]Kd = the rate of degradation of D fraction (%/h); T0 = lag time (h); U = undegradable degradable fractions; D = degradable fractions; RUOM = rumen bypass or undegraded feed OM; EDOM = effective degradability of OM.
[3]RUP = rumen bypass or undegraded feed CP; EDCP = effective degradability of CP.

These findings are in consistent with the CNCPS results (Table 4.1). Protein solubility is considered the major factor in reducing the efficiency of silage protein utilization. A previous study showed a quadratic relationship between silage protein solubility and feed intake and body weight gain where reported as solubility increases above 45% CP, then intake and gains decline (Choung et al., 1990). Thus, our findings indicate that the corn silage (P7213R) would provide the dairy cattle with an adequate content of SCP compared to BS varieties. All barley silage varieties exhibited higher EDCP content compared to corn silage ($P < 0.05$). The CP degradation kinetics values reported in our study for BS and corn silage are in agreement with those of previous studies (Abeysekara et al., 2013b; Rosser et al., 2013). The higher EDCP in barley silage is attributed to the higher *in situ* soluble fraction and/or the rapid rate of degradation of the slowly degradable fraction that resulted in high ruminal degradability of CP (Mustafa et al., 2000).

4.4.5. Intestinal Digestibility of Protein

Results of the intestinal digestibility estimation are presented in Table 4.4. The results showed that corn silage has higher IDP (19 %CP, $P < 0.05$) compared to Cowboy and Xena BS (averaged = 13.6 %CP). The intestinal digestibility of rumen undegradable protein for corn silage obtained in our study was similar to that reported by in the previous study (Abeysekara et al., 2013b). The higher intestinal digestibility of CP in corn silage relative to BS could be attributed to its relatively higher content of RUP (%CP) compared to BS, which could allow the RUP to be more available for digestion in the small intestine. Although the numerical increase in digestibility of RUP for corn silage (dIDP % RUP) in the small intestine, the amount of CP (g/kg DM) digested in small intestine was similar among all the studied forages. For barley silage, the

results of the current study showed that Xena BS tended ($P = 0.10$) to have a lower amount of IDP (16 g/kg DM) compared to other two BS varieties (21 g/kg DM).

The total ruminal and intestinal digestibility of protein (TDP) was calculated in the current study as summation of IDP (g/kg DM) and RDP (g/kg DM). The results showed that corn silage had similar ($P > 0.10$) TDP (%CP) compared to BS varieties. However, when the TDP expressed as g/kg DM, the corn silage exhibited a lower TDP compared to Cowboy and Copeland BS. This reduction in the amount TDP is attributed to the lower CP content in corn silage.

4.4.6. Potential Protein Supply to Dairy Cows

The MP content of a feed is the total protein content that contribute to milk production (NRC, 2001). The total MP in the NRC model is the summation of AECP, ARUP and AMCP (NRC, 2001). The results of the current study showed that the MP content was the same for all studied forages (on average 51 g/kg DM, $P > 0.10$; Table 4.5). On the basis of MP results, all silages exhibited similar FMV (averaged 1.04 g/kg DM). These findings could indicate that barley and corn silages have the potential to provide the same level of MP for dairy cows.

The degraded protein balance (DPB) was used in this study to detect the differences between MCP synthesis resulting from RDP and the MCP synthesis resulting from the available energy (Peng et al., 2014). The DPB value of corn silage was significantly lower (-48.3 g/kg DM) than that of BS (-32 g/kg DM). A lower DPB values in corn silage could indicate to a much shortage in N supply in the rumen, which can impair MCP synthesis (Peng et al., 2014). Thus, it is necessary to provide the corn silage with protein- rich feeds in the ration to increase nitrogen supply in rumen, and, hence, enhancing ruminal synchronization of nitrogen and CHO.

Table 4.4. Intestinal digestibility of RUP and total digestible CP: Comparison different barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Intestinal digestion of protein[2]						
%dIDP, %RUP	39.3	33.8	34.6	31.36	2.39	0.11
IDP, %CP	19.1*a*	14.4*b*	14.9*ab*	12.8b	1.09	0.01
IDP, g/kg DM	20.0	20.3	20.8	16.4	1.36	0.10
The total ruminal and intestinal digestibility of protein[3]						
TDP, %CP	70.7	71.8	71.5	71.7	1.42	0.89
TDP, g/kg DM	74.8*b*	101.3*a*	99.7*a*	92.3*ab*	4.70	<0.01

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$)
[2]IDP = intestinal digestibility of RUP
[3]TDP = the total ruminal and intestinal digestibility of protein

Table 4.5. Predicted truly absorbed metabolizable protein to dairy cows and feed milk value: Comparison different barley silage varieties vs. corn silage

Items	Silage				SEM[1]	*P* value
	Corn	Barley				
	P7213R	Cowboy	Copeland	Xena		
Rumen-synthesized microbial protein truly absorbable in small intestine, g /kg DM[2]						
MCP_{RDP}	44.0	47.8	46.7	51.9	1.87	0.07
MCP_{TDN}	84.8*a*	73.9*d*	77.6*b*	76.0*c*	0.27	<0.01
AMCP	28.2	30.6	29.9	33.2	1.20	0.07
Rumen-undegradable feed protein truly absorbable in small intestine, g/kg DM[3]						
ARUP	20.0	20.3	20.8	16.4	1.36	0.10
Rumen endogenous protein truly digested in small intestine, g/kg DM[4]						
ECP	4.0*a*	3.3*c*	3.7*b*	3.6*b*	0.041	<0.01
AECP	1.6*a*	1.3*c*	1.5*b*	1.5*b*	0.02	< 0.01
Total truly absorbed (metabolizable) protein in small intestine, g/kg DM[5]						
MP	49.8	52.2	52.2	51.1	1.76	0.74
Degraded protein balance, g/kg DM[6]						
DPB	-48.3*b*	-31.0*a*	-36.6*a*	-28.7*a*	2.24	< 0.01
Feed milk value, kg milk/kg feed[7]						
FMV	1.01	1.06	1.06	1.03	0.042	0.77

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).
[2]$MCP_{TDN,}$ = microbial protein synthesized in the rumen based on available energy; MCP_{RDP} = microbial protein synthesized in the rumen based on available protein; AMCP = truly absorbable rumen-synthesized microbial protein in the small intestine.
[3]ARUP = truly absorbable rumen-undegradable protein in the small intestine.
[4]ECP = rumen endogenous protein; AECP = truly absorbed rumen endogenous protein in the small intestine.
[5]MP = total metabolizable protein
[6]DPB = degraded protein balance;
[7]FMV = feed milk value, kg milk/kg feed

4.4.7. Protein Molecular Structural Profiles in Relation to Digestible Protein Contents

Table 4.6 presents the result of correlation between the protein molecular spectral profiles and the protein nutritive profiles, the rumen degradation kinetics of CP, and the potential protein supply to dairy cattle for combined forages (n = 8). The current study showed that CP tended to positively correlate with the amide area (r = 0.63, $P < 0.09$). Several studies reported significant correlation between CP estimated by wet chemistry and amide region (Xin et al., 2016; Yu et al., 2008). Protein molecular spectral profiles were correlated ($P < 0.05$) with ADICP (%DM). Amide area exhibited significant correlation with NDICP. These findings are in agreement with previous studies by (Xin et al., 2016; Theodoridou and Yu, 2013a). To the best of our knowledge, this is the first report describing the correlation between protein spectral molecular profiles (estimated by the univariate analysis) and protein profile (estimated by wet chemistry analysis) in silage.

With regard to the *in situ* degradation parameters, the results indicated the amide I height, amide area, α- helix and α- helix to β-sheet ratio were positively correlated with soluble CP fraction and EDCP (r = 0.75, $P < 0.05$; Table 4.6). For the predicted protein supply values, there was a significant correlation between ARUP and amide II and β-sheet (r = -0.72, $P < 0.05$; Table 4.6). The amide area, amide I height, and β-sheet were significantly correlated with DPB (r = 0.77, $P < 0.05$). These finding would refer to the higher sensitivity of FT/IR technique to detect the difference between silages in terms of their digestion behavior in the rumen.

Table 4.6. Coefficient of correlation between protein molecular intensity characteristics and protein nutrient profiles in barley and corn silages.

Items		Amide I[1]	Amide II	Amide I to Amide II ratio	Amide area	α-Helix	*β*-Sheet	α-helix to *β* sheet ratio
Protein fractions								
CP, %DM	r*	0.54	0.12	-0.17	0.63	0.41	0.69	0.17
	P value	0.17	0.78	0.69	0.09	0.32	0.06	0.68
SCP, %CP	R	0.31	0.01	-0.10	0.44	0.16	0.46	-0.03
	P value	0.45	0.98	0.81	0.27	0.71	0.25	0.95
SCP, %DM	R	0.43	0.04	-0.11	0.54	0.28	0.59	0.06
	P value	0.29	0.92	0.80	0.16	0.51	0.12	0.89
ADICP, %CP	R	0.65	0.89	-0.85	0.51	0.72	0.36	0.68
	P value	0.08	0.00	0.01	0.19	0.05	0.38	0.06
ADICP, %DM	R	0.91	0.85	-0.84	0.85	0.90	0.75	0.73
	P value	0.00	0.01	0.01	0.01	0.00	0.03	0.04
NDICP, %CP	R	0.65	0.54	-0.35	0.59	0.56	0.58	0.37
	P value	0.08	0.17	0.39	0.12	0.15	0.13	0.36
NDICP, %DM	R	0.55	0.60	-0.32	0.86	-0.14	0.67	-0.42
	P value	0.45	0.40	0.44	0.01	0.86	0.33	0.58
In situ rumen degradation kinetics of protein, %CP								
S	R	0.73	0.68	-0.64	0.72	0.81	0.55	0.72
	P value	0.04	0.06	0.09	0.05	0.02	0.16	0.04
D	R	-0.49	-0.29	0.40	-0.55	-0.43	-0.51	-0.28
	P value	0.22	0.49	0.32	0.15	0.29	0.20	0.50
U	R	0.32	0.08	-0.25	0.41	0.21	0.42	0.06
	P value	0.44	0.84	0.55	0.31	0.62	0.30	0.88
EDCP	R	0.78	0.67	-0.64	0.82	0.72	0.70	0.53
	P value	0.02	0.07	0.09	0.01	0.04	0.05	0.18
Absorbed protein supply to dairy cows and feed milk value, g/kg DM								
ARUP	R	-0.65	-0.72	0.63	-0.52	-0.76	-0.40	-0.72
	P value	0.08	0.04	0.09	0.18	0.03	0.33	0.04
DPB	R	0.78	0.64	-0.61	0.84	0.69	0.74	0.47
	P value	0.02	0.09	0.11	0.01	0.06	0.04	0.25
MP	R	0.11	0.01	-0.02	0.26	-0.07	0.23	-0.21
	P value	0.79	0.98	0.96	0.53	0.87	0.59	0.62

*coefficient of correlation.

[1]Baseline for protein spectral peak, (ca. 1706–1487 cm^{-1}); Amide I and Amide II peaks ca 1636 cm^{-1}; and 1542 cm^{-1}, respectively; *β*-Sheet (ca. 1631 cm^{-1}); α-Helix (ca. 1652 cm^{-1}).

4.5. Conclusion

The results of the present study indicated that there was no effect of BS variety on the estimated protein supply in dairy cows. The results of this study showed that corn silage had lower degradable protein in the rumen and had a lower degraded protein balance value compared to barley silage. Despite of this, the new short-season corn silage would provide the dairy cows with a similar amount of MP as barley silage. The results of the molecular structure study for silage samples showed that protein spectral profiles are highly correlated with protein utilization.

Based on the previous studies (Chapter 3 and 4), have been found differences between barley forage varieties or between barley and corn silages in terms of chemical profile and ruminal degradation kinetics, which in turn could affect dairy cows' performance. Thus, in the next studies (Chapter 5 and 6), the effects of barley silage with varying rates of ivNDFD on lactation performance, chewing activity, rumen fermentation, and microbial protein synthesis of high-yield dairy cows in comparison with a new short-season corn silage were evaluated.

5. Effect of Digestible Carbohydrates Content of Barley Silage on Lactation Performance and Chewing Activity of Lactating Dairy Cows in Comparison with Corn Silage

5.1. Abstract

There is limited knowledge on the effect of barley silage with different ruminal *in vitro* NDF digestibility (ivNDFD) on dairy cow performance and chewing activity. The objectives of this study were to assess the effects of barley silage varieties selected for varying rates of ivNDFD on lactation performance and chewing activity of high-yield dairy cows in comparison with a new short-season corn silage hybrid. A 4×4 Latin square design was applied in this study with four mid-lactating multiparous Holstein cows. The cows were fed diets containing 49% barley-based concentrate and 51% forage (DM basis). The results show that cows fed corn silage produced more milk ($P < 0.05$) and had greater ($P < 0.05$) feed efficiency than cows fed barley silages. Cows fed barley silage with relatively higher ruminal ivNDFD did not show significant differences from the cows fed other barley silage varieties in milk yield and total chewing activity. The preliminary results indicated that a univariate approach with only one factor consideration (ivNDFD) might not be a satisfactory method for evaluating and ranking barley silage quality. However, a large-scale animal study with different stages of lactation (early, mid- to late-) is warranted to confirm this finding. The new short-season corn forage hybrid with low heat unit requirement may be an appropriate option as forage source for high producing dairy cows in Western Canada.

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5.2. Introduction

It is important to provide high producing dairy cows with satisfactory dietary nutrients to maintain their high levels of milk production and body maintenance (NRC, 2001). In Western Canada, it is a challenge to produce forage crops due to the restricted growing season that limits the growth range of cropping alternatives (Bootsma and Brown, 1995). Barley silage is well-suited for production in such a growing season with relatively low heat requirement (Wallsten and Hatfield, 2016).

The single time point incubation for 30 h ruminal *in vitro* NDF digestibility (ivNDFD) analysis is widely used for evaluating and ranking forages (Oba and Allen, 1999b). Selecting forages with increased ivNDFD has been reported to improve the lactation performance of dairy cattle. Oba and Allen (1999b) found that one percent unit increase in ivNDFD equated to 0.25 kg/d increase in 4% fat-corrected milk yield (FCM) and 0.17 kg/d increase in DMI. Several studies have confirmed the positive effects of feeding forage with increased ivNDFD on DMI and productivity of dairy cattle (Ferraretto and Shaver, 2015). Effects of forage ivNDFD on milk production have been extensively studied for corn silage. The corn silage hybrids for increased ivNDFD have been developed and commercially available in the US. However, knowledge on the effects of barley silage with different ivNDFD on dairy cattle performance is very limited (Oba and Allen, 2011). Previous research by Nair et al. (2016) showed differences in ruminal *in vitro* digestible fiber concentration among seven varieties of barley silage grown in Western Canada. However, effects of barley with different ruminal *in vitro* digestible fiber concentration on the productivity of lactating dairy cows and chewing activities have not been studied for those varieties.

The corn grown in the Canadian prairies is different from the corn hybrid varieties grown in warmer climates such as Eastern Canada (Abeysekara et al., 2013a, b). These differences are attributed to some limitations during growing season (i.e. lower growing temperatures). Successful growth of corn in Canadian prairies depends on the availability of crop heat units, and corn is considered more suitable to areas receiving a minimum of 2000 to 2100 crop heat units (McCartney et al., 2009). Recently, new hybrids were introduced in Western Canada. These new hybrids require low crop heat unit. However, no lactation performance study was conducted on any of these corn varieties. In order to establish these hybrids as a forage crop, these must be compared against other conventional forage crops such as whole plant barley silage. The objective of this study was to assess the effects of different varieties of barley silage with varying ruminal ivNDFD on lactation performance and chewing activity of high-yield dairy cows in comparison with the corn silage. The hypothesis of this study was that forage with higher ruminal ivNDFD would improve lactation performance and enhance chewing activity.

5.3. Materials and Methods

5.3.1. Preparation of Silage

Three barley varieties were selected based on the screening study by Nair et al. (2016) from seven varieties of barley silage using 30 h ivNDFD as an approach for ranking silage varieties. The seven varieties (Conlon, Copeland, Cowboy, Falcon, Legacy, Metcalfe, and Xena) of barley silages were collected within two years (2012 and 2013). Total number of 80 samples were collected from different dairy and beef operations in Saskatchewan and Alberta. These barley varieties have shown some differences in 30 h ivNDFD (range = 28% to 37%). The varieties with the higher and lower ivNDFD after 30 h of incubation were selected for beef,

sheep, and dairy study in Saskatchewan and Alberta. This study reports dairy work. All barley varieties were of the two-row type. The ivNDFD of CDC Cowboy (Ardell Seeds, Vanscoy, SK), CDC Copeland (Wylie Farms Ltd., Biggar, SK), and Xena (Crop Production Services, Bow Island, AB) were 37%, 31% and 29%, respectively.

One corn forage variety was selected in the current study (P7213R) based on the previous screening study done at University of Saskatchewan (Abeysekara et al., 2013a, b). In the screening study, six corn forage varieties were selected: Pioneer P7443R, Pioneer P7213R, and Pioneer P7535R (Pioneer Hi-Bred International Inc., Johnston, IA), Hyland Baxxos RR, Hyland SR22, and Hyland SR06 (Hyland Seeds, Blenheim, ON, Canada). The results of the previous screening study showed Pioneer P7213R has higher feeding value compared to other corn forage varieties (Abeysekara et al., 2013a, b).

The three barley varieties with different digestible fiber concentrations were grown on the University of Saskatchewan Research Farm (latitude: 52.15 ° N, Longitude: 106.61° W and altitude 491 m) in 2014 under dry land conditions under the same management conditions, to avoid the effect of environment on silage quality, particularly ivNDFD. Barley varieties were seeded on May 20, 2014, in 12 ha of non-irrigated land, and harvested on July 31, 2014, at the mid-dough growth stage. Consideration was taken to seed and harvest all barley varieties at the same maturity stage, to account for the environmental effect and growing duration period, although the optimum harvesting for those forages are different where CDC Cowboy is preferred to be harvested at late maturing while CDC Copeland and Xena are optimum at medium maturing as reported by Fedko (2015). The DM yield for all barley forages averaged 7.8 Mg/ha. The cost of production for each barley forage variety was $28.6 /wet Mg. All barley varieties

were cut at 10-cm above ground and chopped to 10-mm by a forage harvester. The chopped forages stored directly in a pile without using inoculation and covered with plastic for 40 d. The corn variety (hybrid 7213R, Pioneer, Warman, SK), was also grown at the University of Saskatchewan Research Farm and was seeded May 29, 2014, in 18.2 ha of non-irrigated land, and harvested on September 29, 2014, after reaching target heat units at about 33% DM. The DM yield of corn forage was 16.7 Mg/ha. The cost of corn forage was $30.3 /wet Mg. Corn forage was chopped at 1.95-cm theoretical cut length and processed with a 1-mm roller clearance and stored in a pile using double-layer cover without inoculation. All barley forages were ensiled for 40 d (then opened and used for beef cattle performance trial first), while corn silage was ensiled for 120 d (then opened for this dairy trial together with the barley silages). The storage period for barley silage when the dairy trial started was about 200 days.

The chemical composition of the silage as sampled at two different times from the silos before starting the experiment is presented in Table 5.1. Wet chemistry analyses were conducted commercially (Cumberland Valley Analytical Service, Inc. Maugamsville, MD). Samples from the barley and corn silages were taken, and frozen at -20°C until processing. On the day of processing, samples were thawed at 4°C. All samples were processed for measuring pH, lactate, ammonia, and volatile fatty acid (VFA) concentrations. For the ruminal *in vitro* study, the silage samples were collected and dried at 55°C for 48 h. These samples were a composite (4 kg of each) of three months of sampling, collected during our lactation trial. The dried samples were ground to pass a 1-mm screen (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK).

Table 5.1. Chemical and silage fermentation profiles for corn and barley silages

Items	Silages			
	Corn	Barley		
	P7213R-CS	Cowboy-BS	Copeland-BS	Xena-BS
Chemical profile, %DM[1]				
DM	33.8	27.5	31	30.5
Ash	5.7	8.3	7.4	8.2
EE	2.7	2.9	2.8	2.6
CP	9.1	11.7	11.2	10.7
Starch	26.4	8.7	13.3	15.3
NDF	44.4	55	51	48.4
ADF	25.3	34.3	31.9	30.0
Hemicellulose	19.1	20.7	19.0	18.4
Silage fermentation				
pH	3.89	4.03	4.13	4.27
Acetate, % DM	0.98	2.08	2.41	2.44
Propionate, % DM	ND*	0.045	0.083	0.315
Butyrate, % DM	ND	0.108	0.006	0.001
Lactate, % DM	7.43	9.17	6.80	4.98
Ammonia, % DM	0.15	0.31	0.27	0.28

[1]NDF = neutral detergent fiber; ADF = acid detergent fiber; hemicellulose = NDF – ADF
*ND = not detected

5.3.2. Experimental Cows and Diets

Mid-lactating multiparous Holstein cows (n = 4; average BW = 703 ± 78 kg; DIM = 101 ± 25; parities= 2.75 ± 0.83) were used in a 4 × 4 Latin square design. The cows were housed indoors individually in tie-stalls at the Rayner Dairy Research and Teaching Facility, University of Saskatchewan, Saskatoon, SK, Canada. The cows were fed diets containing 49% barley-based concentrate and 51% forage (on DM basis). The forage consisted of 10% alfalfa hay and 41% whole-plant silages. The four whole-plant silages were: corn silage (P7213R), CDC Cowboy BS, CDC Copeland BS, and Xena BS. The nutrient composition of the experimental forages is presented in Table 5.1. For barley silage, the diets were formulated to be similar in ingredient composition to associate changes in cow performance and total chewing activity with change in barley forage variety. For corn silage, the diet was formulated to have similar CP level to barley silage-based diets. The 30-h ruminal ivNDFD of corn silage, CDC Cowboy, CDC Copeland and Xena varieties were 32, 39, 38, and 30%, respectively. The diets were formulated using the NDS Professional (Version 3, RUM&N - NDS Professional, Reggio Nell'Emilia, Emilia-Romagna, Italy) to supply adequate metabolizable energy and metabolizable protein for dairy cattle in 120 days in milk producing 42 kg of milk.

