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Comparison of barley silages with varying digestible fiber content to corn silage on rumen fermentation characteristics and microbial protein synthesis using RUSITEC

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Running head: Digestible fiber content and microbial protein synthesis of silages and TMR

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ABSTRACT:

The objectives of this study were to assess the magnitude of differences among barley silages with different in vitro NDF digestibility (ivNDFD) in comparison with corn silage in 1) predicted carbohydrate digestibility 2) rumen fermentation characteristics and 3) microbial protein synthesis using Rumen Simulation Technique (RUSITEC). The experiment was carried-out in a completely randomized block design with four treatments. The four whole-plant silages utilized in this study were: CS-TMR = corn silage (30h ivNDFD = 32%), HNDFD-TMR = barley silage with high ivNDFD (30h ivNDFD = 37%), INDFD-TMR = barley silage with intermediate ivNDFD (30h ivNDFD = 28%), and LNDFD-TMR = barley silage with low ivNDFD (30h ivNDFD = 26%). Results from RUSITEC showed that nutrient disappearance, rumen fermentation characteristics, and microbial protein synthesis did not differ among diets that contained different varieties of barley silage ($P > 0.1$). However, CS-TMR tended to have a higher microbial protein yield than all barley silage diets ($P = 0.06$). These results imply higher in vitro NDF digestibility of barley silage may not necessarily correspond with greater impact on rumen fermentation and microbial protein synthesis. However, feeding the corn silage had higher microbial protein synthesis in the RUSITEC and might enhance dairy cattle performance compared to barley silage.

Keywords: Bacterial N production, in vitro fiber digestibility, RUSITEC

Abbreviations: ADF, acid detergent fiber; CHO, total carbohydrates; CS, corn silage; GP, gas production ; HNDFD, high NDFD; iNDF, indigestible NDF; INDFD, intermediate NDFD; ivNDFD in vitro digestibility of NDF; K_d /h, the digestion rates; LNDFD, low NDFD; NDF, neutral detergent fiber; NDFD, NDF digestibility; NDIN, insoluble protein; NFC, non-

fiber carbohydrate; **NSC**, non-structural carbohydrate; **pdNDF**, potentially digestible NDF fraction; **RDCB2**, ruminal degraded of soluble fiber fraction; **RDCB3**, ruminal degraded of available NDF; **RUSITEC**, rumen simulation technique; **SCP**, total soluble CP; **TMR**, total mixed ration; **TRDC**, total degraded ruminal CHO fraction; **TRUC**, total escaped CHO fraction; **uNDF**, undigested NDF; **VFA**, volatile fatty acid.

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 1999).

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 1999). Effects of forage ivNDFD on milk production have been extensively 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.

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 high quality 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 RUSSETIC.

MATERIALS AND METHODS

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 (Cowboy, Falcon, Metcalfe, Copeland, Conlon, Xena, and Legacy)

harvested for making silage at the mid-dough stage of maturity were collected within two 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 26 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%, HNDFD), CDC Copeland (28%; INDFD) and Xena (26%; LNDFD). 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 (latitude: 52.154388 ° N, longitude: 106.618689° W and altitude 491m) in 2014 under dry land conditions. Barley varieties were all seeded on May, 20, 2014 and harvested on July, 31, 2014 at the mid-dough stage of maturity and were cut at 10 mm. All the barley forages were packed in three individual piles without inoculation. The corn variety (32%, CS; hybrid 7213R, Pioneer, Warman, SK), was also grown at the University of Saskatchewan research farm, but was seeded May 29, 2014, and harvested on September 29, 2014, after reaching target heat unit at about 33% DM. Corn forage was cut at 9.5 mm theoretical cut length with kernel processing and without inoculation stored in a pile using double-layer cover.

Samples were prepared by sampling the silages from various locations in the pile. Two samples from each silage variety were taken, the first sample was taken after 90 days of ensiling and the second sample was taken after 150 days of ensiling 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 mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK). The detailed chemical analysis are presented in the following section. For silage fermentation analysis, another subsample was taken from each replicate were frozen at -20°C until processing. The frozen samples were thawed at 4°C and about 15g from each sample was used for measuring pH, lactate, ammonia and volatile fatty acid (VFA) concentrations. For the RUSITEC study, shortly before commencing the study, subsample from each pile were collected and dried at 55°C for 48 h. During a lactation trial silages were sampled weekly and composited with each sample around 4 kg collected during four months sampling. The composite sample was used in the RUSITEC study. The dried sample was ground to pass a 4-mm screen (Arthur Thomas Co., Philadelphia, PA).

RUSITEC Trial

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 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 (2009).

The experimental design was fitted in a randomized complete block design with two blocks (RUSITEC apparatuses), sixteen 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: CS-TMR = TMR with CS (P7213R corn silage), HNDFD-TMR = TMR with HNDFD (CDC Cowboy barley silage with high ivNDFD), INDFD-TMR = TMR with INDFD (CDC Copeland barley silage with intermediate ivNDFD), and LNDFD-TMR = TMR with LNDFD (Xena 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. 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 days in milk) that produce 42 kg of milk with 3.95 % milk fat and 3.25% milk 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, one nylon bag containing silage only and one nylon bag containing alfalfa hay and 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%/h.

The experimental period consisted of 16 days including 10 days of adaptation and 6 days 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 re-usable 2 L vinyl collection bag (Curity®; Conviden Ltd, Mansfield, MA) 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). Dry matter disappearance was determined after 48 h of incubation from day 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 disappearance. From day 11 to 14 of the experimental period, liquid effluent was collected into 1 L flasks and samples were taken for VFA and $\text{NH}_3\text{-N}$ analysis.