Diets were offered as a TMR for ad libitum intake (approximately 5% orts on an as-fed basis) and refusals were removed and weighed before each morning feeding. The experimental period lasted for 23 d with each experimental period comprising 2 d diet change-over period, 16 d adaptation period, and 5 d sampling period. During the change-over period, diets were gradually adjusted to the subsequent diet by feeding the dairy cows diet content 25% of new

Table 5.2 Ingredients, chemical composition, and particle size distribution of the total mixed ration (TMR) in the four silage diets.

Items	Diets			
	Corn	Barley		
	P7213R-TMR[1]	Cowboy-TMR	Copeland-TMR	Xena-TMR
Feed ingredients, %DM				
Forage (alfalfa hay)	10.3	10.3	10.3	10.3
Corn silage P7213R	41.0	-	-	-
Barley silage	-	41.0	41.0	41.0
Barley grain	20.7	24.3	24.3	24.3
Corn grain	6.7	6.4	6.4	6.4
Canola meal	11.3	10.2	10.2	10.2
Soybean meal	4.3	2.1	2.1	2.1
Corn gluten meal	0.42	0.40	0.40	0.40
Corn distillers	0.82	0.78	0.78	0.78
Dairy premix[2]	1.33	1.34	1.34	1.34
RP10 palmitic	0.92	0.93	0.93	0.93
Molasses cane	0.54	0.54	0.54	0.54
PotMagSulfate	0.08	0.09	0.09	0.09
Sodium bicarbonate	0.53	0.54	0.54	0.54
Salt white	0.24	0.24	0.24	0.24
Limestone	0.91	0.72	0.72	0.72
Nutrient composition of TMR, %DM (n = 4)				
OM	93.3	92.1	93.1	93.6
CP	16.0	15.6	15.7	16.3
NDF	31.7	35.9	34.9	32.8
ADF	18.1	21.2	20.7	19.0
DM retained for TMR, % [3]				
19.0 mm	5.5	4.8	6.0	6.6
8.0 mm	25.0	38.3	41.4	40.6
Pan	69.5	56.9	52.6	52.8
pefps-2s	0.31	0.43	0.47	0.47
peNDFps- 2s, % of DM	9.7	15.5	16.5	15.5

[1]TMR = total mixed ration.
[2]formulated to contain: 16% Calcium, 6.5% Phosphorus, 10.4% Chloride, 6.3% Sodium, 2% Potassium, 0.4% Sulfur, 1,500 mg of Magnesium, 675 mg of Copper, 2500 mg of Zinc, 1,500 mg of Manganese, 3,000 mg of Iron, 20 mg of Selenium, 80 mg of Iodine, 30 mg of Cobalt, Vitamin A ('000 i.u) = 330, Vitamin D ('000 i.u.) = 60, Vitamin E (i.u.) = 1.
[3]Particle size distribution of diets was measured using the Penn State Particle Separator; pefps-2s = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996); peNDFps-2s = physically effective NDF determined as dietary NDF content multiplied by pefps-2

TMR + 75% of older TMR in the first day and 50% new TMR + 50% of older TMR in the second day. Cows were individually fed a TMR at 0900 and 1600 h and milked three times daily at 0430, 1230 and 1900 h. Samples of silages and hay were collected weekly, and the DM content was used to adjust diets to maintain the specified forage to concentrate ratio.

Feed ingredient and orts samples were collected during the last 5 d of each period and pooled by cow and by period for chemical analysis. Representative samples of the offered TMR were taken during sampling period for particle size determination. Samples for particle size determination were sieved using the 2-screen (19- and 8-mm) Penn State Particle Separator (PSPS; Lammers et al. 1996). The separated fractions were weighed and dried prior to calculation of physically effective NDF (peNDF) as described by Lammers et al. (1996). The method of the calculation of peNDF consisted of the sum of particles retained on sieves of 19- and 8-mm of PSPS multiplied by the NDF concentration of the diet. The amount of feed offered and refused was recorded in last 10 d of each period for feed intake determination.

Chewing activity (i.e. eating, ruminating) for cows was monitored over 24 h period on d 19 and 20 of each period using cameras with digital video recorder (QSEE, QC908 HD DVR, China). Each recording commenced before the morning feed. Observations were made at five-minute intervals for time spent eating and ruminating as described by Mugerwa et al. (1973).

The yield of milk was recorded at each milking during the last 10 d of each experimental period. Milk samples were collected in the last 3 d of each sampling period (21, 22 and 23 d), preserved with potassium dichromate, and tested at CanWest DHI (Edmonton, AB) for milk fat (%, fat yield), protein (%, protein yield), lactose (%, lactose yield), milk urea, and total solids (%). Somatic cell count was determined using a near infrared analyzer (Foss System 4000, Foss

Electric, Hillerød, Denmark). Solids-not-fat was calculated as follow: protein + lactose + other solids factor, where, in Canada, other solids factor is taken as 0.9759 % of milk. This comprises minerals plus components not measured by the near infrared analyzer. Minerals in milk are about 0.72%. Energy-corrected milk (ECM) was calculated according to Bernard (1997): ECM [kg] = (0.3246 × milk yield [kg]) + (12.86 × milk fat [kg]) + (7.04×milk protein [kg]). Intake of NEl was estimated from DMI and estimated NEl contents (NRC, 2001; Neal et al., 2014).

5.3.3. Cumulative Gas Production of Silages

Cumulative gas production of silages was measured in batch culture (mL/g OM) as described by Eun et al. (2007a). Approximately 0.5 g sample (1-mm) was weighed in triplicate into acetone washed filter bags (F57, Ankom Technology, Macedon, NY). The bags were sealed and placed into 100-ml serum bottles. Two different rumen-cannulated Holstein cows were used, and rumen fluids were collected 2 h after feeding from two rumen-cannulated Holstein cows.

The ruminal *in vitro* experimental runs were also performed on two different days. The cows were fed a total mixed ration twice daily at 0800 and 1600. The total mixed ration feed (kg DM feed/d) was formulated with 6.3 kg barley silage, 3.4 kg corn silage, 4.5 kg chopped alfalfa hay, and 13.8 kg barley-based concentrate to meet the NRC requirement for lactating dairy cows. Each serum bottle received 15 mL of strained ruminal fluid and 45 mL of McDougall's buffer, during which oxygen-free CO_2 was flushed. The bottles were capped with a 14-mm butyl rubber stopper and crimp sealed. Three blanks which contained 60 mL of medium without feed samples were used for each incubation time. Sealed bottles were incubated in an oscillating shaker at 39°C with an oscillation speed of 125 rpm for 30 h.

Headspace gas production was measured at 3, 6, 9, 12, 24 and 30 h by inserting a 23-gauge (0.6 mm) needle attached to a pressure transducer (Model PX4200-015GI; Omega Engineering, Inc., Laval, QC, Canada) connected to a visual display device (Data Track, Christchurch, UK). Pressure values, corrected for the gas released from the blanks, were used to generate volume estimates using the equation described by Mauricio et al. (1999).

5.3.4. Chemical Analysis

All the dried forages, silage and diet samples, were ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments Canada Ltd., Mississauga, ON, Canada) for use in chemical analyses. Dry matter (method 930.15), ash (method 942.05), crude fat (method 2003.05), and CP (method 990.03) were analyzed according to AOAC (2000). For estimation of CP, N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI). The acid detergent fiber (ADF), NDF, and acid detergent lignin (ADL) values were also analyzed according to Van Soest et al. (1991) method combined with an ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, USA). Amylase and sodium sulfite were used in the NDF analysis.

For silage fermentation measures, the frozen samples were thawed overnight at 4°C and used for measuring pH, VFA, lactate, and ammonia concentrations according to Zahiroddini et al. (2004) and Chaves et al. (2011). Concentration of VFA was quantified using a gas chromatograph (Model 5890, Hewlett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mm i.d., 1-µm Phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame ionization detection, and crotonic acid (trans-2-butenoic acid) was used as the internal

standard. Ammonia-N concentrations were measured according to the method described by Rhine et al. (1998).

5.3.5. Statistical Analysis

The Latin square data were analyzed using Proc Mixed SAS 9.4 (SAS Institute, Cary, NC). Data for intake, milk production and chewing activity were analyzed with the following model: $Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk}$, where: Y_{ijk} = the observation of the dependent variable; μ = the overall mean; T_i = the fixed effect of the i^{th} treatment; P_j = the fixed effect of the j^{th} period; C_k = the random effect of the k^{th} cow; e_{ijk} = the random error associated with the observation. The final variance and covariance structure model were selected based on AIC and BIC values in the variance and covariance structure assumption models testing results (Tempelman et al., 2001).

The cumulative gas production and ruminal ivNDFD data were analyzed as randomized complete block design. The statistical model used to analyze these parameters was $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where, Y_{ijk} = an observation of the dependent variable; μ = the population mean for the variable; T_i = the treatment effect, as a fixed effect; B_j = block effect of two ruminal *in vitro* runs with two fistulated dairy cows, as a random effect; e_{ijk} = the random error associated with the observation. Results are reported as least squares means, compared using the Tukey correction for multiple comparisons.

Significance was declared at $P \leq 0.05$ and a trend at $0.05 < P < 0.10$ unless otherwise stated. Comparison between the corn silage vs. average all barley silages-based diets was carried out using a SAS Contrast Procedure of SAS 9.4.

5.4. Results and Discussion

5.4.1. Chemical Profile of the Silage and Cumulative Gas Production of Silages

Chemical characteristics of barley and corn silages are presented in Table 5.1. Starch concentration was higher in CS (26.4 %DM), intermediate for Copeland and Xena BS (average 14.3 %DM), and lower for Cowboy BS (8.7 %DM). A previous study by Nair et al. (2016) reported a lower starch concentration for Cowboy (14.7%), compared to Copeland (21.0 %DM) and Xena (20.0 %DM). The fiber concentration differs between silages, where Cowboy BS had higher NDF concentration, compared with other barley forages (55 vs. 50 % DM). The fiber concentration for all barley varieties in the present study was greater than that reported by Nair et al. (2016). The lower starch and higher NDF concentration for barley silage varieties in the present study may be attributed to effect of maturity and/or environmental conditions that altered the chemical profile of the selected forages (Yu et al., 2004). In the present study, the DM yield of all barley forage varieties was lower than corn forage (7.8 vs 16.7 Mg/ ha). These results would suggest that new corn hybrids with low crop heat unit may produce high quality forage to meet the nutrient requirements of high producing dairy cattle. Further research is warranted to confirm this finding.

Ruminal *in vitro* gas production and ruminal ivNDFD of silages are presented in Table 5.3. There were no differences among barley silages in gas production during 30 h of incubations ($P > 0.10$). However, CS had higher cumulative gas production after 30 h of incubation than barley silage varieties (147 vs. 132 mL/g OM; $P = 0.03$). The average ivDMD in CS was greater than all barley silage varieties (60 vs. 57 %; $P = 0.02$).

Table 5.3. Cumulative gas production and *in vitro* nutrient digestibility for barley silages with different digestible carbohydrates contents compared with corn silage

Items	Silages[1]				SEM[1]	*P* value	*P* value Contrast
	Corn (C)	Barley (B)					
	P7213R	Cowboy	Copeland	Xena			C × B
Asymptotic cumulative gas volume, mL/g OM							
3 h	18.9	24.8	20.8	17.6	6.06	0.37	0.53
6 h	45.0	41.9	43.4	40.4	6.55	0.86	0.51
9 h	69.7	57.7	60.8	58.7	4.96	0.23	0.05
12 h	86.9	73.2	75.9	72.5	4.73	0.11	0.02
24 h	131.2	117.2	119.0	113.5	6.91	0.12	0.02
30 h	146.6	133.2	134.1	128.7	6.36	0.16	0.03
In vitro ruminal nutrient digestibility, %							
DM-30 h	59.5*a*	54.8*b*	57.5*ab*	56.5*ab*	1.12	0.03	0.02
NDF-30 h	32.4*ab*	39.0*a*	38.0*ab*	29.7*b*	4.67	0.02	0.26

[1]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).

The greater digestibility and gas production of CS are attributed to the large portion of non-structural carbohydrates in CS compared with barley silage varieties. The difference in ensiling time between CS and BS may have some effect in ivDMD (Der Bedrosian et al., 2012). The results showed Cowboy BS had a higher ruminal ivNDFD compared to Xena BS (39 vs. 29 %, $P = 0.02$; Table 5.3). It has been reported that forage fiber digestibility could be influenced by many variables, such as the stage of maturity (Yu et al., 2004), soil type, climate conditions, growing conditions (Chow et al., 2008). To our opinion, the stage of maturity is the most important. A previous study also showed ruminal ivNDFD was higher in Cowboy BS than Xena and Copeland BS (Nair et al., 2016).

5.4.2. Feed Intake

The results in the current study (Table 5.4) showed the cows fed corn silage-based diet tended to improve the DM and OM intakes ($P < 0.10$), compared with cows fed barley silage-based diets. These findings are in agreement with Benchaar et al. (2014), where they found that replacing barley silage-based diet with corn silage-based diet is associated with increase in DMI in dairy cattle. The higher DMI in corn silage could be attributed to the lower and higher NDF and starch concentrations, respectively, and therefore higher energy density in the corn silage-based diet than barley silage-based diets. The corn silage-based diets had a lower peNDF compared to barley silage diets (9.7 vs. 15.8 % DM, Table 5.2); the reduction in peNDF would in turn enhance feed intake. Fischer et al. (1994) observed greater DMI when multiparous cows were fed short alfalfa silage, compared with long alfalfa silage.

Intakes of DM, OM, CP and NDF were not affected by the barley silage-based diets (Table 5.4). Feeding dairy cattle diets with high fiber concentrations is likely to limit feed intake by physical fill and ruminal distension. Oba and Allen (1999b) found forages with higher ruminal ivNDFD could increase DM intake by 0.17 kg for every one-unit increase in ivNDFD. The lack of effect of Cowboy BS-TMR with increased ruminal ivNDFD on DM intake in the present study is in agreement with previous short-term studies on dairy cows fed barley silage-based TMR or corn silage-based TMR with higher ruminal ivNDFD (Weiss and Wyatt 2006; Chow et al., 2008; Holt et al., 2010, 2013; Oba and Swift, 2014). The higher indigestible fiber content of Cowboy BS relative to Xena BS would be the main reason of the limited effect of this variety on DMI.

5.4.3. Body Weight gain, Milk Production and Milk Composition

In the present study, there was no effect of barley and corn silage-based diets on BW gain (averaged 0.29 kg/cow/d; $P = 0.14$, Table 5.5). The effect of forages with enhanced ivNDFD on BW gain has been inconsistency in the literature. Some studies have found that forage with higher ivNDFD had no effects on BW gain (Aydin et al., 1999; Ballard et al., 2001). However, other studies reported that cows fed corn silage (Block et al., 1981; Castro et al., 2010), or barley silage (Chow et al., 2008) with the highest NDFD have partitioned extra metabolizable energy to BW gain instead of increasing milk production. Tine et al. (2001) noted that cows did not improve their production levels equally to the higher energy intake when the cows fed corn silage with enhanced ivNDFD.

Table 5.4. Feed intake in lactating dairy cows fed diets containing corn or barley silages

Items	Diets				SEM[2]	*P* value	*P* value Contrast
	Corn (C)	Barley (B)					C × B
	P7213R-TMR[1]	Cowboy-TMR	Copeland-TMR	Xena-TMR			
Intake, kg/cow/d							
DM	28.08	24.76	24.93	27.46	1.350	0.14	0.10
OM	25.61	23.04	23.59	25.62	1.348	0.13	0.07
CP	4.03	3.72	3.63	4.04	0.198	0.36	0.31
NDF	8.94	8.88	8.71	9.04	0.475	0.30	0.55

[1]TMR = total mixed ration
[2]SEM = standard error of means.

Table 5.5. Milk yield, milk compositions and predicted energy values in lactating dairy cows fed diets containing corn or barley silages

Items	Diets				SEM[2]	*P* value	*P* value Contrast
	Corn (C)	Barley (B)					C × B
	P7213R-TMR[1]	Cowboy-TMR	Copeland-TMR	Xena-TMR			
BW gain, kg/cow/d	0.44	0.29	0.35	0.08	0.126	0.14	0.11
Milk Yield, kg/d[3]							
Milk	40.14*a*	35.25*b*	35.94*b*	34.78*b*	1.711	0.04	0.01
FCM	42.16	36.53	37.25	37.29	2.455	0.08	0.02
ECM	40.21*a*	36.35*b*	36.16*b*	36.10*b*	1.994	0.03	0.06
Fat	1.51	1.33	1.35	1.36	0.116	0.35	0.10
Protein	1.17	1.02	1.06	1.02	0.049	0.06	0.02
Lactose	1.78*a*	1.52*b*	1.56*b*	1.60*b*	0.068	0.04	<0.01
Solids-not-fat	3.37*a*	2.84*b*	2.92*b*	3.02*b*	0.129	0.05	0.01
Milk composition[4]							
Fat, %	3.46	3.70	3.71	3.89	0.221	0.37	0.20
Protein, %	2.91	2.89	2.93	2.93	0.062	0.91	0.91
Lactose, %	4.42	4.38	4.45	4.45	0.058	0.77	0.86
Total solids, %	11.91	11.88	12.18	12.25	0.241	0.24	0.36
Solids-not-fat, %	8.31	8.26	8.35	8.35	0.111	0.80	0.91
MUN, mg/dL	12.69	12.83	13.03	12.95	1.096	0.99	0.83
SCC, cells /ml	105.2	40.3	112.2	39.7	18.48	0.27	0.25
Efficiency							
FCM/DMI	1.61	1.37	1.43	1.44	0.079	0.14	0.07
ECM/DMI	1.57	1.32	1.40	1.39	0.071	0.12	0.03
Predicted energy values, (NEl; NRC-2001)							
NEl, Mcal/kg DM	1.51	1.36	1.44	1.38	0.063	0.23	0.09

[1]TMR = total mixed ration

[2]SEM = standard error of mean; Means with different letters in the same row differ ($P < 0.05$).

[3]FCM = fat-corrected milk; ECM = Energy-corrected milk.

[4]MUN = milk urea nitrogen; SCC = somatic cell count.

They concluded that the metabolizable energy primarily partitioned to body tissues by 45%, heat production by 36%, and the least portion was toward to the milk synthesis by 18%.

Cows fed CS had higher milk, FCM, ECM, protein, lactose, and solids-not-fat yields than cows fed barley silages (Cowboy BS-TMR, Copeland BS-TMR, Xena BS-TMR; $P < 0.05$; Table 5.5). The cows fed a corn silage-based diet had improved feed efficiency compared with cows fed barley silage-based diets (1.57 vs. 1.37 ± 0.07 ECM/DMI, $P = 0.03$, Table 5.5). Due to differences in DMI and yields of milk and its components, predicted NEl was higher in Cows fed CS than the cows fed BS diets (1.51 vs 1.39 Mcal/kg of DMI, $P = 0.09$). The higher milk yield is attributed to the higher *in vitro* DM digestibility in CS, compared to cows fed barley silage-based diets. The difference in ensiling time between CS and BS might affect ivDMD and consequently might also affect milk production (eg. milk, FCM, ECM; Der Bedrosian et al., 2012).

There was no difference among all forages in milk fat concentration. There was no effect of P7213R CS-TMR on protein and milk urea N compositions compared to other diets. However, the cows fed P7213R CS-TMR produced more milk protein, compared to barley silage diets (1.17 vs. 1.03 kg/d; $P = 0.02$). Benchaar et al. (2014) found replacing barley silage with corn silage increased the protein concentration and yield. They attributed these increases to higher N utilization by animals that fed corn silage-based diet compared to the cows that fed barley silage-based diets.

There were no differences among all barley silage-based diets on milk yield or milk composition and feed efficiency (Table 5.5). Oba and Allen (1999b) found a one-unit increase in ruminal ivNDFD equated to 0.25 kg/d increase in 4% FCM yield. The lack effect of Cowboy BS-TMR on milk production or composition and feed efficiency are attributed to its limited

effect on DM intake. Tine et al. 2001 observed brown midrib corn silage (bmr) with higher ruminal ivNDFD could provide greater energy concentration to dry cows at maintenance, due to the higher fiber digestibility, but this effect was alleviated when cows fed bmr corn silage during lactation. Those authors suggested any increases in milk production because of feeding bmr corn silage should be driven by increasing DM intake. They also suggested most of additional metabolizable energy due to feeding bmr corn silage diets was partitioned toward tissue energy gain but not milk energy. Oba and Allen (1999b) concluded dairy cows took more advantage from the increased NDF digestibility of corn silage because it allowed them to consume more DMI that supported higher milk yield.

There was no effect of the barley silage-based diets on milk fat percentage or milk fat yield ($P > 0.10$). Milk fat percentage is primarily related to the supply of digestible carbohydrates concentration in the diets. The amount of forage, NDF concentration, and peNDF has been also reported to affect rumen microbiota and milk fat percentage (Santos, 2002). In the current study, all barley silages have similar milk fat concentration because they have similar peNDF content.

5.4.4. Eating and Ruminating

Time spent eating, ruminating, or total chewing activity, either expressed as min/d, min/kg DM or, min/kg NDF were not affected by feeding cows barley silage with increased ruminal ivNDFD (Table 5.6). The effects of increased ruminal ivNDFD of forages on eating and ruminating time are not consistent in literature; higher ruminal ivNDFD of forages was revealed to influence positively (Zebeli et al., 2006), negatively (Taylor and Allen, 2005), or not (Oba and Allen, 2000) the chewing activities in dairy cattle. The lack effect of Cowboy BS-TMR with the

Table 5.6. Total chewing activity in lactating dairy cows in lactating dairy cows fed diets containing corn or barley silages

Items	Diets				SEM[1]	*P* value	*P* value Contrast
	Corn (C)	Barley (B)					
	P7213R-TMR	Cowboy-TMR	Copeland-TMR	Xena-TMR			C × B
Eating							
Min/d	318	321	297	316	26.5	0.90	0.78
Min/kg DM	11.9	11.9	11.6	12.4	0.83	0.90	0.96
Min/kg NDF	37.9	32.8	33.2	38.0	2.36	0.32	0.22
Ruminating							
Min/d	507.5	590.5	590	554.8	31.0	0.26	0.07
Min/kg DM	19.3	22.4	22.6	22.1	2.11	0.50	0.16
Min/kg NDF	60.9	62.6	64.9	67.3	6.19	0.72	0.50
Total chewing activity							
Min/d	826	911	887	871	37.2	0.46	0.18
Min/kg DM	31.3	34.2	34.3	34.5	2.46	0.68	0.27
Min/kg NDF	98.7	95.3	99.2	104.3	6.99	0.73	0.89

[1]SEM = standard error of mean.

higher ruminal *in vitro* digestible fiber concentration on chewing activity is attributed to its limited effect on the DM intake.

In this study, P7213R CS-TMR had a lower level of peNDF (by 48%; Table 5.2) compared with the barley silage-based TMR. The mean time of ruminating tended to lower in P7213R CS-TMR compared to other diets (508 vs. 578 min/d; P =0.07). This reduction in ruminating time reflects the peNDF level in P7213R CS-TMR compared to other diets. Yang and Beauchemin (2006) observed that reducing peNDF by decreasing the chop length of barley silage from 9.5 to 4.8 mm did not affect DMI or time spent eating, but reduced time spent ruminating (by 80 min/d).

5.5. Conclusion

This study showed that cows fed corn silage produced significantly more milk and had greater feed efficiency than cows fed barley silages. Cows fed barley silage with relatively higher ruminal ivNDFD did not show significant difference from the cows fed other barley silage varieties with lower ruminal ivNDFD in milk yield and total chewing activity. The barley forage selected, based on its higher ruminal *in vitro* NDF digestibility did not correspond with a greater effect on lactation performance due to its limited effect on feed intake compared to other barley forage varieties. It is a challenge to select barley forage based on single time point NDF digestibility; the year-to-year effect could alter NDF and rumen fermentable neutral detergent fiber levels. It is necessary to breed new hybrids with higher fermentable neutral detergent fiber concentration that would tolerate any climatic condition. These results indicate feeding a corn silage-based diet increased energy density (less lignin and higher starch) in the rumen, compared to other barley silage-based diets.

6. Comparison of Barley Silages with Varying Digestible Fiber Content to Corn Silage on Rumen Fermentation Characteristics and Microbial Protein Synthesis Using Rumen Simulation Technique

6.1. Abstract

The objectives of this study were to assess the magnitude of differences among barley silages with different *in vitro* neutral detergent fiber digestibility (ivNDFD) in comparison with corn silage in (1) carbohydrate digestibility, (2) rumen fermentation characteristics, and (3) microbial protein synthesis using rumen simulation technique (RUSITEC). The experiment was carried out in a randomized complete block design with four treatments. The four whole-plant silages utilized in this study were P7213R-TMR = corn silage (30-h ivNDFD = 32%), Cowboy-TMR = barley silage with high ivNDFD (30 h ivNDFD = 37%), Copeland -TMR = barley silage with intermediate ivNDFD (30 h ivNDFD = 31%), and Xena-TMR = barley silage with low ivNDFD (30 h ivNDFD = 29%). Results from RUSITEC showed that nutrient digestibility, rumen fermentation characteristics, and microbial protein synthesis did not differ among diets that contained different varieties of barley silage ($P > 0.10$). However, P7213R -TMR tended to have a higher microbial protein yield than all barley silage diets ($P = 0.06$). These results show higher ivNDFD of barley silage may not necessarily correspond with greater impact on rumen fermentation and microbial protein synthesis. However, feeding the corn silage had higher

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microbial protein synthesis in the RUSITEC and might enhance the dairy cattle performance compared with barley silage.

6.2. Introduction

During lactation, it is important to maximize digestible carbohydrate intake and/or improve fiber digestibility from forage, as the energy demand for maintenance and milk production often exceed the amount of energy that high producing cows consume, particularly in early lactation (NRC 2001). Using high-quality silage with enhanced *in vitro* NDF digestibility (ivNDFD) in dairy cattle rations could reduce physical gut fill, allowing cattle to consume more feed and produce more milk (Oba and Allen 1999a, b).

Whole-crop barley (*Hordeum vulgare* L.) silage is the primary forage source for dairy producers in Western Canada. Although there are many barley varieties available, information on their nutritional quality are limited. Recently, Nair et al. (2016) had selected seven varieties of barley silage that are grown in Western Canada, and found significant differences between them regarding chemical composition and ivNDFD for instance, Cowboy barley silage had exhibited the highest NDF and lowest starch content compared to other six barley silage varieties. Moreover, Cowboy barley silage had shown the greatest ivNDFD followed by, Copeland, Falcon and Metcalfe barley silages, while Legacy and Xena barley silages had the lowest ivNDFD. Previous work has shown that average 5-unit difference in ivNDFD was associated with a 0.85 kg/d increase in DMI and 1.25 kg/d increase in 4% fat-corrected milk yield (Oba and Allen, 1999b). Effects of forage ivNDFD on milk production have been studied for corn silage, and corn silage hybrids with enhanced ivNDFD have been developed and are commercially available in the USA (Oba and Allen, 2011). However, there is limited information on the effect

of feeding barley silage with enhanced ivNDFD on dairy cattle performance, particularly for relatively new barley varieties.

The corn grown in Canadian Prairies has a shorter growing season and lower growing temperature compared to the corn grown in warmer region i.e. The USA. Many corn varieties have been developed in Western Canada with lower crop heat unit (2100), one of which (P7213R) exhibited a higher yield and available energy content as compared to other corn varieties grown in Western Canada (Abeysekara et al. 2013a, b). However, no metabolic or *in vitro* studies have been done on this potentially short-season corn variety. The main objective of this study was to investigate the differences among barley silages produced from different varieties in comparison with corn silage. The specific objectives were as follow 1) to predict carbohydrate and total-tract NDF digestibility, 2) to assess rumen fermentation characteristics, and 3) to characterize microbial protein synthesis, when fed as silage alone or in a total mixed ration (TMR) using the RUSETIC.

6.3. Materials and Methods

6.3.1. Preparation of Silage

Three barley varieties were selected based on the screening study by Nair et al. (2016) on seven different varieties of barley silage using 30 h ivNDFD as an approach for ranking the silages. The seven different varieties ('CDC Cowboy', 'Falcon', 'Metcalfe', 'CDC Copeland', 'Conlon', 'Xena', and 'Legacy') harvested for making silage at the mid-dough stage of maturity were collected within 2 years (2012 and 2014; n = 80) from dairy and beef operators in Saskatchewan and Alberta. These barley varieties exhibited a significant difference in 30 h ivNDFD that ranged from 28% to 37%. The varieties with the highest, lowest, and intermediate

NDFD after 30 h of incubation were selected for this study. All varieties were of the two-row type with an ivNDFD ranking based on Nair et al. (2016) of 'CDC Cowboy' (37%, high NDFD), 'CDC Copeland' [31%; intermediate NDFD], and 'Xena' [29%; low NDFD]. The P7213R corn silage (CS) with 32% NDFD was chosen based on its nutritive contents and NDF degradation rate as noted by Abeysekara et al. (2013b).

The three barley varieties with different digestible fiber contents were grown at the University of Saskatchewan research farm in 2014 under dry land conditions. The detailed seeding, growing and harvesting management were described before in Refat et al. (2018). Samples were prepared by sampling the silages from various locations in the pile. Two samples from each silage variety were taken such that each silage was represented by two replicates. One subsample from each replicate was used for the chemical, *in situ* indigestible NDF (iNDF) and silage fermentation analyses. For the chemical and *in situ* iNDF analyses, the samples were oven-dried at 55 °C for 48 h. The dried samples were ground through a 1 mm screen (Christy & Norris 8″ Laboratory Mill, Christy Turner Ltd., Suffolk, UK). The detailed chemical analysis is presented in the following section. During the lactation trial, silages were sampled weekly and composited, with each sample around 4 kg collected during 4 months sampling. The composite sample was used in the RUSITEC study. The dried samples were ground to pass a 4-mm screen (Arthur Thomas Co., Philadelphia, PA, USA).

6.3.2. Experimental Diets and Design

Two units of RUSITEC apparatus (Czerkawski and Breckenridge 1977) equipped with eight 920 mL volume anaerobic fermenters per unit were used. Each fermenter had an infusion in-port and an effluent out-port. Collectively, the fermenters were placed in a water bath at

39 °C. Rumen fluid inoculum (i.e., solid and liquid) collected at 2 h after feeding from three ruminally fistulated cows fed a diet containing 75% barley silage, 20% dry-rolled barley grain, and 5% mineral and vitamin supplement (DM basis). All experimental procedures with the animals were performed following the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

The experimental design was fitted in a randomized complete block design with two blocks (RUSITEC apparatuses), 16 experimental units (fermenters), and four treatments. The four treatments were randomly assigned to fermenters within each RUSITEC apparatus so that each treatment had four replicates (fermenters). The experimental diet contained 49% barley-based concentrate, 10% alfalfa hay, and 41% silage. The four whole-plant silages were P7213R-TMR = new short-season corn silage (P7213R), Cowboy-TMR = barley silage with high ivNDFD, Copeland-TMR = barley silage with intermediate ivNDFD, and Xena-TMR = barley silage with low ivNDFD. The diets were formulated with the assumption that the dairy cows would consume approximately 28 kg DM, and with the same forage to concentrate ratio among the diets (Table 6.1). The diets were formulated using the NDS software with the updated CNCPS 6.5 (RUM&N, Reggio Nell'Emilia, Emilia-Romagna, Italy) to supply adequate metabolizable energy and metabolizable protein for 680 kg dairy cattle (120 d in milk) that produce 42 kg of milk with 3.95% milk fat and 3.25% milk crude protein (CP).

Each fermenter initially filled with 200 mL of warm buffer (artificial saliva, pH 8.2; McDougall 1948), 700 mL of rumen fluid, one nylon bag containing 20 g of wet solid rumen digesta (source solid-phase bacteria), one nylon bag containing silage only, and one nylon bag containing alfalfa hay and

Table 6.1. Ingredients and chemical composition of the total mixed ration (TMR) in the four silage diets fed to rumen simulation technique

Items	Diets			
	Corn	Barley		
	P7213R-TMR[1]	Cowboy-TMR	Copeland-TMR	Xena-TMR
Ingredient, %DM				
Forage (alfalfa hay)	10.3	10.3	10.3	10.3
Corn silage P7213R	41.0	-	-	-
Barley silage	-	41.0	41.0	41.0
Barley grain	20.7	24.3	24.3	24.3
Corn grain	6.7	6.4	6.4	6.4
Canola meal	11.3	10.2	10.2	10.2
Soybean meal	4.3	2.1	2.1	2.1
Corn gluten meal	0.42	0.40	0.40	0.40
Corn distillers	0.82	0.78	0.78	0.78
Dairy premix[2]	1.33	1.34	1.34	1.34
RP10 palmitic	0.92	0.93	0.93	0.93
Molasses cane	0.54	0.54	0.54	0.54
PotMagSulfate	0.08	0.09	0.09	0.09
Sodium bicarbonate	0.53	0.54	0.54	0.54
Salt white	0.24	0.24	0.24	0.24
Limestone	0.91	0.72	0.72	0.72
Nutrient composition of TMR, %DM				
OM	93.3	92.1	93.1	93.6
CP	16.0	15.6	15.7	16.3
NDF	31.7	35.9	34.9	32.8
ADF	18.1	21.2	20.7	19.0
Fermentation profile of silage				
pH	3.89	4.03	4.13	4.27
Acetate, %DM	0.98	2.08	2.41	2.44
Propionate, %DM	0.00	0.045	0.083	0.315
Butyrate, %DM	0.00	0.108	0.006	0.001
Lactate, %DM	7.43	9.17	6.80	4.98
Ammonia-N, %DM	0.15	0.31	0.27	0.28

[1]TMR = total mixed ration

[2]Formulated to contain (DM basis): 16% Calcium, 6.5% Phosphorus, 10.4% Chloride, 6.3% Sodium, 2% Potassium, 0.4% Sulfur, 1,500 mg of Magnesium, 675 mg of Copper, 2500 mg of Zinc, 1,500 mg of Manganese, 3,000 mg of Iron, 20 mg of Selenium, 80 mg of Iodine, 30 mg of Cobalt, Vitamin A ('000 i.u) = 330, Vitamin D ('000 i.u.) = 60, Vitamin E (i.u.) = 1.

concentrate. After the first day, the bag containing solid rumen digesta was removed and replaced with one bag containing silage and one containing alfalfa hay and concentrate. Thereafter, two bags were replaced every morning so that each bag remained in the fermenter for 48 h. Artificial saliva was infused into the fermenter continuously at a dilution rate of 2.9% per hour.

The experimental period consisted of 16 d including 10 d of adaptation, 6 d of sampling, and data collection. Effluent was collected into a 1 L flask, and the total volume was recorded daily. Gas produced (GP) from each fermenter was collected into a reusable 2 L vinyl collection bag (Curity®; Conviden Ltd., Mansfield, MA, USA) attached to each effluent flask. Daily total GP was recorded using a gas meter (Model DM3A, Alexander-Wright, London, England, UK). Daily pH of each fermenter was recorded at the time of feed-bag exchange with a pH meter (B20PI, Symphony Benchtop Meters; VWR, Edmonton, AB, Canada). The DM digestibility was determined after 48 h of incubation from days 11 to 14 of the experimental period. All the bags were rinsed under cold water, dried at 55 °C for 48 h (AOAC 1995; method 930.15) and weighed to determine DM digestibility. From days 11 to 14 of the experimental period, liquid effluent was collected into 1 L flasks and samples were taken for VFA and NH_3-N analysis.

Microbial protein synthesis was estimated as described by Wang et al. (2001). Bacteria in the fermenters were labeled using ^{15}N. The $(NH_4)_2SO_4$ in the McDougall's buffer solution was replaced on day 10 with ^{15}N-enriched $(NH_4)_2SO_4$ (Sigma Chemical Co., St. Louis, MO, USA; minimum ^{15}N enrichment 1 g L^{-1}) until the end of the experiment. Before labeling on day 10, effluent and feed residue were collected to determine the background ^{15}N. On days 15 and

16, ^{15}N concentration of feed particle-associated, feed particle-bound, and effluent bacterial fractions were prepared from feed bag residues and effluent.

6.3.3. Predicting Rumen and Total-Tract Fiber Digestibility

The digestion rates (Kd, h^{-1}) of potentially digestible NDF content (pdNDF; CB3 Kd) of the forages were estimated using 48 h NDFD, a fixed lag of 3 h and uNDF-288, following the method of Van Amburgh et al. (2003). The available NDF fraction Kd rates derived from *in vitro* analysis were entered in the CNCPS 6.5 model for predicting RDCB3 which was calculated according to the following equation: CB3 subfractions × Kd /(Kd + Kp), where Kp is the fractional rate of passage. Total degraded ruminal carbohydrates fraction (TRDC) and total escaped carbohydrates fraction (TRUC) were calculated as described by Tylutki et al. (2008). The total-tract CB3 was estimated assuming intestinal digestibility of available NDF (CB3) amount escaping rumen digestion was 20% (Sniffen et al. 1992; Fox et al. 2004).

6.3.4. Chemical Analysis

The dried forage and diet samples were ground through a 1 mm screen (Christy & Norris 8″ Laboratory Mill, Christy Turner Ltd., Suffolk, UK) for use in chemical analyses. Concentrated samples were ground to pass through a 1 mm screen using a Retsch ZM100 grinder (Retsch, Haan, Germany). Dry matter (method 930.15), ash (method 942.05), crude fat (method 2003.05), and CP (method 990. 03) were analyzed according to AOAC (2000). The estimation of CP and N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA). Acid detergent fiber (ADF) was analyzed according to AOAC (2000; method 973.18). Neutral detergent fiber (NDF) and acid detergent lignin (ADL) were analyzed according to Van Soest et al. (1991). Amylase was used in the NDF analysis but sulfite was omitted. The

total soluble crude protein (SCP) was analyzed by incubating the sample with a bicarbonate–phosphate buffer and filtering it through Whatman 54 filter paper. The ADF insoluble protein and NDF insoluble protein values were measured as described by Licitra et al. (1996). Ethanol soluble carbohydrate and starch were determined using the methods described by Hall et al. (1999). Non-structural carbohydrate content, including sugars, organic acids, and other reserve carbohydrates, was estimated using non-fiber carbohydrate (NFC) and calculated according to NRC (2001). Total carbohydrate was calculated as $100 - (\%EE + \%CP + \%ash)$, where EE is ether extract, and NFC was calculated as
$NFC = 100 - [\%CP + (\%NDF - \%NDICP) + \%EE + \%ash]$, where NDICP is the neutral detergent insoluble protein. Feed residues obtained from RUSITEC were ground through a 1 mm screen (standard model 4, Arthur Thomas Co., Philadelphia, PA, USA). Total N was determined using a combustion analyzer (990.03; AOAC 2006) and atom percent excess of ^{15}N.

For silage fermentation measures, the frozen samples were thawed overnight at 4 °C and a subsample was used for measuring pH, VFA, lactate, and ammonia concentrations according to Zahiroddini et al. (2004) and Chaves et al. (2012). Silage pH was measured using an Accumet Research AR 50 dual channel pH meter (Fisher Scientific, Waltham, MA, USA). Concentration of VFA was quantified using a gas chromatograph (5890, Hewlett-Packard Lab, Palo Alto, CA, USA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA, USA) and flame ionization detection, and crotonic acid (trans-2-butenoic acid) was used as an internal standard. Ammonia N concentrations were measured according to the method described by Rhine et al. (1998).

The iNDF concentration of each feed sample was determined following *in situ* incubations for 288 h in the rumen using two lactating dairy cows fed a TMR twice a day at 0800 and 1600 with 5% refusal and water was available ad libitum. The TMR was formulated on a DM basis with barley silage, chopped alfalfa hay, and barley-based concentrate to meet the nutrient requirements according to NRC (2001). All animals were housed at the Rayner Dairy Research and Teaching Facility farm, University of Saskatchewan, Saskatoon, SK, Canada, and cared for according to the guidelines of the CCAC (2009). Two experimental runs in each time were carried out. Samples of 3 g were weighed in triplicate into 5 cm × 10 cm size custom made *in situ* bags (6 µm pore size, part no. 07-6/5, Sefar America Inc., Depew, NY, USA) and were randomly assigned to cows. After removal from the rumen, the bags were rinsed, oven-dried at 55 °C for 48 h and subsequently the NDF of residues was calculated according to Van Soest et al. (1991).

6.3.5. Statistical Analysis

The parameters measured in the RUSITEC trial were analyzed as randomized complete block design using the MIXED procedure of SAS version 9.4, in which experimental units (fermenters) had been grouped into two blocks (because two RUSITEC apparatus unit) and the four treatments were randomly assigned to two blocks such that each treatment has two replicates (fermenters) in each block (RUSITEC apparatus). Total experiments units for each treatment are four (four fermenters: two fermenters from RUSITEC UNIT I as block 1 and other two fermenters from RUSITEC UNIT II as block 2). The statistical model used to analyze the measured parameters in RUSETIC was $Y_{ijk} = \mu + T_i + R_j + e_{ijk}$, where Y_{ijk} was the dependent

variable, μ was the general mean, T_i was the fixed effect of treatment ($i = 4$), R_j was the random effect of RUSITEC apparatus unit ($j = 2$, two different units), and e_{ijk} was the residual error.

The predicted RDCB3, TRDC, TRUC, and total-tract CB3 were analyzed as randomized complete block design using the MIXED procedure of SAS version 9.4. The statistical model used to analyze the predicted parameters was $Y_{ijk} = \mu + T_i + R_j + e_{ijk}$, where Y_{ijk} was the dependent variable, μ was the general mean, T_i was the fixed effect of treatment ($i = 4$), R_j was the random effect of RUSITEC apparatus unit ($j = 2$), and e_{ijk} was the residual error. Total experiments units for each treatment are four (four fermenters: two fermenters from RUSITEC UNIT I as block 1 and other two fermenters from RUSITEC UNIT II as block 2).

Three model assumptions in randomized complete block design were checked by doing residual analysis with SAS: (1) the treatments had a common variance, (2) the model residual data were normally distributed, and (3) the random block effects were normally distributed. The normality check was carried out by using PROC UNIVARIATE with Normal and Plot options in SAS. Contrast statements were performed to detect differences among barley silages (Cowboy-TMR = barley silage with high ivNDFD, Copeland-TMR = barley silage with intermediate ivNDFD, and Xena-TMR = barley silage with low ivNDFD), and between barley silage and corn silage [corn × average (Cowboy-TMR, Copeland-TMR, and Xena-TMR)]. Statistical significance was declared at $P < 0.05$ and trends were noted at $0.05 \leq P \leq 0.10$.

6.4. Results and Discussion

6.4.1. Characteristics of Corn Silage and Barley Silage

The protein and carbohydrate profiles of the barley and corn silages are presented in Table 6.2. Barley silage and corn silage composition differed as expected and there were also some differences in the chemical composition among all barley silage varieties. The CP content among barley silage varieties appeared to be similar (averaged 11.2% DM). However, CP content differed between barley and corn silages, in that the barley silage varieties had greater CP than corn silage by 21%. The CP of barley and corn silage in the present study is similar to values reported in previous studies (Mustafa et al. 2000; Chow et al. 2008; Chaves et al. 2012; Abeysekara et al. 2013b). In the present study, CS had less SCP than all barley silage varieties. Starch content also varied between barley and corn silages, with CS as the greatest at 26% of DM, whereas Cowboy barley silage had the least starch content at 8.7% DM. There were also differences among barley silage varieties in starch content with Cowboy barley silage starch content lower than Xena barley silage by 55%. On the other hand, Cowboy barley silage had the highest NDF and Xena barley silage had the lowest NDF (49% vs. 43% DM, respectively). All barley silages showed higher ADF compared with the corn silage, 35% vs. 26% DM, respectively. In the present study, iNDF concentration (%DM) was highest in the Cowboy barley silage (25.8% DM), intermediate in the Copeland barley silage, and lowest was reported in the Xena barley silage (17.8% DM).

6.4.2. Rumen Fermentation

Rumen fermentation characteristics in RUSITEC are given in Table 6.3. The diet with Cowboy barley silage showed no effects on rumen fermentation characteristics in comparison to

Table 6.2. Protein and carbohydrate chemical profiles and *in situ* undigested NDF-288 h (mean ± standard deviation) for barley and corn silages.

Items	Silages			
	Corn	Barley		
	P7213R	Cowboy	Copeland	Xena
Basic chemical profile of silage[1]				
DM, %	33.80 ± 0.66	27.51 ± 1.21	30.95 ± 0.98	30.50 ± 0.17
Ash, %DM	5.71 ± 0.49	8.39 ± 0.11	7.43 ± 0.30	8.24 ± 0.93
EE, %DM	2.66 ± 0.23	2.88 ± 0.06	2.82 ± 0.16	2.61 ± 0.04
Protein profile[2]				
CP, %DM	9.05 ± 0.21	11.70 ± 0.85	11.20 ± 0.14	10.65 ± 0.78
SCP, % CP	51.85 ± 3.47	65.40 ± 0.70	64.35 ± 4.60	59.45 ± 4.29
ADICP, %CP	9.95 ± 1.48	9.25 ± 0.49	10.05 ± 1.48	11.80 ± 1.98
NDICP, %CP	12.00 ± 1.31	11.55 ± 2.47	11.65 ± 0.35	12.40 ± 1.41
Carbohydrates profile, %DM[3]				
CHO	82.59 ± 0.51	77.03 ± 0.91	78.56 ± 0.61	78.51 ± 1.68
NFC	44.03 ± 0.92	27.83 ± 3.28	35.43 ± 0.26	32.89 ± 1.12
NSC	27.45 ± 2.33	11.10 ± 0.71	15.80 ± 0.99	17.22 ± 0.97
Starch	26.35 ± 2.76	8.65 ± 0.35	13.25 ± 1.77	15.25 ± 1.99
NDF	38.56 ± 0.41	49.20 ± 2.47	43.13 ± 0.34	45.63 ± 2.80
ADF	26.25 ± 0.92	35.95 ± 2.62	33.95 ± 0.35	34.15 ± 2.19
ADL	2.85 ± 0.08	5.20 ± 0.18	4.85 ± 0.16	4.50 ± 0.32
Hemicellulose	12.31 ± 1.31	13.25 ± 0.15	9.18 ± 0.70	11.48 ± 0.60
Cellulose	23.39 ± 0.83	30.74 ± 2.43	29.10 ± 0.19	29.70 ± 1.87
In situ undigested NDF (288-h)[4]				
uNDF-288, %DM	15.50 ± 1.51	25.80 ± 0.30	20.87 ± 3.45	17.80 ± 2.13

[1]DM, dry matter; EE, ether extract;
[2]CP, crude protein; SCP, total soluble crude protein; ADICP, acid detergent insoluble protein; NDICP, neutral detergent insoluble protein; 30 h ivNDFD, in vitro NDFD after 30 h of incubation;
[3]CHO, carbohydrate (CHO = 100 − EE − CP – ash); NFC, nonfiber CHO [NFC = 100 − (NDF − NDICP) − EE − CP − ash]; NSC, nonstructural carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Hemicellulose = NDF – ADF; Cellulose = ADF − ADL.
[4]uNDF, undigested NDF (288 h in situ incubation).

Table 6.3. Ruminal fermentation characteristics in rumen simulation technique for four barley and corn silage-based diet treatments.

Items	Diets*				SEM[1]	Contrasts *P* value			
	Corn	Barley				Corn vs. Barley	Cowboy vs. Copeland	Cowboy vs. Xena	Copeland vs. Xena
	P7213R-TMR	Cowboy-TMR	Copeland-TMR	Xena-TMR					
pH	6.66	6.72	6.74	6.70	0.029	0.01	0.65	0.15	0.31
NH_3-N, mg/dL	8.89	8.70	8.57	8.60	0.532	0.63	0.86	0.90	0.90
Total VFA, mmol, L	83.51	81.14	77.06	81.88	3.048	0.32	0.34	0.86	0.27
VFA, mol /100 mol									
Acetate (C2)	50.32	52.15	51.49	51.69	1.013	0.03	0.40	0.56	0.80
Propionate (C3)	28.27	24.50	23.70	23.35	0.265	<0.01	0.07	0.01	0.37
Butyrate	12.45	14.57	15.35	15.30	0.490	<0.01	0.17	0.20	0.94
Caproate	0.73	1.04	1.27	1.34	0.091	<0.01	0.09	0.04	0.64
Valerate	5.45	5.37	5.84	5.92	0.466	0.57	0.40	0.33	0.90
BCVFA[2]	2.78	2.43	2.36	2.41	0.107	0.01	0.65	0.92	0.72
C2/C3	1.78	2.13	2.18	2.21	0.044	<0.01	0.15	0.01	0.20

*TMR = total mixed ration
[1]SEM = standard error of mean.
[2]BCVFA = branched-chain volatile fatty acids (isobutyrate + isovalerate).

Copeland-TMR and Xena-TMR for molar proportion of acetate, butyrate, and the total concentration of VFA ($P > 0.10$). Similarly, studies done by Ivan et al. (2005), Weiss and Wyatt (2006), and Chow et al. (2008) showed no significant effects of feeding silage with higher ivNDFD on total VFA concentrations. In a meta-analysis study (Ferraretto and Shaver 2015), it has been found that the ruminal pH, total VFA, and acetate concentration are unaffected by corn silage hybrid type that differs in ivNDFD. There were differences between Cowboy -TMR and Xena-TMR in the molar proportion of propionate and acetate to propionate ratio, in which Cowboy-TMR had a higher molar proportion of propionate than Xena-TMR ($P < 0.01$). The previous study showed that higher fiber digestibility level in silage can increase the passage rate of feed through dairy cattle rumen which promotes the production of VFAs in the dairy cow's rumen, in particular the concentration of propionic acid (Ramirez-Ramirez, 2011).

There were significant differences between P7213R-TMR and the average of all barley silage-based diets, in which P7213R-TMR had a lower ruminal pH (6.66 vs. 6.72, $P < 0.05$), higher molar proportion of propionate (28% vs. 23%, $P < 0.01$), lower acetate to propionate ratio (1.8 vs. 2.2, $P < 0.01$), and lower molar proportion of butyrate (13% vs. 15%) compared to all barely silage-based diets. These findings are in agreement with a previous study by Benchaar et al. (2014) in which dairy cows fed a corn silage-based diet had a higher proportion of ruminal propionate compared with those fed a barley silage-based diet. The effects of corn silage on the VFA pattern currently observed are mainly attributed to the changes in composition of ruminally fermented carbohydrates. The higher propionate production and lower pH can be attributed to an increase in starch fermentability in corn silage-based diets compared with barley silage-based diets, whereas the lower butyrate can be related to the adverse effect of P7213R-TMR on fiber digestion. There was a significant difference in branched-chain volatile fatty acids (BCVFAs)

between P7213R-TMR and the average of all barley silage-based TMR (2.4 vs. 2.8 mol /100 mol, $P < 0.05$). The BCVFA consists of isovalerate and isobutyrate, which are derived from microbial deamination of branched amino acids. In this study, P7213R-TMR had a higher BCVFA and no significant difference on ammonia compared with the average of all barley silage-based TMR. This can happen only if efficiency of microbial protein synthesis increases (Goel et al. 2009).

6.4.3. Microbial Protein Production

Microbial N production for feed particle-associated, feed particle-bound, and effluent fraction of the barley silages with different levels of ivNDFD and of the corn silage in RUSITEC, are given in Table 6.4. The results of this study showed that Cowboy-TMR did not increase microbial colonization of fiber, as microbial N production in feed particle-associated and silage feed particle-bound fractions were similar among TMR diets that contained barley silage. In contrast to these findings, Oba and Allen (2000) observed that feeding diets containing corn silage with higher ivNDFD could increase the microbial N flow to the duodenum as well as microbial efficiency. Microbial yield in the rumen depends on the availability of carbohydrate and N in the rumen (Chumpawadee et al. 2006). The diet containing P7213R-TMR tended to be higher ($P = 0.06$) in total microbial protein production 52.74 (mg d^{-1}) compared with the average of Cowboy-TMR, Copeland-TMR, and Xena-TMR silage diets, suggesting a higher potential of corn silage-based diet to provide adequate amounts of energy for the synthesis of microbial N compared with barley silage-based diets. Although these findings were from an *in vitro* study which could not simulate adequately an *in vivo* system, but it would give an indication about the higher nutritive value of corn silage compared with barley silage.

Table 6.4. Bacterial nitrogen production in rumen simulation technique for four barley and corn silage-based diet treatments.

Items	Diets*				SEM[1]	Contrasts *P* value			
	Corn	Barley							
	P7213R-TMR	Cowboy-TMR	Copeland-TMR	Xena-TMR		Corn vs. Barley	Cowboy vs. Copeland	Cowboy vs. Xena	Copeland vs. Xena
Bacterial nitrogen production, mg/d									
Total	52.74	49.59	49.52	48.56	1.513	0.06	0.92	0.63	0.62
Feed particle-bound (TMR)	16.82	17.90	18.70	17.37	1.287	0.44	0.66	0.76	0.48
Feed particle-bound (silage)	10.35	8.93	10.62	10.27	1.024	0.77	0.26	0.36	0.81
Feed particle associated (TMR)	6.28	5.57	4.78	5.94	0.735	0.32	0.44	0.72	0.27
Effluent	29.65	26.13	26.04	25.25	1.439	0.07	0.96	0.68	0.71

*TMR = total mixed ration

[1]SEM = standard error of mean.

6.4.4. Nutrients Digestibility

Nutrients digestibility of TMR in RUSITEC are presented in Table 6.5. There was no significant effect of Cowboy-TMR ($P > 0.10$), which contained Cowboy silage, on digestibility of DM, organic matter (OM), and CP in the TMR when compared with Copeland-TMR and Xena-TMR. Furthermore, there was no significant effect of P7213R-TMR on nutrient digestibility (DM, OM, and CP) of TMR compared with the barley silage treatments.

The nutrient digestibility of barley and corn silages were also evaluated in this study (Table 6.6). The results showed that Cowboy had a higher ivNDFD compared with Xena (51% vs. 45%, $P = 0.02$). However, the DM digestibility of Cowboy was not significantly different compared with Xena. This might be attributed to the presence of lower content of starch and a higher proportion of NDF compared with Xena. Furthermore, Cowboy exhibited a lower starch digestibility compared with Copeland and Xena barley silages. In this study, CS had a higher DM digestibility when compared with barley silage varieties (69.1% vs. 64.8%, $P < 0.05$). The greater digestibility of CS is attributed to the existence of a significant portion of digestible nutrients, higher NFC and lower content of NDF compared with the barley silages. In the present study, CS showed a lower NDF digestibility than all barley silages varieties (41.5 vs. 48.1, $P < 0.01$).

6.4.5. Predicted Total-Tract Fiber Digestibility Based on *In Vitro* Results

It has been found that when modeling to accurately predict NDF digestibility, the model should partition NDF into iNDF and pdNDF, and fractionates feed particles by their retention and passage in the rumen using a predicted Kd by an *in vitro* system (Huhtanen et al. 2008). Considering the estimation of the degradation kinetics (Kd h^{-1}) of all silages, the results showed that there were significant differences ($P < 0.05$) in the degradation rate of NDF among barely

varieties. The value was highest in Cowboy, intermediate in Copeland, and lowest in Xena (Table 6.7). The *in vitro* Kd values were used for the prediction of the dietary rumen NDFD using CNCPS model version 6.5 (Higgs et al. 2015; Van Amburgh et al. 2015). The results showed that Cowboy had the highest estimated ruminal NDFD (12.6% DM) when compared with Copeland and Xena barley silages (9.7% DM, $P < 0.05$). On the other hand, the predicted TRDC values were greater in Copeland (35% DM) than Xena and Cowboy (averaged 31% DM). The predicted TRDC was greater in CS compared with all barley silage varieties. Thus, corn silage has a greater potential than barley silage to provide the dairy cattle with higher energy content required for milk production and to support the energy demands during lactation period.

Single time point ivNDFD can be used for ranking the forages when the NDF content of forages are similar, but in the current study, the forages are different in NDF and starch contents. These differences may influence the feeding value rather than the digestible fiber content. The results of Oba and Allen (1999b) were from trials in which diets had similar NDF content; hence, ivNDFD was the main variable among treatments. However, this is not common when evaluating forages where both the NDF and ivNDFD can vary.

Table 6.5. Gas production and nutrient digestibility in rumen simulation technique for four barley and corn silage-based diet treatments.

Items	Diets*				SEM[1]	Contrasts *P* value			
	Corn	Barley							
	P7213R-TMR	Cowboy-TMR	Copeland-TMR	Xena-TMR		Corn vs. Barley	Cowboy vs. Copeland	Cowboy vs. Xena	Copeland vs. Xena
Gas production									
Total, mL/d	1845.0	1711.9	1636.9	1628.8	138.27	0.27	0.71	0.67	0.95
Gas, ml/g OM fermented	270.0	248.2	237.7	239.9	19.87	0.25	0.72	0.77	0.94
Nutrient digestibility of TMR, %									
DM	72.98	74.26	73.43	72.02	1.168	0.61	0.85	0.40	0.62
OM	74.12	74.68	73.76	72.24	1.187	0.53	0.69	0.39	0.60
CP	79.87	80.36	78.93	76.56	2.061	0.54	0.59	0.40	0.61

*TMR = total mixed ration

[1]SEM = standard error of mean.

Table 6.6. Nutrient digestibility in rumen simulation technique for barley and corn silages.

Items	Silages				SEM[1]	Contrasts *P* value			
	Corn	Barley				Corn vs. Barley	Cowboy vs. Copeland	Cowboy vs. Xena	Copeland vs. Xena
	P7213R	Cowboy	Copeland	Xena					
Nutrient digestibility of silage, %									
DM	69.13	64.62	65.56	64.23	1.438	0.02	0.65	0.85	0.53
OM	69.66	65.52	66.38	65.11	1.289	0.02	0.63	0.82	0.50
NDF	41.47	51.20	48.36	44.98	1.698	<0.01	0.26	0.02	0.19
ADF	37.39	46.69	43.80	37.62	2.155	0.05	0.36	0.01	0.06
Hemicellulose[2]	48.69	59.47	56.93	56.66	1.465	<0.01	0.24	0.21	0.90
CP	79.96	77.06	80.74	78.40	2.120	0.63	0.24	0.87	0.45
Starch	98.83	95.41	98.26	98.70	0.925	0.20	0.04	0.02	0.72

[1]SEM = standard error of mean.
[2]Hemicellulose = NDF – ADF

Table 6.7. Total carbohydrates and fiber digestibility predictions based on *in vitro* results for barley and corn silages.

Items	Silages				SEM[1]	Contrasts *P* value			
	Corn	Barley							
	P7213R	Cowboy	Copeland	Xena		Corn vs. Barley	Cowboy vs. Copeland	Cowboy vs. Xena	Copeland vs. Xena
Predicted ruminal fiber digestibility[2]									
RDCB3, % DM	6.71	12.63	10.54	8.93	0.390	<0.01	<0.01	<0.01	<0.01
Degradation rate of available NDF[3]									
Kd, %/h	2.24	5.74	4.26	2.65	0.312	<0.01	0.01	<0.001	0.04
Predicted ruminal carbohydrates digestibility[4]									
TRDC, %DM	38.47	30.18	34.59	31.18	0.376	<0.01	<0.01	0.07	<0.01
TRUC, % DM	43.31	51.54	46.72	47.33	0.898	<0.10	<0.01	<0.01	0.19
Predicted total tract fiber digestibility[5]									
Total-tract CB3, %DM	10.14	16.25	14.10	13.01	1.164	<0.01	<0.01	<0.01	0.10

[1]SEM = standard error of mean.
[2]RDCB3 = rumen degraded CB3 fraction.
[3]Degradation rate for of available NDF fraction (RDCB3) using 48-h NDFD, a fixed lag of 3-h and uNDF-288 following the method of Van Amburgh et al. (2003)
[4]TRDC (total degraded CHO fraction); TRUC (total escaped CHO fraction).
[5]Total-tract CB3 = [RDCB3 (rumen degraded CB3 fraction) + IntDigCB3 (B3 carbohydrate intestinal digestibility; default = 0.20)].

6.5. Conclusion

The results of the present study indicate that higher *in vitro* NDF digestibility of barley silage might not necessarily correspond with significantly greater effect on rumen fermentation characteristics (VFA and pH) and microbial protein synthesis. *In vitro* NDF digestibility assessment of forages could be used as an analytic tool for evaluating forage quality, when NDF concentrations are similar. The results of this study show that feeding corn silage could have higher feeding value compared with barley silage. However, the results from the current study were from *in vitro* technique which could not completely mimic the *in vivo* system. Further work including animal trial is required to confirm these findings.

On the basis of results in previous chapters (Chapter 5, 6), it would be concluded that selecting barley silage varieties based on their ivNDFD level is not a satisfactory method for improving the milk yield and DMI. Therefore, for my next experiment (Chapter 7, 8) I focused on increasing ivNDFD of barley silage or barley silage-based diet using an exogenous fibrolytic enzymes as a recognized approach to enhance ivNDFD, and to correlate this increase with dairy cows' performance during mdi-lactation (Chapter 7) or early-lactation (Chapter 8), to see whether or not it corresponds with greater dairy cow performance.

7. Effect of Fibrolytic Enzymes on Lactational Performance, Feeding Behavior, and Digestibility of in High Producing Dairy Cows Fed a Barley Silage-Based Diet

7.1. Abstract

The objectives of this study were to evaluate the effects of pre-treating dairy cow rations with a fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK) on lactation performance, digestibility, and feeding behavior in response to feeding barley silage-based diet. Before starting the dairy trial, in vitro incubations were conducted to determine whether the addition of FETR would have a response on these animal performance characteristics when applied to a barley silage-based diet for dairy cows. The dairy trial was performed using eight Holstein dairy cows. The cows were blocked by their parity and assigned randomly to one of 4 treatments: 0, 0.5, 0.75, and 1 mL of FETR / kg DM of diet in a replicated Latin square design. The pre-treatment was applied to the complete diet during the mixing process. The experimental period continued for 22 d with each experimental period consisting of 16-d adaptation period and 6-d sampling period. The daily feed intake of each individual cow was monitored using the Insentec feed bins. Feeding behavior characteristics were measured during the entire sampling period using the feed bin attendance data. Milk samples were

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collected in the last 3 d of each experimental period. The addition of FETR linearly increased in the vitro DM digestibility and tended to improve the in vitro neutral detergent fiber (NDF) digestibility in barley silage. There was a cubic effect of the enzyme levels on the total tract DM or NDF digestibility. Maximal digestibility was reached at 0.75 mL FETR / kg of TMR. The milk fat yield, fat corrected milk and the energy corrected milk quadratically responded to the incremental levels of FETR. The milk protein percentage linearly (improved in response to FETR. Increasing FETR levels resulted in a quadratic effect on feed efficiency. There was no effect of FETR level on feeding behavior. In conclusion, pre-treating dairy cow barley silage-based diet with FETR increased the feed efficiency without affecting DMI. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common.

7.2. Introduction

Dairy cows are able to utilize the digestible nutrients from forages and convert them into milk and meat products for humans. The rate and extent of forage digestion in dairy cows are lower than those of concentrates, which limits feed intake and performance of dairy cows (Reynolds, 2000). Thus, it is important to improve forage digestibility to increase milk production level. Fibrolytic enzymes are used as feed additives in ruminant diets to enhance forage fiber digestibility and lactational performance of dairy cows. Enzyme addition directly to the feed enhances the digestibility of DM and NDF (McAllister et al., 1999; Kung et al., 2000; Yang et al., 2000). However, there are inconsistent results regarding the effect of providing of

fibrolytic enzymes to ruminant diets on dairy cow performance. Thus, the use of fibrolytic enzymes as feed additives have not yet been extensively adopted in commercial dairy farms. Nevertheless, due to continuous increase in feed costs, it is essential to reconsider and refine the use of enzymes as feed additives in ruminant diets as a strategy to improve feed efficiency and decrease the cost of milk production.

Whole-crop barley (*Hordeum vulgare* L.) silage is a main forage component of dairy and beef rations in Western Canada, because the crop is well adapted for production in this region (Wallsten and Hatfield, 2016). In a previous study, three relatively new developed barley forage varieties were selected based on their varying rate of in vitro NDF digestibility (ivNDFD) to study the effects of barley silage on growing performance of beef cattle (Nair et al., 2017). From the results of this study, it was found that all barley varieties, despite differences in NDFD, have similar impact on feed efficiency. However, other factors did substantially affect forage quality and digestibility, such as the environmental temperature and the stage of maturity at harvest. The large differences in nutritional quality among forage barley varieties suggest that there is a necessity to apply other approaches to improve the forage digestibility such as using fibrolytic enzymes products with high activity (xylanase and cellulose) for improving the digestibility of barley silage. To our knowledge, there is little information that has documented the lactation performance response by cows to with fibrolytic enzymes applied to these forage barley varieties. This study aimed to evaluate the effects of supplementing fibrolytic enzyme product applied directly to a barley silage-based diet fed to dairy cows during mid-lactation on milk yield, milk composition, nutrient intake and digestibility, predicted energy content, and feeding behavior.

7.3. Materials and Methods

7.3.1. In Vitro Study

In vitro study was conducted to determine whether the addition of fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK) would have a response on these animal performance characteristics when applied to a barley silage-based diet for dairy cows. The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute). The enzyme was tested on barley silage harvested at mid dough stage with 41% DM content. The samples of barley silage were oven dried at 55°C for 48 h. The dried samples were ground through a 1 mm screen for the chemical analysis and the in vitro study (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK). The detailed chemical composition of barley silage is shown in Table 7.1. The cumulative gas production of silage treated with different doses of enzyme was measured using batch culture technique as described by Eun et al. (2007). Six doses of enzyme were applied at 0.0, 0.25, 0.50, 0.75, 1.00, and 1.25 mL of FETR/kg DM of silage. Approximately 0.5 g sample (1-mm) of dried silage was weighed in triplicate into acetone-washed filter bags (F57, Ankom Technology, Macedon, NY). The bags were sealed and placed into 100 mL serum bottles. All dosages of enzymes were diluted with distilled water (0.1% FETR) and pipetted in serum bottles. The volume of

diluted enzyme solution was equal for all doses as well an equal volume of distilled water was added to serum bottles to serve as control. Two experimental runs were performed on different days. Ruminal fluid was collected 2-h after feeding from 2 rumen-cannulated Holstein cows. The cows were fed diets formulated with 34.1% barley silage, 16.1% chopped alfalfa hay, 30.1% lactation concentrate pellet, and 19.7% barley grain to meet the requirements for lactating dairy cows producing 40 kg milk (NRC, 2001). Each serum bottle received 15 mL of strained ruminal fluid and 45 mL of McDougall's buffer, during which oxygen free CO_2 was flushed. Three blanks which contained 60 mL of medium without feed samples were used for each incubation time. Sealed bottles were incubated in an oscillating shaker at 39°C with an oscillation speed of 125 rpm for 48 h. Headspace gas production was measured at 3, 6, 9, 12, 24 and 48 h by inserting a 23-gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI; Omega Engineering, Inc., Laval, QC, Canada) connected to a visual display device (Data Track, Christchurch, UK). Pressure values, corrected for the gas released from the blanks were used to generate volume estimates using the equation described by Mauricio et al. (1999).

7.3.2. Lactation Study

A lactational performance study was conducted using 8 mid-lactating Holstein dairy cows (average parity = 2.8 ± 1.2) consisting of 4 primiparous cows (average BW = 618 ± 61 kg; DIM = 118 ± 17) and 4 multiparous cows (average BW = 738 ± 13 kg; DIM = 137 ± 5.4). All experimental procedures used in this experiment were approved by the University of Saskatchewan Animal Care Committee. All cows were housed at the

Rayner Dairy Research and Teaching Facility farm, University of Saskatchewan (Saskatoon, SK, Canada).

The experimental design was a double 4 × 4 Latin square with four 22-d periods. The cows were housed in one pen in a free-stall barn. The pen was equipped with 8 Insentec feed bins (Insentec BV, Marknesse, the Netherlands). The Insentec system was set to allow one cow to access one feed bin during the entire experiment and the feed intake and frequency were automatically recorded.

The cows were fed ad libitum a TMR consisting of 34.0% barley silage, 16.0% alfalfa hay, 19.7% barley grain, and 30.0% concentrate on a DM basis. The diets were formulated using the NDS software (Version 3, RUM&N, NDS Professional, Reggio Nell'Emilia, Emilia-Romagna, Italy) to provide an adequate amount of metabolizable energy and metabolizable protein for dairy cows 120 DIM producing 40 kg of milk. Animals were fed twice daily at approximately 0900 and 1700 h and were milked 3 times a day at approximately 0430, 1230, and 1830 h. The experimental period lasted for 22 d with each experimental period comprising 16-d adaptation period and 6-d sampling period.

Animals within each square were assigned to 4 experimental treatments. The experimental treatments consisted of the basal TMR (control, Table 7.1) and the basal TMR supplemented with 3 levels of FETR: 0.5, 0.75, and 1 mL of FETR/kg DM of TMR. The enzyme mixture was prepared twice a week. The enzyme liquid was sprayed onto the TMR with the TMR kept for approximately 1 h before feeding to increase enzyme efficacy.

The yield of milk was recorded at each milking during the last week of each experimental period. Milk samples were collected in the last 3 d of each sampling period (d 20, 21, and 22), preserved with potassium dichromate, and tested at CanWest DHI (Edmonton, AB) for milk fat, protein, lactose, milk urea-N, and total solid using infrared spectroscopy (MilkoScan 605, Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16).

7.3.2.1. Nutrient Intake and Digestibility

Feeding time and daily feed intake of each individual cow was monitored using Insentec feed bins. Feed samples and orts were collected twice weekly for DM adjustment. From d 19 to 22, TMR and ort samples were collected and stored at -20°C for later analysis. The refusal and TMR samples were thawed and dried at 60°C for 48 h for nutrient analysis. Samples were ground through a 1-mm screen (Standard Model 4; Arthur H. Thomas Co., Philadelphia, PA) and composited by period for analysis of OM, NDF, indigestible NDF (iNDF), and CP. The iNDF content of each sample was determined following in situ incubations for 12d in the rumen (Ahvenjärvi et al., 2000).

A fecal grab sample (300 to 500 g fresh basis) was collected immediately before feeding for 4 d at the end of each period. All fecal samples were mixed and pooled per cow, and a subsample stored at −20°C for later analysis. Total tract digestibility of DM, OM, NDF, and potentially digestible NDF (pdNDF) were estimated using iNDF as an internal marker (Ahvenjärvi et al., 2000).

Table 7.1. Ingredient and chemical composition of total mixed ration and barley silage used for the four exogenous fibrolytic enzyme-supplemented diet treatments.

Item	%
Ingredient composition, % (DM basis)	
Barley silage	34.1
Alfalfa hay	16.1
Barley grain	19.7
Barley grain	6.6
Corn grain	2.8
Canola meal	7.8
Soybean meal	2.8
Peas	1.7
Corn distillers dehydrated	0.6
Wheat ground	1.1
Corn gluten meal	0.6
Acid buffer	0.3
Sodium bicarbonate	0.5
Calcium phosphate	0.0
Limestone	0.8
Fat tallow	0.6
Premix[1]	0.8
Salt white	0.3
Beet pulp pellets	1.8
Rp10 palmitic	1.0
Chemical composition of silage	
Dry matter, %	41.9
Crude protein, %DM	12.3
NDF, %DM	45.8
ADF, %DM	27.3
Lignin (ADL), %DM	4.4
In vitro NDFD 24 h, %	19.6
Starch, %DM	21.7
Chemical composition of diet[2]	
DM, %	55.1
OM, %DM	93.3
NDF, %DM	29.5
iNDF, %DM	14.3
pdNDF, %DM	15.2
CP, %DM	16.2

[1]Composition of the premix: Calcium=16%; Phosphorus=8.0%; Chloride=10.4%; Sodium=7.6%; Potassium= 1.8%; Sulfur=1.0%; Magnesium= 4.5%; Copper=535 ppm; Zinc= 2100 ppm; Manganese= 1500 ppm; Iron= max 1050 ppm; Selenium=16 ppm; Iodine =45 ppm; Cobalt=16 ppm; Vitamin A ('000 i.u.) = 330; Vitamin D ('000 i.u.) = 60; Vitamin E (i.u.) = 2500.

[2] iNDF = indigestible NDF; pdNDF = potentially digestible NDF.

7.3.2.2. Feeding Behavior

Feeding behavior characteristics were assessed in this study in the last 6 d of each experimental period using feed bin attendance data. The parameters recorded included feed intake, daily eating time, duration and frequency of bunk attendance, and meal frequency. The meal was defined as a visit to the bunk followed by an absence of 5 min or greater. The Intsentec data included individual intake, duration of bunk attendance, meal frequency, and the duration and size of each meal. The eating rate was calculated by dividing the amount of individual intake by the eating time. The system was monitored daily for proper operation.

7.3.3. Chemical Analysis

All frozen TMR, refusals, and fecal samples were thawed overnight at room temperature and dried at 55°C for 72 h. All samples were ground through a 1-mm screen using a Christy-Norris mill (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK) for use in chemical analyses. DM (method 930.15), ash (method 942.05), crude fat (method 2003.05), and CP (method 990.03) were analyzed according to AOAC (2000). For estimation of CP, N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI). ADF, NDF, and ADL values were also analyzed according to the Van Soest et al. (1991) method combined with an ANKOM A200 filter bag technique (ANKOM Technology Corp., Macedon, NY). Amylase and sodium sulfite were used in the NDF analysis.

7.3.4. Calculations and Statistical Analysis

Total-tract pdNDF digestibility was calculated according to Ferraretto et al. (2015). The digestibility of pdNDF was expressed as follows: pdNDF digestibility, % pdNDF =100 − [(dietary iNDF/fecal iNDF) × (fecal pdNDF concentration/dietary pdNDF)].

Energy partitioning was calculated by using milk yield, milk composition, and BW gain data (NRC, 2001). Body weight was measured at the beginning of each experimental period on two consecutive days. The net energy for maintenance was calculated as described by NRC (2001) according to the following equation: $BW^{0.75}\times$ 0.08. Energy of BW gain was assumed to be 4.924 Mcal/kg for BW loss or 5.114 Mcal/ kg for BW gain (NRC, 2001). Milk energy was calculated according to the following equation: 0.0929 × milk fat concentration + 0.0563 × milk true protein concentration + 0.0395 × milk lactose concentration (NRC, 2001). Ultimately, the predicted NE_L was calculated by summation of net energy (maintenance, BW gain, and milk) divided by DMI (Neal et al., 2014).

The data were analyzed using Proc Mixed SAS 9.4 (SAS Institute, Cary, NC). Data for intake, digestibility, milk production, and feeding behavior were analyzed with the following model: $Y = \mu + T_i + P_j + S_k + C_{l(k)} + e_{ijkl}$, where: μ = the overall mean; T_i = the fixed effect of the i^{th} treatment (i = 1 to 4); P_j = the fixed effect of the j^{th} period (j = 1 to 4); S = the random effect of the k^{th} square (k = 1 to 2); $C_{l(k)}$ = the random effect of l^{th} cow within square (l = 1 to 4); e_{ijkl} = random residual error, assumed to be normally distributed..

The cumulative gas production, in vitro DM or NDF digestibility data was analyzed as a randomized complete block design. The statistical model used to analyze

these parameters was $Y_{ij} = \mu + T_i + B_j + e_{ij}$, where Y = an observation of the dependent variable; μ = the population mean for the variable; T_i =the treatment effect, as a fixed effect; B_j = block effect with experimental run (n = 2), as a random effect; e_{ij} = the random error associated with the observation. Results are reported as least squares means. Polynomial contrasts were used to determine the effect (linear, quadratic and cubic) of increasing enzyme application.

7.4. Results and Discussion

Table 7.2 shows the effect of FETR levels on in vitro gas production and DM and NDF digestibility. There was no effect ($P > 0.10$) of FETR levels on in vitro gas production. There was a linear effect ($P = 0.02$) of enzyme levels on in vitro DM digestibility over 48 h of incubation. The results showed that enzyme level also tended to have linear effect ($P = 0.08$) on in vitro NDF digestibility over 48 h of incubation.

The effect of FETR levels on intake and digestibility are presented in Table 7.3, which indicates that FETR levels did not alter DM, OM or NDF intake ($P > 0.10$) in dairy cows. These results are in line with previous studies which noted no effect of exogenous fibrolytic enzyme addition on DMI of dairy cows during different stages of lactation (Bernard et al., 2010; Chung et al., 2012; Dean et al., 2013).

Table 7.2. Effect of exogenous fibrolytic enzyme level of FETR on asymptotic cumulative gas volume (mL/g OM) and nutrient degradability in barley silage

Item	Enzyme level (ml FETR/ kg DM silage)						SEM[1]	Polynomial contrast values		
	Control	0.25	0.50	0.75	1.00	1.25		Linear	Quadratic	Cubic
Asymptotic cumulative gas volume, mL/g OM										
3-h	18.8	34.3	30.6	26.0	23.6	24.7	4.96	0.870	0.174	0.163
6-h	36.3	55.4	53.4	46.0	39.4	44.2	7.50	0.811	0.218	0.175
9-h	53.3	74.1	73.7	65.1	56.6	65.7	9.17	0.990	0.289	0.177
12-h	68.2	88.9	89.0	81.1	70.5	82.3	9.98	0.930	0.343	0.190
24-h	98.9	119.3	121.4	113.3	102.0	114.5	12.08	0.852	0.375	0.236
48-h	133.1	150.5	140.1	151.4	131.3	138.7	6.77	0.760	0.184	0.304
Nutrients digestibility, %										
DM (48-h)	58.5	62.0	60.5	62.5	61.7	62.9	4.95	0.018	0.303	0.133
NDF (48-h)	34.7	40.8	36.8	39.9	39.1	40.8	2.96	0.081	0.561	0.223

[1]SEM = standard error of mean

However, some studies (Beauchemin et al., 2000; Gado et al., 2009) reported a positive effect of exogenous fibrolytic enzyme with high cellulase or xylanase activity on DMI. The intake of forage-based diets by dairy cows is often controlled by rumen fill and the rate of degradation (Allen, 1996; Poppi, 2011). The forage-based diets with higher NDF would be more filling than diet with lower NDF concentration, causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996; Poppi, 2011). Improving the DMI in dairy cows may be attributed to decrease gut fill, which allows the cows to eat more to meet their requirements during early lactation period. However, this mechanism was not conclusive from previous studies, for example Chow et al. (2008) found that the cows fed barley silage with high NDFD had no effect on DMI, but the cows tended to gain more weight. Other studies have found feeding forages with higher NDFD improved the feed efficiency (same DMI and high milk yield) for dairy cows during early lactation (Oba and Swift, 2014).

The response of DM, OM or NDF digestibility to the increasing level of FETR was cubic ($P < 0.01$; Table 7.3), where the intermediate dosage (0.75 ml FETR / kg DM TMR) has exhibited the best effect on nutrients digestibility. These findings are in agreement with both *in vitro* (Kung et al., 2002; Elwakeel et al., 2007) and *in vivo* studies (Gado et al., 2009; Tager and Krause, 2011; Salem et al., 2013). The effect of fibrolytic enzyme on nutrient digestibility in the current study would be attributed to the ability of FETR to increase the number of cellulolytic bacteria and alter the rumen population (Beauchemin and Holtshausen, 2010).

Table 7.3. Effect of exogenous fibrolytic enzyme levels of FETR on nutrient intake and total tract nutrient digestibility in dairy cows

Item	Enzyme levels (ml FETR/ kg DM TMR)				SEM[1]	Polynomial contrast values		
	Control	0.50	0.75	1.00		Linear	Quadratic	Cubic
Intake, kg/d								
DM	27.26	27.34	27.64	27.24	0.442	0.798	0.527	0.391
OM	25.46	25.56	25.84	25.42	0.412	0.808	0.448	0.380
NDF	8.31	8.35	8.46	8.27	0.131	0.789	0.223	0.285
pdNDF[2]	4.55	4.62	4.74	4.56	0.074	0.357	0.072	0.068
Digestibility, %								
DM	68.42	66.09	70.83	69.05	1.070	0.280	0.322	0.011
OM	66.42	65.00	68.51	67.00	0.924	0.299	0.548	0.024
NDF	47.30	44.17	53.44	49.72	1.947	0.110	0.479	0.007
pdNDF[3]	79.59	72.94	88.64	83.08	3.946	0.226	0.406	0.023

[1]SEM = standard error of mean.
[2]pdNDF = potentially digestible NDF.
[3]pdNDF digestibility (%) =100 − [(dietary iNDF/fecal iNDF) × (fecal pdNDF concentration/dietary pdNDF)].

Table 7.4 shows the effects of fibrolytic enzyme level of FETR on milk production and composition. There were quadratic and cubic ($P < 0.05$) effect of FETR level on FCM and ECM with highest FCM and ECM at 0.75 mL FETR / kg of TMR. In some studies, applying fibrolytic enzymes increased milk production (Lewis et al., 1999; Yang et al., 1999, 2000; Mohamed et al., 2013), but no response was reported in others studies (Elwakeel et al., 2007; Arriola et al., 2011; Peters et al., 2015). The effect of FETR supplementation on milk yield in the current study could be attributed to improved total tract NDF digestibility, hence increased energy density.

There was a quadratic response ($P = 0.02$; Table 7.4) of milk fat yield to FETR levels. Dietary differences in the composition of milk are normally related to changes in ruminal digestion. The higher milk fat yield resulted from an enhancement of fiber digestibility. At the highest enzyme dosage, the dairy cows exhibited lower milk fat percentage and milk fat yield. This finding is in line with a previous study that showed a reduction in milk fat content when applying enzymes (rich in xylanase and cellulase) to dairy cow diets (Yang et al., 1999). These authors explained this decrease as a result of a reduction in the ratio of acetate to propionate in the rumen (Yang et al., 1999).

Table 7.4. Effect of exogenous fibrolytic enzyme levels of FETR on milk yield and milk composition of lactating dairy cows

Item	Enzyme level (ml FETR/ kg DM TMR)				SEM[1]	Polynomial Contrast values		
	Control	0.50	0.75	1.00		Linear	Quadratic	Cubic
Yield, kg/d[2]								
Milk	38.63	38.22	39.33	38.42	2.074	0.783	0.814	0.036
FCM	36.35	37.32	38.87	33.81	1.793	0.365	0.013	0.056
ECM	36.76	37.26	38.94	34.72	1.619	0.406	0.017	0.028
Fat	1.21	1.28	1.35	1.07	0.083	0.398	0.017	0.122
Protein	1.23	1.19	1.26	1.22	0.051	0.635	0.505	0.033
Lactose	1.72	1.74	1.79	1.73	0.103	0.286	0.136	0.040
SNF	3.32	3.30	3.43	3.32	0.169	0.437	0.630	0.019
Milk composition[3]								
Fat, %	3.12	3.05	3.28	2.85	0.195	0.359	0.189	0.062
Protein, %	3.16	3.18	3.24	3.21	0.087	0.039	0.795	0.150
Lactose, %	4.46	4.52	4.54	4.49	0.038	0.147	0.085	0.396
Total solids, %	11.58	11.50	11.83	11.39	0.242	0.759	0.275	0.025
SNF, %	8.62	8.63	8.73	8.72	0.099	0.104	0.791	0.315
MUN, mg/dL	11.89	11.72	12.16	11.82	0.384	0.905	0.960	0.403
Efficiency								
ECM/DMI	1.38	1.38	1.42	1.28	0.057	0.190	0.066	0.105
FCM/DMI	1.35	1.38	1.44	1.28	0.068	0.584	0.065	0.110

[1]SEM = standard error of mean
[2] FCM = fat corrected milk; ECM = energy-corrected milk; SNF = solids-not-fat
[3]MUN = milk urea nitrogen.

There was a linear increase ($P = 0.04$) in milk protein content, but not yield with applying enzyme to the TMR (Table 7.4). It has been reported that milk protein could be influenced by energy content in the diet due to the high energy requirement for protein synthesis (Reynolds et al., 1994). In the current study, addition of the enzymes did increase the fermentable carbohydrate supply to ruminal bacteria due to increasing the digestibility of forage (Fondevila et al., 1990; Newbold et al., 1992).

The percentage of milk lactose tended to quadratically respond ($P = 0.09$) with increasing FETR levels (Table 7.4). The dosage of 0.75 mL/kg DM of TMR exhibited the best milk lactose percentage. The higher milk lactose content is related to an increase in fermentable carbohydrates amount in the rumen as a consequence of enzyme supplementation. Any increase in the fermented OM in the rumen would in turn enhance delivery of glucogenic precursors to the mammary gland and hence increase the milk lactose. In agreement with this finding, Yang et al. (1999) reported an increase in milk lactose when applying fibrolytic enzymes to dairy cows during early lactation.

Applying the enzymes tended to linearly ($P = 0.10$) increase the SNF percentage (Table 7.4). The SNF contains lactose, caseins, whey proteins, and minerals (ash content). This increase in SNF appeared to be entirely accounted for by the increase in milk protein and lactose. The milk urea content shown in this study is within the normal range resulting from a diet with balanced protein supply and energy (Kirchgessner et al., 1986). The averaged MUN concentration was 11.9 mg/dL of milk, and application of enzymes did not affect MUN concentration.

Enzyme application tended to affect feed efficiency in a quadratic pattern (P = 0.07; Table 7.4). The diet with the highest dosage showed lower feed efficiency as expressed as FCM/DMI or ECM/DMI. The diet supplemented with 0.75 mL/kg of TMR exhibited higher feed efficiency and higher FCM yield compared to the other doses. These findings indicate that supplementing the forage with moderate enzyme levels could enhance the dairy cow's performance.

There was numerical (P = 0.13) improvement in the net energy of lactation (Table 7.5; averaged NE_L for enzyme treatments vs. control, 1.51 vs. 1.29 Mcal/kg of DMI) when the enzymes were applied directly to the dairy cow diet. This increment in the energy content was attributed to a numerical increase in BW gain + milk compared with the control (averaged enzyme treatments vs. control, 32.0 vs. 26.9; P = 0.18). The numerical difference in the energy between the control diet and enzyme-treated diets in the current study may have been caused by the lack of effect on DMI during mid-lactation.

The effect of enzymes on feeding behavior is presented in Table 7.6. There was no effect (P > 0.10) of FETR level on feeding behavior. The lack effect is attributed to no effect of enzymes on feed intake. Nielsen (1999) observed the change of daily feed

Table 7.5. Effect of exogenous fibrolytic enzyme levels of FETR on BW gain and predicted energy values of lactating dairy cows

Item	Enzyme levels (ml FETR / kg DM TMR)				SEM[1]	Polynomial contrast values		
	Control	0.50	0.75	1.00		Linear	Quadratic	Cubic
BW gain, kg/d	0.44	1.13	0.91	1.64	0.704	0.316	0.924	0.414
Calculated net energy values, Mcal/ d [2]								
Maintenance	11.21	11.33	11.18	11.13	0.175	0.406	0.154	0.465
BW gain	2.02	7.74	5.14	9.02	3.357	0.163	0.811	0.417
Milk	24.66	24.04	26.37	23.76	0.933	0.705	0.901	0.002
BW gain + Milk	26.85	31.41	31.70	32.92	3.479	0.183	0.744	0.860
Total	38.03	42.70	42.86	44.03	3.528	0.189	0.719	0.849
$NE_{l,}$ Mcal/kg DMI	1.29	1.44	1.47	1.57	0.136	0.133	0.992	0.852

[1]SEM = standard error of mean

[2]Net energy value used for maintenance, BW gain, and milk; Calculated NE_l = calculated total net energy, Mcal / DMI (kg/ d).

Table 7.6. Effect of level of exogenous fibrolytic enzyme of FETR on feeding behavior in dairy cows

Item	Treatment effect (ml FETR/ kg DM TMR)				SEM[1]	Polynomial Contrast values		
	Control	0.50	0.75	1.00		Linear	Quadratic	Cubic
Meal bouts (d)	9.1	8.4	8.7	9.0	0.39	0.726	0.213	0.761
Eating time (min/d)	225.5	233.3	225.6	232.8	9.09	0.432	0.847	0.223
Meal length (min)	26.3	28.8	27.3	27.2	1.95	0.727	0.367	0.548
Eating rate (g/min)	124.6	122.3	125.1	124.9	4.84	0.866	0.607	0.629
Meal size (kg)								
DM	3.2	3.5	3.4	3.3	0.18	0.626	0.294	0.660
OM	3.0	3.3	3.1	3.1	0.17	0.642	0.301	0.662
NDF	1.0	1.1	1.0	1.0	0.06	0.621	0.285	0.720
PdNDF	0.5	0.6	0.6	0.6	0.03	0.581	0.231	0.810

[1]SEM = standard error of mean

intake is consequence of increasing the amount of feed ingested per meal or the number of meal per day. The results in the current study agree with previous studies that reported fibrolytic supplementation had no effect on eating or rumination activities (Bowman et al., 2002; Peters et al., 2015). Other studies have reported positive effects of enzyme addition on feeding behavior; for example, Gandra et al. (2017) reported an increase in eating time for Jersey heifers fed either corn silage- or sugarcane silage-based diets treated with fibrolytic enzymes. Further study by He et al. (2015) found eating time and bunk attendance frequency increased when fibrolytic enzymes were added to the diet of beef heifers. Silva et al. (2016) found that total chewing activities (rumination and eating) increased with fibrolytic enzyme supplementation.

7.5. Conclusion

Pre-treating dairy cow barley-based TMR with FETR improved dairy cow performance during the mid-lactation phase. Based on the current study, the optimum dosage of the fibrolytic enzymes was 0.75 mL FETR/DM kg of TMR. Applying this dosage improved NDF digestibility, and FCM yield. The feed efficiency increased by up to 7% compared to the control diet. Further studies are warranted to evaluate the effect of the fibrolytic FETR on lactation performance of dairy cows during early lactation phase.

8. Evaluating the Effects of Fibrolytic Enzymes Derived from *Trichoderma reesei*-Fungal Extraction on Rumen Fermentation, Omasal Nutrient Flow and Production Performance in Dairy Cows during Early Lactation

8.1. Abstract

This study was performed to evaluate the effects of pre-treating a barley silage-based diet with an exogenous fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK) on lactation performance, omasal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile in Holstein dairy cows during early lactation. The dairy trial was conducted using nine Holstein dairy cows (averaging 46 ± 24 DIM and 697 ± 69 kg BW, six cows were fitted with a rumen cannula and three were non-cannulated). Two groups of cows were randomly assigned to each of the dietary treatments in a crossover design: control (without FETR supplementation) and supplemented (with 0.75 mL of FETR/kg DM of diet based on our previous study). The pre-treatment was applied to barley silage-based diet one hour before feeding by mixing FETR with the diet. The experiment consisted of two consecutive experimental periods of 27 days each. Within each period, the first 18 days were used for adaptation to the treatments, followed by three days of milk sampling, three days for the collection of the ruminal, omasal and fecal samples, and the last three days for measuring the pH profile using indwelling pH probes. The application of FETR tended to decrease the DM intake compared to control (32.8 vs. 33.7; $P = 0.08$). There were no effects of FETR ($P > 0.10$) on omasal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile. There was a significant decrease ($P =$

0.05) in milk urea nitrogen by 7% as a consequence of adding FETR to the diet. In conclusion, barley silage-based diet pre-treated with FETR had maintained the milk yield of dairy cows with less feed intake. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common

8.2. Introduction

Fibrolytic enzymes are commonly used in ruminant diets to improve forage fiber digestibility and the production performance of lactating dairy cows. Enzyme supplementation has been found to improve the digestibility of forage (Yang et al., 1999; Gado et al., 2009). Nevertheless, the limited usage of fibrolytic enzymes was attributed to the inconsistent dairy cow response (Adesogan et al., 2014). Many factors may explain the inconsistent results in animal response such as enzyme activity, application rate, method of enzyme application, stage of lactation, and differences in energy status of experimental cows (Adesogan et al., 2014). Thus, it is important to consider all these factors before testing the fibrolytic enzymes on dairy cows.

Whole-plant barley silage is the primary forage used by dairy producers in Western Canada. Previous research by Nair et al. (2016) showed a significant difference in *in vitro* NDF digestibility (ivNDFD) of barley silage. However, when these forages were fed to dairy or beef cattle, the performance was not affected by level of forage ivNDFD (Nair et al., 2017). It was concluded from those studies that other important aspects need to be considered when interpreting the effect of level of ivNDFD for barely silage varieties on dairy cows' performance such as the maturity stage and environmental conditions. Due to the large variations among barley silage varieties in their nutritional

quality, thus there is a necessity to develop other approaches to improve the forage quality and decrease the impact of environmental conditions. One of these approaches is the usage of new generation of fibrolytic enzymes products with high xylanase and cellulase activity for improving the barley silage digestibility.

Previous studies identified several fibrolytic enzymes are able to improve ivNDFD of common forage crops such as corn silage and alfalfa hay (Eun et al., 2007b; Lynch et al., 2014). However, there are only a limited number of studies that found any positive effect of fibrolytic enzymes product on ivNDFD of barley silage. Beauchemin et al. (1995) found adding commercial fibrolytic enzymes product to barley silage had no effect on feedlot performance. They also tested the same product on alfalfa hay and timothy hay and reported a significant improvement in feedlot performance. Holtshausen et al. (2011) tested fibrolytic enzymes on ivNDFD of alfalfa hay, barley silage, and alfalfa silage. They found that the fibrolytic enzymes improved the ivNDFD of alfalfa hay, but there was no effect of fibrolytic enzymes on barley silage. As the barley silage is main ingredient in dairy cows' ration in Western Canada (about 40% DM basis of TMR), thus it is important to develop new generation of fibrolytic enzymes to enhance the ivNDFD of barley silage or barley silage-based diet.

The hypothesis in this study was that adding fibrolytic enzymes improves nutrient digestion, digestible energy intake, milk production, milk composition and feed efficiency due to improved forage digestion in dairy cows during early lactation. This experiment aimed to identify the effects pre-treating barley silage based-diet with an exogenous fibrolytic enzyme derived from *Trichoderma reesei* (FETR) on lactational

performance, duodenal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile in Holstein dairy cows during early lactation.

8.3. Materials and Methods

8.3.1. Animals and Experimental Design

At the end of the 6th week of postpartum, nine Holstein cows (averaging 46 ± 24 DIM and 697 ± 69 kg) were assigned randomly to two treatments in a crossover design (4-wk periods). Six of these cows were ruminally fistulated. All cows were housed and fed individually in tie stalls at the Rayner Dairy Research and Teaching Facility farm, University of Saskatchewan (Saskatoon, SK, Canada). Two groups of cows randomly assigned to two treatments: control (without supplementation) and supplemented (ENZ; with 0.75 mL of FETR / kg DM of diet, ABVista, Wiltshire, UK). The FETR was sprayed onto TMR hour before feeding. The commercial enzyme is derived from a fermentation extract of *Trichoderma reesei*. The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birch xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute).

Dietary ingredient composition of TMR is presented in Table 8.1. Diets contained 54% forage (two-thirds barley silage and one-third alfalfa hay) and 46% concentrate (DM basis). Diets were formulated using NDS Professional ration formulation software (Version 3, RUM&N, NDS Professional, Reggio Nell'Emilia,

Emilia-Romagna, Italy) to provide an adequate amount of metabolizable energy and metabolizable protein for dairy cows in early lactation. All cows were fed twice daily at approximately 0900 and 1700 h and were milked 3 times a day at approximately 0430, 1230, and 1830 h.

8.3.2. Data Collection and Sampling

The experimental period lasted for 27 d with each experimental period comprising 18-d adaptation period and 9-d sampling period. Body weights were taken at the beginning and the end of each experimental period. Feed intake and milk yield were recorded throughout the experiment. The milk samples were taken from three milk times in the first 3d of sampling period (d 19, 20, and 21) to estimate milk composition. The milk samples were stored into containers having 2-bromo-2-nitropropane-1–2-diol as a preservative. All milk samples were submitted to CanWest DHI (Edmonton, Alberta, Canada) for CP, fat, lactose, and MUN analyses.

The triple markers method was used to measure omasal nutrients flow (France and Siddons, 1986). Indigestible NDF (iNDF; Reynal et al., 2005), $YbCl_3$ (Siddons et al., 1985) and CrEDTA (Udén et al., 1980) were used as digesta markers for the large particle (LP), small particle (SP) and fluid (FP) phases, respectively as described by Chibisa et al. (2012).

Table 8.1. Ingredient and chemical composition of total mixed ration for early lactation dairy cows

Ingredient composition	% DM
Barley silage	37.0
Alfalfa hay	17.4
Rolled barley grain	15.2
Canola meal	8.6
Corn grain	6.4
Soybean meal.	5.0
Peas	3.8
Soybean hulls pellet	1.7
Urea	0.1
Fat canola oil	0.9
Rp10 palmitic	1.3
Premix[1]	2.6
Chemical profile	
DM, %	59.5
OM, %DM	93.0
NDF, %DM	34.0
iNDF, %DM	15.7
pdNDF, %DM	18.3
CP, %DM	16.9
$NE_{l,}$ Mcal/kg DM	1.61

[1]Composition of the premix: Calcium=16%; Phosphorus=8.0%; Chloride=10.4%; Sodium=7.6%; Potassium= 1.8%; Sulfur=1.0%; Magnesium= 4.5%; Copper=535 ppm; Zinc= 2100 ppm; Manganese= 1500 ppm; Iron= max 1050 ppm; Selenium=16 ppm; Iodine =45 ppm; Cobalt=16 ppm; Vitamin A ('000 i.u.) = 330; Vitamin D ('000 i.u.) = 60; Vitamin E (i.u.) = 2500.

Briefly, before marker infusing start on d 18, a priming dose (500 ml) of the $YbCl_3$ and Cr-EDTA, which equivalent to the half of daily dose (~1 L) was added directly into the rumen via the rumen cannula. Then, the marker solution was administered into the rumen using a peristaltic pump (Model 205U, Watson-Marlow, Cornwall, UK) for 7 d (d 18 to 24) at a constant rate of 1 L/d, to provide daily amount of 2.77 g of Cr (Binnerts et al., 1968) and 3.35 g of Yb (Brito et al., 2006). The daily infused amounts were recorded every day. In each period, subsample from each marker solution was taken and stored at room temperature for later analysis.

The omasal sampling was performed as described by Huhtanen et al. (1997). Once the sampling tube had been inserted in the omasal canal, about 400-mL omasal digesta sample was taken from each cow eight times over three days (0900, 1500, and 2100 h on d 22, 0300, 1200, and 1800 h on d 23, and 0000 and 0600 h on d 24) to ensure that the collected samples are representative of a 24-h feeding cycle. After mixing the omasal digesta, the collected amount was dived into two subsamples (100 and 200 mL). Then, 100 and 200 mL subsamples were immediately stored at – 20 ºC, and were pooled by cow through the collection period in 1.2 and 2.4 L containers, respectively. The large containers were used for marker analysis and the smaller ones were kept as reserve.

Samples of TMR and refusals were collected from d 22 to 25 of each experimental period. The samples of orts were composited by cow and period. Then all samples were stored at −20°C for later analysis. The apparent total-tract nutrient digestion, VFA concentration, and ammonia concentration were measured using 6 ruminally cannulated cows from d 22 to 24. The total-tract digestibility was measured

using iNDF as internal marker. An equal amount of fecal grab samples (approximately 300 g wet matter for each sample) were collected from each cow at 0900, 1500, and 2100 h on d 22, 0300, 1200, and 1800 h on d 23, and 0000 and 0600 h on d 24, such that the collected samples were representative of a 24-h feeding cycle. Then all samples were pooled by cow and collection period and stored at −20°C for later analysis.

At the same time points as fecal grab sampling, rumen fluid was collected from different sites in the rumen (the cranial ventral, caudal ventral, central, and cranial dorsal rumen) through the cannula. The rumen fluid was used to measure the ruminal NH_3-N and VFA concentrations. About 1,000 mL of ruminal fluid was collected from each cow. The rumen samples were strained through four layers of cheesecloth. Then, two 10-mL subsamples of ruminal fluid were taken and added to 25% (wt/vol) metaphosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at −20°C for later estimation of VFA and NH_3-N, respectively.

In the last 3-d of each experimental period, the ruminal pH was continuously measured every minute using the Lethbridge Research Centre Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006). Probes were standardized using two buffers (pH 7 and 4). In the last day of pH measurement, all probes were collected from each cow, washed, and the data was downloaded. The electrode signal in mV was converted to pH data using calibration slope. The pH probes determine the slope by measuring the difference in the mV reading of two different buffers (pH 7 and 4) and divides it by the difference in pH of the buffers.

8.3.3. Sample Analysis

The diet ingredients and fecal samples were dried in a forced air oven at 55ºC. All feed ingredient samples were ground using a Christy and Norris Lab mill (Christy Turner Ltd., Chelmsford, UK) with a 1-mm screen, while the fecal samples were ground using Retsch ZM (Brinkmann Instruments Canada Ltd., Mississauga, ON, Canada) with 1-mm screen. The ground samples were used for the chemical analysis of DM (method 930.15), ash (method 942.05), crude fat (method 2003.05), and CP (method 990.03; AOAC, 2000). For estimation of CP, N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI). The NDF was analyzed according to the Van Soest et al. (1991) method combined with an ANKOM A200 filter bag technique (ANKOM Technology Corp., Macedon, NY). Amylase and sodium sulfite were used in the NDF analysis.

The omasal digesta composite samples were separated into three phases (i.e., LP, SP and FP) as described by Brito et al. (2009) and Chibisa et al. (2012). Then, all fractions were freeze-dried and ground through a 1-mm screen (Retsch ZM, Brinkmann Instruments Canada Ltd., Mississauga, ON, Canada). All omasal digesta fractions were analysed for Cr and Yb. Briefly, one gram of omasal digesta were weighed in 50-mL crucible and ashed at 550 ºC in a muffle furnace for 8 hours. Then, all samples were digested using 15 ml of 1.5 mol/ L HNO_3 containing 0.2% KCl with boiling for 3 min. Finally, the mixture was diluted to 100 ml with double distilled water and filtered using Whatman No 1 filter paper. The resulting supernatant was stored at room temperature in 50 ml plastic vials until analyzed by atomic absorption spectrophotometry (ice 3000

series, Thermo scientific, Waltham, MA) equipped with a Cetac ASX 260 autosampler (Cetac technologies, Omaha, NE). The concentration of iNDF was determined in TMR, LP, and SP samples according to (Ahvenjärvi et al., 2000) and Chibisa et al (2012). After marker analysis, the omasal true digesta was reconstituted from the LP, SP and FP as described by France and Siddons (1986).

Ammonia-N concentration in rumen fluid was determined following the procedure proposed by Broderick and Kang (1980) using a phenolhypochlorite assay. Ruminal VFA samples were separated and quantified by gas chromatography (Agilent 6890, Mississauga, ON) as described by (Erwin et al., 1961).

8.3.4. Statistical Analysis

The PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC) was used to analyze the data. Milk yield, milk composition, feed intake, digestibility, omasal nutrient flow, ruminal fermentation, feed efficiency, milk net energy, and ruminal pH profile were analyzed assuming a crossover design. The final model included the fixed effects of period, order of treatment, treatment, and treatment period interaction. The random effect was cow within order.

Means effects were determined using the LSMEANS procedure. Normality was tested using univariate procedure of SAS software with the Shapiro-Wilk test. Differences were declared significant if $P < 0.05$ and values of $0.05 < P < 0.10$ were interpreted as tendencies towards significance.

8.4. Results and Discussion

The effects of FETR supplementation on nutrients intake are shown in Table 8.2. Adding the FETR tended to decrease the DM and OM intakes for dairy cows. On other hand, there was no effect of FETR supplementation on NDF or pdNDF intakes. Consistent with these findings, Holtshausen et al. (2011) found that adding fibrolytic enzyme rich in xylanase to diet can decrease the DMI without altering the level of milk yield in dairy cows during the mid-lactation stage. They attributed this reduction in DMI to the positive effect of fibrolytic enzymes in enhancing the available energy from the diet to dairy cows. The effect of fibrolytic enzymes addition on DMI is inconsistent across previous studies, some studies reported increased (Gado et al., 2009), or no effect (Arriola et al., 2011; Chung et al., 2012; Peters et al., 2015). Improving forage fiber digestibility usually associated with improving the feed intake and milk production (Oba and Allen, 1999b). Improving the DMI in dairy cows may be attributed to decrease gut fill, which allows the cows to eat more to meet their requirements during early lactation period. However, this mechanism was not conclusive from previous studies, for example Chow et al. (2008) found that the cows fed barley silage with enhanced ivNDFD had no effect on DMI, but the cows tended to gain more weight. Other studies have found that feeding forages with higher ivNDFD improved the feed efficiency (same DMI and high milk yield) for dairy cows during early lactation (Oba and Swift, 2014).

Table 8.2. Effect of novel fibrolytic enzyme supplementation on nutrient intake in early lactating dairy cows

Items	Control	ENZ	SEM[1]	*P* value
Intake, kg/d [2]				
DM	33.65	32.83	1.408	0.087
OM	31.29	30.52	1.309	0.087
NDF	11.18	11.02	0.471	0.352
pdNDF	5.94	5.85	0.251	0.384

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).
[1]SEM = standard error of mean; values are least squares means obtained from 9 cows.
[2] pdNDF = potentially digestible NDF.

Thus, pretreating barley silage with fibrolytic enzyme would exert different effects on dairy cows compared to other forages.

The effect of FETR supplementation on ruminal nutrient digestion is shown in Table 8.3. Adding the FETR numerically improved the ruminal OM and NDF digestion, where the amount of OM digested in the rumen was 8% higher for cows fed ENZ than the cows fed control diet, meanwhile the amount of NDF digested in the rumen was 20% higher for ENZ diet than control diet. However, this increased ruminal NDF digestibility for cows fed FETR was not statistically significant ($P > 0.10$). Several studies reported a positive effect of fibrolytic enzymes on ruminal digestion of nutrients during mid-lactation *in vivo* (Beauchemin et al., 1999; Bowman et al., 2002; Peters et al., 2010) or *in situ* (Romeo et al., 2015). However, there is very limited information regarding evaluation the effect of fibrolytic enzymes on ruminal nutrients digestion in dairy cows during early lactation using *in vivo* method. Yang et al. (1999) found that adding fibrolytic enzymes to alfalfa hay cubes improved the OM and NDF digestion in the rumen during early lactation. The numerical improvement in ruminal NDF digestion in the current study is consistent with the findings in our previous study (Chapter 7), where the ruminal ivNDFD of barley silage was 14% higher for ENZ treatment (barley silage pre-treated with 0.75 ml of FETR/ kg DM) than control treatment.

There was no effect ($P > 0.10$) of FETR on the total tract digestibility of DM, OM, and NDF (Table 8.4). These findings are in agreement with Yang et al. (1999), where reported that, the addition of fibrolytic enzymes could enhance the nutrient digestion in the rumen rather than in the intestine during early lactation.

Table 8.3. Effect of novel fibrolytic enzyme supplementation on ruminal nutrient digestion and omasal nutrient flow in early lactating dairy cows

Item	Control	ENZ	SEM[1]	*P* value
Omasal fluid flow, kg DM/d	6.26	6.20	0.570	0.937
Omasal particle flow, kg DM/d	17.84	17.06	0.746	0.481
DM				
Omasal flow, kg/d	24.08	23.28	1.059	0.327
Apparent digestion, kg/d	9.95	10.85	0.921	0.509
Apparent digestion, %	29.27	31.75	2.592	0.517
OM				
Omasal flow, kg/d	20.47	19.72	0.863	0.556
Apparent digestion, kg/d	11.20	11.98	0.830	0.523
Apparent digestion, %	35.38	37.77	2.368	0.500
NDF				
Omasal flow, kg/d	8.27	7.63	0.530	0.215
Apparent digestion, kg/d	3.29	4.04	0.611	0.337
Apparent digestion, %	28.00	34.32	4.898	0.300

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).
[1]SEM = standard error of mean; values are least squares means obtained from 6 cows.

In contrast, Beauchemin et al. (1999) found that fibrolytic enzymes was mainly caused by postruminal digestion. The variability in in mode of actions of enzymes between studies was likely due to methods of application, stage of lactation, and diet composition (Adesogan et al., 2014). Many studies have reported positive effects of applying fibrolytic enzymes to corn silage and alfalfa hay (Yang et al. 1999; Arriola and Adesogan, 2011). However, there is limited study that have reported any positive effect of fibrolytic enzymes on barely silage-based diet digestibility. Holtshausen et al. (2011) reported no effect of fibrolytic enzymes on ruminal ivNDFD of barley silage.

There was no effect of FETR addition on ruminal fermentation characteristics in early lactating dairy cows (Table 8.5). Several studies have reported adding fibrolytic enzymes could increase the total concentration of VFA (Pinos-Rodríguez et al., 2002; Giraldo et al., 2007). However, other studies have shown adding fibrolytic enzymes had no effect on total VFA concentration (Beauchemin et al., 1999; Sutton et al., 2003; Romero et al., 2016). The inconstancy effects of fibrolytic enzymes on total VFA concentration across studies seem to be influenced by the diet composition, the type of enzymes, the application rate of fibrolytic enzymes, and the effect fibrolytic enzymes on DMI (Beauchemin et al., 2003; Romero et al., 2016). In the current study, the limited effect of fibrolytic enzymes on the total VFA concentration is likely attributable to the low DMI levels in the cows fed ENZ diet compared to the cows fed CTR diet. Other factors could also affect the mode of action of fibrolytic enzymes on ruminal fermentation characteristics such as method of fibrolytic enzymes delivery and the forage to concentration ratio. Eun and Beauchemin (2005) observed feeding cows on high-forage diet supplemented with fibrolytic enzymes decreased the molar proportions of acetate, however

when the concentrate to forage ratio was increased, the molar proportions of butyrate decreased. Sutton et al. (2003) found the ratio of acetate, butyrate and propionate varied depending on method of fibrolytic enzymes application (sprayed onto the TMR vs. added to the concentrate portion vs. infused into the rumen).

There was no effect of FETR on ruminal pH profile in early lactating dairy cows (Table 8.6). The lack effect of FETR supplementation is due to its limited effect on ruminal fermentation characteristics. The total-tract rate of passage is also an important factor that could affect the ruminal pH (Mouriño et al., 2001). During early lactation, the passage rate increases due to the effect of gut fill on ruminal retention time, but the passage rate in this study was the same for all cows (Table 8.3).

Table 8.4. Effect of novel fibrolytic enzyme supplementation on total tract digestibility of nutrients in early lactating dairy cows.

Item	Control	ENZ	SEM[1]	*P* value
Digestibility, %[2]				
DM	58.00	59.00	0.993	0.413
OM	59.56	60.27	0.966	0.480
NDF	39.02	40.11	2.245	0.614
pdNDF	77.32	77.62	3.089	0.920

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).

[1]SEM = standard error of mean; values are least squares means obtained from 6 cows.

[2] pdNDF = potentially digestible NDF, pdNDF digestibility (%) =100 − [(dietary iNDF/fecal iNDF) × (fecal pdNDF concentration/dietary pdNDF)].

Table 8.5. Effect of novel fibrolytic enzyme supplementation on ruminal fermentation characteristics in early lactating dairy cows

Item	Control	ENZ	SEM[1]	*P* value
Total VFA, mmol, L	116.1	114.4	1.22	0.338
Ruminal VFA, mol /100 mol				
Acetate (C2)	55.50	55.05	0.895	0.735
Propionate (C3)	24.02	24.86	1.125	0.610
Butyrate	12.00	11.62	0.379	0.487
Isobutyrate	0.68	0.64	0.035	0.494
Valerate	1.40	1.45	0.046	0.427
Isovalerate	1.10	1.02	0.048	0.291
C2: C3 ratio	2.41	2.33	0.150	0.742
BCVFA[2]	1.77	1.66	0.074	0.311
Ruminal ammonia				
NH_3-N, mg/dL	16.21	13.87	1.312	0.242

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).
[1]SEM = standard error of mean; values are least squares means obtained from 6 cows.
[2]BCVFA = branched chain VFA, BCVFA = isovalerate + isobutyrate.

Table 8.6. Effect of novel fibrolytic enzyme supplementation on ruminal pH profile in early lactating dairy cows.

Item	Control	ENZ	SEM[1]	*P* value
Ruminal pH				
Mean	6.02	6.00	0.037	0.737
Minimum	5.55	5.50	0.039	0.376
Maximum	6.78	6.79	0.041	0.844
pH < 5.8				
Area, pH × min/d	35.54	34.25	10.490	0.907
Time, min/d	265.8	290.8	73.17	0.676
pH < 5.5				
Area, pH × min/d	0.042	1.089	0.681	0.512
Time, min/d	16.7	19.2	13.07	0.895

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).
[1]SEM = standard error of mean; values are least squares means obtained from 6 cows.

The effect of enzymes on milk production and composition in early lactating dairy cows are presented in Table 8.7. There was no significant effect of FETR on milk yield, milk fat or milk protein yields and feed efficiency. Our previous study (Chapter 7) showed that the milk yield and FCM were 1.8% and 6.8% higher, respectively in ENZ (0.75 ml/ kg DM) treatment versus control during mid-lactation period. The lower effectiveness of FETR supplementation on milk production of early lactating dairy cows in the current study could be due to the milk yield was not limited by the intake of digestible energy.

Lack of effect of FETR supplementation on in milk fat content between the treatment groups in the current study may attributed to the absence effect of FETR on ruminal fermentation characteristics as the dietary differences in milk composition are generally reflective of differences in ruminal fermentation. These findings are in agreement with the obtained effect of FETR addition on ruminal fermentation where there was no effect of ENZ application on rumen fermentation (Table 8.5).

Information on the concentration of MUN and ammonia nitrogen in the rumen in dairy cows allows to assess energy balance and protein rations supplied, which in turn could be useful in reducing feed costs and nitrogen emission to the environment (Guliński et al., 2016). The MUN is an important indicator as management tool for dairy farmers and nutritionist to assess how dietary protein is being utilized including form of protein, level of rumen fermentable carbohydrate, and rumen efficiency (Guliński et al., 2016). The increase in protein level in dairy cows' diet could result in increasing of total N excreted in the urine in relation to the N excreted in the feces, causing greater N

excreted in the milk (Broderick, 2003). The effect of FETR on MUN was more pronounced in the current study in early lactating dairy cows compared with previous study (Chapter 7) in mid-lactating dairy cows because of different level of milk yield. Jonker et al. (1998) found a positive correlation between MUN and milk yield levels. This correlation is attributed to the energy balance, where the cows with high milk production have a low energy balance and difficulty in satisfying energy requirements required for MCP synthesis.

There was no effect of FETR in ENZ diet on feed efficiency (1.58 vs. 1.63 Mcal/d, $P > 0.10$; Table 8.8) compared to control diet. In contrast to our results, Holtshausen et al. (2011) found a significant improved in feed efficiency (1.50 vs. 1.67 FCM/DMI; $P = 0.02$) in diet supplemented with fibrolytic enzymes compared to control diet. However, the higher efficiency in the later study is attributed to applying higher dosage (1.0 ml / kg DM TMR) of fibrolytic enzymes compared to our study. A negative effect of fibrolytic enzymes supplementation on feed efficiency was found by (Dean et al., 2013), while other studies report no differences between treatments (Peters et al., 2015; Romeo et al., 2015). Results across studies did not give a clear conclusion on fibrolytic enzymes effects on feed efficiency, mostly due to high inconsistency in DMI and milk production response.

Table 8.7. Effect of novel fibrolytic enzyme supplementation on milk yield and milk composition in early lactating dairy cows

Item	Control	ENZ	SEM[1]	*P* value
Yield, kg/d[2]				
Milk	53.83	55.53	2.943	0.280
FCM	57.35	58.31	3.112	0.707
ECM	55.30	56.61	3.014	0.599
Fat	2.10	2.12	0.139	0.912
Protein	1.54	1.62	0.091	0.274
Lactose	2.45	2.53	0.140	0.241
Solids-not-fat	4.51	4.69	0.245	0.248
Milk composition[3]				
Fat, %	3.82	3.73	0.225	0.578
Protein, %	2.86	2.92	0.100	0.301
Lactose, %	4.55	4.56	0.027	0.440
Total solids	12.22	12.22	0.307	0.981
Solids-not-fat, %	8.38	8.46	0.102	0.259
MUN, mg/dL	14.97	13.97	0.846	0.050
Efficiency				
ECM/DMI	1.64	1.72	0.064	0.392
FCM/DMI	1.70	1.77	0.068	0.466

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).
[1]SEM = standard error of mean; values are least squares means obtained from 9 cows.
[2] FCM = fat corrected milk; ECM = energy-corrected milk.
[3]MUN = milk urea nitrogen.

Table 8.8. Effect of novel fibrolytic enzyme supplementation on weight gain and predicted net energy values of early lactating dairy cows

Item	Control	ENZ	SEM[1]	*P* value
Body weight				
Mean body weight, kg	720.9	725.0	27.75	0.874
Weight gain, kg/d	0.85	0.74	0.275	0.769
Calculated net energy values[2]				
Maintenance, Mcal/d	11.12	11.17	0.323	0.865
Milk, Mcal/d	37.48	38.45	2.201	0.582
BW gain, Mcal/d	4.30	3.71	1.388	0.761
BW gain + Milk, Mcal/d	41.78	42.17	2.419	0.903
Total, Mcal/d	52.89	53.33	2.597	0.895
NEl, Mcal/ kg DMI	1.58	1.63	0.056	0.530

Note. Two groups of cows were randomly assigned to each of the dietary treatments in a cross-over design: CTR (without supplementation) and ENZ (with 0.75 mL of FETR/ kg DM of diet).

[1]SEM = standard error of mean; values are least squares means obtained from 9 cows.

[2]Net energy value used for maintenance, BW gain, and milk; Calculated NEl = calculated total net energy, Mcal/ kg DMI (kg/ d).

8.5. Conclusion

Pre-treating barley silage-based diet with FETR would decrease DMI, meanwhile maintaining the milk production. The use of fibrolytic enzymes did not have a major effect on ruminal degradation of DM or NDF and ruminal fermentation characteristics in dairy cows during early lactation. The positive effect of FETR may depend on diet composition, lactation stage and milk yield level.

9. GENERAL DISCUSSION

Barley silage is the most common forage in Western Canada. Barley is appropriate for swath grazing, green feed, or silage production in all soil zones at Saskatchewan. Several relatively new varieties of barley forage are grown for silage in Western Canada (Nair et al., 2016). However, there is limited data regarding their overall feeding value, molecular structure features, and nutrient availability and utilization in dairy cows. It is necessary to conduct studies to reveal their feeding value on the lactation performance of dairy cows. The corn forage that grows in Western Canada differs from corn in warmer climates in terms of feeding value and yield. In recent years, several varieties have been developed, but to establish these varieties as silage crop, they should be compared with other conventional forages such as barley silage. The hypothesis of this research was that selecting barley silage with high ivNDFD will improve the DMI and milk yield as a consequence of increasing the dietary energy relative to other barley silage varieties with low ivNDFD. The new short-season corn silage could show high feeding value and would result in improving the dairy cows' performance relative to the conventional forage such as barley silage. The objectives of the research were 1) to estimate the effect of different barley silage varieties potentially varying in ivNDFD on milk production efficiency, rumen degradation kinetics, rumen fermentation and microbial protein synthesis in dairy cows; 2) to assess the effects of feeding with barley silage and short-season corn silage upon the production performance of dairy cows; 3) to quantify the association between the molecular makeup related to protein and carbohydrate of barley and corn silages in relation to their chemical composition and

ruminal degradability in dairy cows and 4) to measure the effect of supplementing a new fibrolytic enzyme on production performance of dairy cows.

The chemical fractions, molecular structure, and degradation kinetics of DM and NDF of different barley silage varieties and a new short-season corn silage in dairy cows were assessed (Chapter 3). The three varieties of barley silage that were selected for this study were based on screening study done by Nair et al. (2016) on seven varieties. The studied three varieties were harvested at mid-dough stage, the selection of these varieties was based on using 30h-ivNDFD as a univariate approach for evaluating the barley silage. The new short-season corn silage variety was selected based on other screening study by Abeysekara et al. (2013a, b), where this variety had shown a higher feeding value and yield relative to other corn forage varieties that grown in Saskatchewan.

The results in this study showed that all barley silages had similar digestible fiber (CB3, %CHO) contents, which is different from the earlier study by Nair et al. (2016). Furthermore, the barley silage that was selected based on its higher ivNDFD showed a higher indigestible fiber fraction (CC, %CHO) compared to other barley silage varieties. Cowboy BS had exhibited a lower EDDM and EDNDF compared with Xena. These changes in digestible carbohydrate between the two studies are possibly due to genotype–environment interaction. This means that some varieties of barley forage (i.e. Cowboy BS) would perform contrarily in some adverse growing conditions. All barley forage varieties that were used in the current study have received the same management during growing. Thus, other variables such as soil type and fertility could affect the quality of barley forage varieties (Saskatchewan Ministry of Agriculture, 2016).

Using single time incubation (30 or 48-h ivNDFD) as a method for evaluating forages would have some disadvantages because it would only indicate to the residual NDF after a specific time and these residues might include some potential fiber fraction in addition to the indigestible fraction of NDF (Lopez et al. 2015). Furthermore, ivNDFD would not able to give any indication about the degradation kinetics of forages (Lopez et al. 2015). It has been found that when modelling in order to accurately predict NDF digestibility, the model should partition NDF into iNDF and pdNDF, and fractionate feed particles by their retention and passage in the rumen using a predicted K_d by an in vitro system (Huhtanen et al. 2008). Therefore, for the upcoming studies it is recommended using a multivariate approach to assess the barley silage varieties. This approach should consider the indigestible fiber content and the degradation rate of forage. The indigestible fiber fraction would be estimated by incubating the feed samples in rumen for 240h, while the degradation rate would be predicted accurately by using 2-time points ivNDFD (30h and 120h; Lopez et al. 2015). Furthermore, it is possible to include FT/IR spectroscopy analysis in this approach by analyzing feed, rumen and intestinal digesta, and fecal samples for discovering the spectrum data that are associated with the digestible fiber content of forage and the degradation rate of fiber (Yu, 2012). Ultimately, it is possible to develop new equations that could predict the in vivo ruminal and total-tract digestibility of NDF of forage by considering spectrum data along with the multiple time-point ivNDFD (30h, 72h, and 240h).

The new short-season corn silage had a higher content of starch and a lower indigestible fiber content (CC, %CHO) relative to barley silage, which would result in increasing the digestible carbohydrates content available for dairy cows (NRC, 2001). All

the predicted energy values of NRC model (NRC, 2001) were significantly greater in the new short-season corn silage variety relative to barley silage. Corn forage could produce high yield when moisture, nutrients and heat are not limiting (Saskatchewan Ministry of Agriculture, 2016). The new short-season corn silage that selected in the current study would be a good alternative to the conventional crop forages grown in Western Canada such as barley silage.

The FT/IR spectroscopy has been used to detect structural changes induced by feed processing and to evaluate the nutritional-related structure of forages and grain (Damiran and Yu, 2011; Abeysekara et al., 2013a, b; Huang et al., 2017). However, there is no reported study related to the application of FT/IR spectroscopy for predicting the nutrient availability and utilization of ensiled barley and corn forages. Chemometric analysis of molecular spectral data usually include univariate and multivariate analyses. These two methods are commonly used to improve the understanding of the relation between feed quality or physical properties of feed and the molecular structure (Yu et al., 2004b). Studying the associations between molecular structures related to carbohydrate and lignin with the nutrient availability and utilization of barley and corn silages showed some significant correlations. These associations could reflect the accuracy of molecular information that collected by the FT/IR (functional groups) spectroscopy to reveal the nutritional value of barley and corn silage.

The multivariate analysis (using whole spectrum) of barley and corn silage were not very distinguishable in spectral characteristics of all carbohydrates regions such as structural carbohydrates, non-structural carbohydrates, and total carbohydrates regions.

The results of these study suggest that the original spectra are not able to distinguish between structures of feed samples. Thus, in the future it is recommended to perform a pre-processing to whole spectrum using a second derivative method which could offer a practical and more specific method than routinely used absorption spectrum analysis methods to obtain clear compositional information on forage sample with FT-IR spectroscopic imaging (Yu, 2005; Malek et al., 2014). This would offer an advantageous usage for resolving the absorption bands, which are mainly overlapped. Processing the spectral by the second derivative analysis would highlight band positions and separations (Malek et al., 2014). In a second derivative spectral profile, the overlapping absorption components in the spectral profile are shown as negative bands. The second derivative analysis needs spectra with high signal-to-noise ratio where the second derivative band intensities are contrariwise relative to the square of the original band half-width, this would in turn cause great enhancements of the relative contribution of sharp lines due to noise and vapour (Malek et al., 2014).

Using diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) along with the multivariate spectral analysis would also detect carbohydrates and protein molecular structure differences of feed (Liu and Yu, 2010). DRIFT spectroscopy could be used to understand the inherent chemical structures of plant tissues or plant parts. In future, it is suggested to use this technique for detecting the plant internal structure and to reveal the variances between different genotypes of barley forage in terms of their carbohydrates molecular structure (Liu and Yu, 2010).

The nutritional features, molecular structure, and metabolic characteristics of protein in dairy cows of barley silage in comparison with a new short season-corn silage were evaluated (Chapter 4). *In situ* ruminal degradation of CP showed all barley silage varieties had the same content of EDCP. The intestinal digestibility of corn silage was higher than barley silage, this was due to the higher RUP content in corn silage compared to barley silage.

Studying the predicted truly absorbed MP to dairy cows and FMV showed that all silages would provide the dairy cow with a similar content of MP (average 51.3 g/kg DM). The degraded protein balance was lower in the new short-season corn silage relative to all barley silage varieties, which would in turn impair the microbial protein synthesis in the rumen. Thus, it is important to provide more degradable protein source with corn silage to provide adequate amount of N for microbial protein synthesis.

The study on association between molecular structures of barley and corn silages and the metabolic characteristic of protein in dairy cows showed that there was a significant correlation between the amide I height, amide area, α- helix or α- helix to *β*-sheet ratio and soluble CP fraction and EDCP. It has been observed the changes in the ratio of α -helix: *β*-sheet ratio could induce alterations in protein molecular makeup (Yu, 2005). The high proportion of *β* -sheet structure could limit the access of gastrointestinal digestive enzymes, which results in a low protein value (Yu, 2005). In this current study, there was a negative correlation between ARUP and *β*-sheet. The amide area, amide I height, and *β*-sheet were significantly correlated with DPB.

The effects of feeding different barley varieties potentially varying in ivNDFD on lactation performance and chewing activity in comparison with a new short-season corn silage in lactating dairy cows were assessed (Chapter 5). Feed intake was not influenced by feeding different barley silage-based diets. The lack of an effect of CDC cowboy (selected based on its high ivNDFD) is attributed to its comparable content of CB3 to other barley silage varieties. All barley silage-based diets had the same milk yield, milk composition, and feed efficiency. In agreement with these findings, Nair et al. (2017) did not observe any improvement in feedlot performance of steers during backgrounding and finishing phases with feeding Cowboy BS compared to Xena BS (low ivNDFD). The lacking effect of CDC Cowboy compared to other BS varieties on milk production, milk composition and feed efficiency is attributed to the limited effect of this variety on feed intake (Oba and Allen, 1999b) due to the lower content of starch (8.7% vs. 14.3%) and the higher NDF content (55% vs. 49.8) relative to other BS varieties.

Feeding cows with a new short-season corn silage improved milk, FCM, ECM, protein, lactose, and solids-not-fat yields compared to cows fed barley silage-based diets. The cows fed corn silage-based diet had a higher feed efficiency compared with cows fed barley silage-based diets. The higher performance of dairy cows fed corn silage-based diet is attributed to the lower CC (17 vs. 24%) and higher starch (26 vs. 12%) in corn silage relative to barley silage. In this study, the corn silage-based diet tended to decrease the ruminating time compared with the barley silage-based diet. This reduction in ruminating time may be attributed to lower peNDF level in corn silage diet compared to barley silage diets (Yang and Beauchemin, 2006). There were no significant differences among BS diets on chewing activity, the lack effect of a BS diet on chewing activities is

attributed to the limited effect of barley silage varieties on feed intake (Oba and Allen, 2000).

The effects of feeding different barley varieties on rumen fermentation characteristics and microbial protein synthesis have been evaluated using the rumen simulation technique (Chapter 6). There was no effect of BS diets on rumen fermentation characteristics and mean ruminal pH. The corn silage-based diet decreased the ruminal pH and acetate to propionate ratio. These findings are consequences of the higher content of fermented carbohydrates in CS compared to BS (Benchaar et al., 2014).

There was no difference among BS diets in synthesis of microbial protein, which may be explained by the similar contents of fermentable CHO and N in the rumen in all diets as previously reported by Chumpawadee et al. (2006). The diet containing CS improved the microbial protein production compared to BS diets. These differences between BS and CS are attributed to the higher energy available for MCP. These findings suggest a higher nutritive value for short-season corn silage compared to other barley silages.

Based on the results in previous two chapters, it is concluded that selecting barley silage varieties based on their ivNDFD level is not satisfactory for improving dairy cows' performance. Therefore, in the next two experiments (Chapter 7 and 8) I focused on increasing ivNDFD of barley silage or barley silage-based diet using a novel exogenous fibrolytic enzyme as an approach to improve ivNDFD, and to correlate this increase with dairy cows' performance during mdi-lactation (Chapter 7) or early-lactation (Chapter 8).

In Chapter 6, the effects of exogenous fibrolytic enzyme supplementation to barley silage-based diet on lactation performance and feeding behavior in mid-lactation dairy cows were studied. Firstly, *in vitro* incubations were performed to examine the effect of fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK) on the digestibility of barley silage. The results showed that ivNDFD tended to increase linearly by increasing the FETR level. Thus, we selected three levels of this product to be tested on mid or early lactating dairy cows. The FETR did not alter the DMI, but improved the nutrient digestibility, where there was a cubic response ($P < 0.01$) of total-tract digestibility of DM, OM, NDF, and pdNDF to increasing FETR levels. The intermediate dosage (0.75 ml / kg DM) exhibited the highest total-tract nutrient digestibility.

By adding the FETR to TMR, the milk production and composition were improved but this was dependant on the level of enzyme. The moderate level of enzyme (0.75 ml FETR/ kg DM) improved the FCM, ECM, and milk fat yields. In line with these findings, Peters et al. (2015) reported a tendency to improve ECM by fibrolytic enzymes in dairy cows during mid-lactation. Improving the FCM, ECM, and milk fat yields in the current study could be attributed to improving fiber digestion due to the FETR supplementation. It has been reported that any increase in forage NDF digestibility could improve milk yield (Oba and Allen, 2000). The high producing dairy cows which are in negative energy balance, the spared acetate (resulting from higher ruminal fiber digestion) is more likely to be used by the mammary gland for lactose and protein synthesis and subsequently increase milk yield (de Veth et al., 2006).

The effect of adding FETR on feeding behavior of dairy cows was evaluated in the current study (Chapter 6). Eating time, meal size, the number of daily feeding episodes in the current study were not significantly different among treatments. The lacking effect of FETR on feeding behavior could be attributed to the limited effect of FETR on feed intake. Effect of FETR on feeding behavior in dairy cows is not consistent across previous studies. Beauchemin et al. (2000) found that fibrolytic enzyme supplementation decreased the time spent eating per unit of NDF. They attributed this change to pre-ingestive effects of the fibrolytic enzymes on the feed intake, indicating that enzyme application directly to the feed could alter the fragility of the feed and, hence, its ability to increase chewing activity. In contrast, Beauchemin et al. (2003) and Peters et al. (2015) found that supplementing the diet with fibrolytic enzymes did not alter feeding behavior during early and mid-lactation stages, respectively. Based on the findings in this study, the best level of the fibrolytic enzymes was the 0.75 mL FETR/ kg DM of TMR. Applying this dosage to TMR, enhanced the digestibility of NDF, milk yield and milk fat yield in dairy cows. Furthermore, the feed efficiency increased by up to 7% relative to the control diet

Based on the findings in this study (Chapter 6), the next study (Chapter 7) was performed to assess the effects of the best FETR dosage (0.75 ml / kg DM) adding to silage-based diet on lactation performance, digestibility, and ruminal fermentation characteristics in dairy cows during early lactation. Adding the FE tended to decrease the DM intake. The effect of FETR on feed intake of dairy cows during early lactation stage is not consistent across studies. Previous published studies did not detect effect of fibrolytic enzymes (rich in xylanase and cellulase) supplementation on DMI of dairy

cows during different stages of lactation (Arriola et al., 2011; Chung et al., 2012; Peters et al., 2015). Holtshausen et al. (2011) detected a reduction in DMI in dairy cows supplemented with fibrolytic enzymes during early lactation, but the milk yield was maintained at the same level as control diet. They attributed this reduction in DMI to the positive effect of fibrolytic enzymes in improving the energy availability of the diet.

The lower level of MUN in FETR diet could be attributed to increasing nitrogen utilization in dairy cows. Consistent with previous results, the level of ammonia in the rumen was numerically lower in FETR diet compared to CTR diet (13.9 vs. 16.2 mg/dL). In agreement with this finding, Yang et al. (1999), found that the fibrolytic enzymes supplementation increased the MCP synthesis as a result of increasing the fermented organic matter in the rumen. The effect of FETR on MUN is more pronounced in the current study compared with previous study (Chapter 6), due to the high level of milk yield (Jonker et al., 1998).

Based on the findings form this study, FETR did not exhibit a major effect on ruminal degradation of NDF and animal performance compared to what was found in the previous trial. The positive effect of FETR could rely on diet composition, lactation stage and milk yield level. For the upcoming studies, it is important to consider not only the quality of forage but also the level of feed intake, diet composition, and forage inclusion level in diet to better evaluate the effects of fibrolytic enzyme on dairy cows' performance.

10. GENERAL CONCLUSION

The barley silage that selected based on its high ruminal in vitro NDF digestibility did not correspond with a greater effect on lactational performance, ruminal fermentation characteristics, and microbial protein synthesis compared to the barley silage variety with low ivNDFD due to its limited effect on feed intake. There was no effect of barley silage variety on the estimated metabolizable protein supply to dairy cows. The results suggested that the univariate approach with only one factor consideration such as 30h in vitro NDF digestibility seems to be non-satisfactory method for evaluating barley silage varieties. Further research is warranted to assess barley silage varieties using other methods of evaluation such as 288h undigested NDF.

The new short-season corn silage had a greater energy density (low indigestible fiber and high starch contents) relative to barley silage. The findings of this study showed that new short-season corn silage had less degradable protein content in the rumen and degraded protein balance. Feeding the new short-season corn silage could has a great potential to increase the milk yield, microbial protein synthesis, and feed efficiency in dairy cows compared with barley silage. Thus, the new short-season corn silage would be a good alternative to conventional forages in Western Canada.

The spectral profiles of barley and corn silages were highly related to their nutrient availability and utilization in dairy cows.

Pre-treating barley silage-based diet with fibrolytic enzyme (rich in xylanase and cellulase) would improve the performance of dairy cows. However, the positive effect of

fibrolytic enzyme depends on diet composition, lactation stage and the level of milk production.

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12. APPRNDIX

Table 1. Basic chemical profile of barley silages with lowest, intermediate and highest NDFD compared with corn silage

Items	Silages				SEM[1]	Contrasts *P* value			
	Corn	Barley				Corn vs. Barley	Cowboy vs. Copeland	Copeland vs. Xena	Cowboy vs. Xena
	P7213R	Cowboy	Copeland	Xena					
Basic chemical profile									
Ca (%DM)	0.25*b*	0.45*a*	0.40*a*	0.40*a*	0.017	<0.01	<0.01	0.58	0.29
Phosphorous (%DM)	0.31*b*	0.33*ab*	0.30*b*	0.37*a*	0.008	0.01	0.09	0.04	0.00
DCAD[2]	29.8*a*	40.85*b*	31.2*a*	31.45*a*	0.953	0.01	0.01	<0.01	0.86

[1]SEM = standard error of mean
[2]DCAD = dietary cation-anion difference (meq/100 g DM).

Table 2. Predicted energy values of lactating dairy cows for four barley and corn silage-based diet treatments.

Items	Diets			
	Corn	Barley		
	P7213R-	Cowboy-	Copeland-	Xena-
Maintenance	10.86	10.94	10.95	10.87
BW gain	2.24	1.47	1.79	0.38
Milk	26.54	24.09	24.50	24.44
BW gain + Milk	28.77	25.56	26.29	24.82
Total	39.63	36.49	37.24	35.69
NE_l (Mcal / kg DMI) [1]	1.51	1.36	1.44	1.38

[1]Net energy value used for maintenance, BW gain, and milk; Calculated NE_l = calculated total net energy, Mcal / DMI (kg / d)

Table 3. Prediction ruminal degradation of CHO for barley silages with different digestible carbohydrates contents compared with corn silage using the updated CNCPS model

Items	Silages[1]				SEM[2]	P- value	P- value Contrast
	Corn (C)	Barley (B)					
	P7213R	Cowboy	Copeland	Xena			C × B
Predicted ruminal degradation of fiber[3]							
Kd, hr	2.79*b*	4.33*a*	3.58*ab*	2.61*b*	0.197	0.02	0.05
CC, %DM	15.41*c*	25.42*a*	21.83*b*	16.77*c*	0.584	0.002	0.01
CB3, %DM	29.00	29.60	29.18	31.63	0.584	0.07	0.12
RDCB3, %DM	11.90*b*	15.37*a*	13.76*ab*	12.43*b*	0.589	0.03	0.02
TRDC, %DM	39.39	32.00	34.08	32.76	0.811	0.25	0.01

[1]P7213R-CS = P7213R corn silage; Cowboy-BS = CDC Cowboy barley silage with a higher ivNDFD; Copeland-BS= CDC Copeland barley silage with an intermediate ivNDFD; Xena-BS= Xena barley silage with a lower ivNDFD.

[2]SEM = standard error of mean

Means with different letters in the same row differ ($P < 0.05$).

[3]Kd = degradation rate for of available NDF fraction using 3-time point NDFD; CC = unavailable cell walls; CB3 = available aNDF; RDCB3 = ruminal degradation of available NDF calculated by applying kd rates obtained from three time points NDFD; TRDC = total ruminally degraded carbohydrate

Table 4. Coefficient of correlation between the predicted digestible CHO contents by the updated CNCPS model and milk yield and composition

Items	CC (%DM) [1]		CB3 (%DM)		RDCB3 (%DM)		TRDC (%DM)	
	r[2]	*P* value	r	*P* value	r	*P* value	r	*P* value
Milk, kg/d	-0.55	0.15	-0.54	0.17	-0.32	0.44	0.95	0.01
FCM, kg/d[3]	-0.72	0.05	-0.37	0.37	-0.51	0.20	0.95	0.01
Milk fat, %	0.21	0.62	0.73	0.04	-0.04	0.93	-0.82	0.01
Milk protein, %	-0.50	0.21	0.35	0.40	-0.59	0.12	0.03	0.95
Milk lactose, %	-0.55	0.16	0.32	0.44	-0.63	0.09	0.10	0.82

[1]CC = unavailable cell walls; CB3 = available aNDF; RDCB3 = ruminal degradation of available NDF calculated by applying Kd rates obtained from three time points NDFD; TRDC = total ruminally degraded carbohydrate
[2]r = correlation coefficient
[3]FCM = Fat-corrected milk

Table 5. Differences between the older and updated CNCPS models in predicting the available energy and potential milk yield

Items	Diets				SEM[1]	P- values contrasts			
	Corn (C)	Barley (B)							
	P7213R	Cowboy	Copeland	Xena		Corn vs. Barley	Cowboy vs. Copeland	Copeland vs. Xena	Cowboy vs. Xena
Predicted NDFD, %[2]									
ttNDFD (ADL input)	63.9	58.1	53.2	60.2	-	-	-	-	-
ttNDFD (uNDF288hr input)	50.51	55.39	53.25	49.60	0.742	0.07	0.21	0.03	0.01
Predicted available energy[3]									
ME Mcal/day (ADL input)	71.6	63.0	60.2	61.0	-	-	-	-	-
ME Mcal/day (uNDF288 input)	63.78	59.9	61.3	60.7	0.872	0.01	0.14	0.48	0.32
MP g/day (uNDF288 input)	3018	2764	2821	2803	18.06	0.01	0.11	0.52	0.23
Predicted Milk yield, kg/d[4]									
Milk yield (ADL input)	47.4	38.8	36.1	39.3	-	-	-	-	-
Milk yield (uNDF288 input)	39.8	36.7	37.3	36.7	0.72	0.02	0.56	0.59	0.99
Actual milk yield[5]	40.0	35.3	35.9	34.8	1.71	-	-	-	-

[1]SEM= Standard error of mean.
[2]ttNDFD = total-tract NDFD.
[3]ME = metabolizable energy; MP = metabolizable protein.
[4]Predicted milk yield based on allowable ME.
[5]Actual milk yield obtained from dairy trail using the same diet that used for predicting milk yield by CNCPS models.

Table 6. Total revenue of milk yield of mid lactating dairy cows fed a novel fibrolytic enzyme

Items	Control	Enz (0.75 ml FETR/kg DM TMR)
Price of milk components[1]		
Fat ($)	10.93	10.93
CP ($)	7.93	7.93
Other solids ($)	1.19	1.19
Revenue		
Revenue Fat ($)	12.88	14.03
Revenue CP ($)	9.73	9.96
Revenue Other solids ($)	2.41	2.50
Total revenue $ (Cow/day)	25.01	26.50

[1]data obtained from SASKMILK, available online: http://www.saskmilk.ca/index.php/publications/newsletters

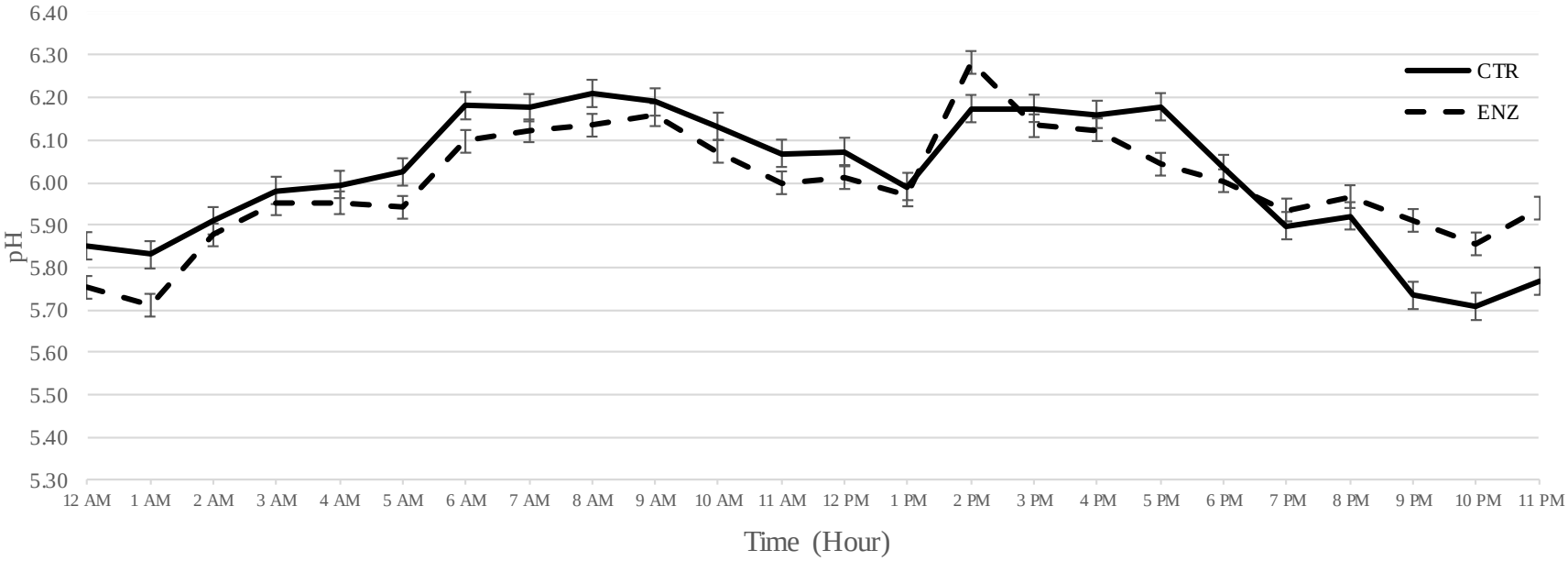

Figure 1. Effect of fibrolytic enzyme supplementation on rumen pH profile using in-dwelling pH probes, averaged over 24 h feeding period.

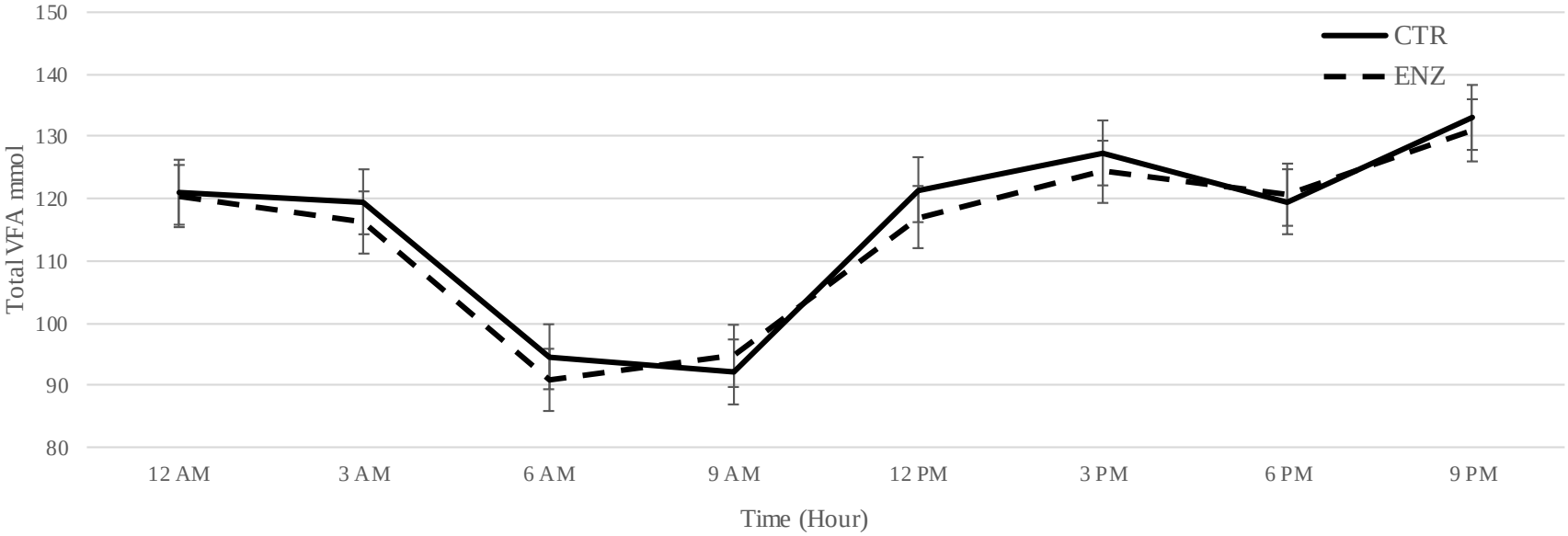

Figure 2. Effect of fibrolytic enzyme supplementation on total volatile fatty acid concentration (mmol/L), averaged over 24 h feeding period.

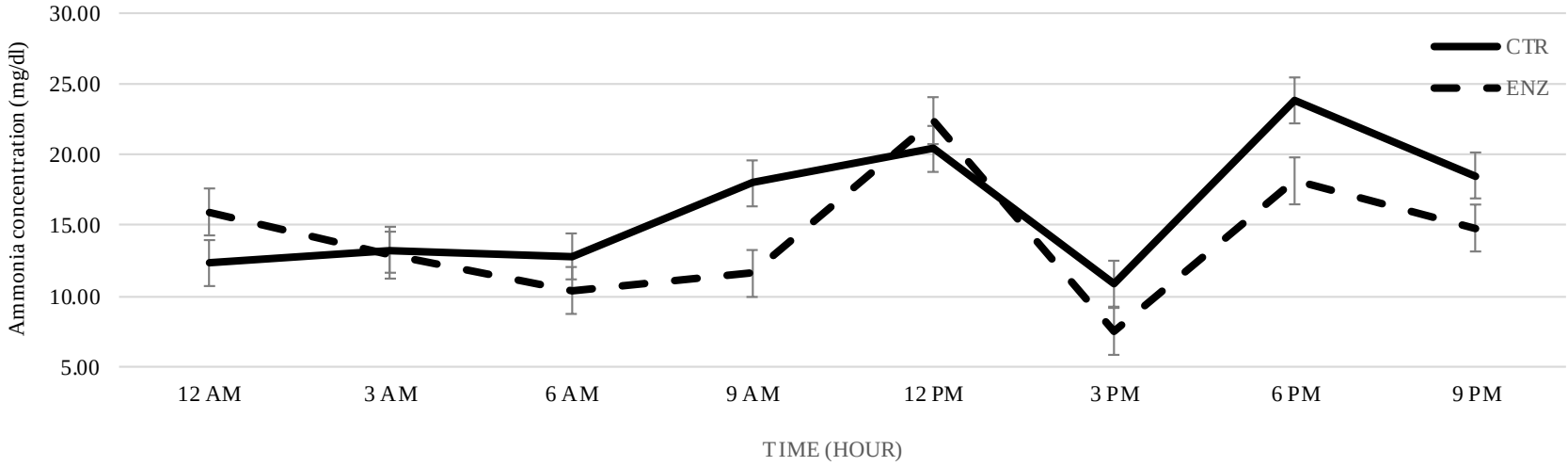

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Figure 3. Effect of novel fibrolytic enzyme supplementation on ruminal ammonia concentration (mg/dL) averaged over 24 h feeding period for early lactating dairy cows.