Microbial protein synthesis was estimated as described by Wang et al. (2001). Bacteria in the fermenters were labeled using ^{15}N . The $(\text{NH}_4)_2\text{SO}_4$ in the McDougall's buffer solution was replaced on day 10 with ^{15}N -enriched $(\text{NH}_4)_2\text{SO}_4$ (Sigma Chemical Co., St. Louis, MO; minimum ^{15}N enrichment 1 g / L) until the end of the experiment. Before labeling on day 10, effluent and feed residue were collected to determine background ^{15}N . On day 15 and 16, ^{15}N concentration of feed particle-associated, feed particle-bound and effluent bacterial fractions prepared from feed bag residues and effluent.

Predicting Rumen and Total-Tract Fiber Digestibility

The digestion rates (K_d /h) of potentially digestible NDF content (pdNDF; CB3 K_d) of the forages were estimated using 48-h NDFD, a fixed lag of 3-h and iNDF at 288-h following the method of Van Amburgh et al. (2003). The available NDF fraction K_d 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; $\text{CB3 sub-fractions} \times K_d / (K_d + K_p)$, where K_p is fractional rate of passage. Total degraded ruminal CHO fraction (TRDC) and total escaped CHO 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).

Chemical Analysis

The dried forage and diet samples were ground through a 1-mm screen (Christy & Norris mill 8" Lab mill, Christy Turner Ltd, Chemsford, UK) for use in chemical analyses. Concentrate 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. For estimation of CP, N was determined using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St Joseph, MI). ADF was analyzed according to AOAC (2000; method 973.18). NDF and ADL were analyzed according to Van Soest et al. (1991). Amylase was used in the NDF analysis but sulfite was omitted. The total soluble CP (SCP) was analyzed by incubating the sample with a bicarbonate-phosphate buffer and filtering it through Whatman 54 filter paper. The acid detergent fiber insoluble protein (ADIN) and neutral detergent fiber insoluble protein (NDIN) 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 (NSC) content, including sugars, organic acids, and other reserve carbohydrates, were estimated using non-fiber carbohydrate (NFC) and calculated according to NRC (2001). Total CHO was calculated as: $100 - (\% \text{ EE} + \% \text{ CP} + \% \text{ ash})$, and NFC was calculated as $\text{NFC} = 100 - (\% \text{ CP} + (\% \text{ NDF} - \% \text{ NDIP}) + \% \text{ EE} + \% \text{ ash})$. Feed residues obtained from RUSITEC were ground through a 1-mm screen (standard model 4, Arthur Thomas Co., Philadelphia, PA).

Total N was determined using a combustion analyzer (method 990.03; AOAC, 2006) and atom per cent excess (APE) 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). 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-butenic acid) was used as the 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 daily 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, Canada and cared for according to the guidelines of the Canadian Council on Animal Care (CCAC 2009). Two experimental runs in each time were carried out. Samples of 3 g were 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 cows. After removal from the rumen, the bags were rinsed, oven dried at 55°C for 48h and subsequently the NDF of residues was calculated according to Van Soest et al. (1991).

Statistical Analysis

The parameters measured in the RUSITEC trial were analyzed as randomized complete block design using the MIXED procedure of SAS 9.4, where 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 4 (4 fermenters: 2 fermenters from RUSITEC UNIT I as block 1 and other 2 fermenters from RUSITEC UNIT II as block 2). The statistical model used to analyze the measured parameters in RUSITEC 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 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 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 random effect of RUSITEC apparatus unit ($j=2$), and e_{ijk} was the residual error. Total experiments units for each treatment are 4 (4 fermenters: 2 fermenters from RUSITEC UNIT I as block 1 and other 2 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 (HINDFD-

TMR $\times$ INDFD-TMR, HNDFD-TMR $\times$ LNDFD-TMR and INDFD-TMR $\times$ LNDFD-TMR) and between barley silage and corn silage (Corn $\times$ average (HNDFD, INDFD, LNDFD-TMR). Statistical significance was declared at $P < 0.05$, and trends were noted at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Characteristics of Corn Silage and Barley Silage

The protein and carbohydrate profiles of the barley and corn silages are presented in Table 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. However, these differences among barley varieties and between barley and corn silages were not statistically tested. The CP content among barley silage varieties appeared to be similar (averaged 11.2% DM). However, CP content differed between barley and corn silages, where 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; Abeysekara et al. 2013b; Chaves et al. 2012). In the present study, CS had less SCP than all barley silage varieties. Starch content also varied between barley and corn silages, with CS was the greatest at 26% of DM, while HNDFD had the least starch content at 8.7% DM. There were also differences among barley silage varieties in starch content with HNDFD barley silage starch content was lower than LNDFD barley silage by 55%. On the other hand, HNDFD had the highest NDF and INDFD had the lowest NDF (49 vs 43% DM, respectively). All barley silages showed higher ADF compared with the corn silage 34.7% vs 26.3% DM, respectively. In the

present study, iNDF concentration (%DM) was highest in the HNDFD silage (25.8% DM), intermediate in the INDFD silage, and lowest was reported in the LNDFD silage (17.8% DM).

Rumen Fermentation and Microbial Protein Production in RUSITEC

Rumen fermentation characteristics in RUSITEC are given in Table 3. The diet HNDFD-TMR with HNDFD barley silage showed no effects on rumen fermentation characteristics in comparison to INDFD-TMR and LNDFD-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 (2002) and Chow et al. (2008) showed no significant effects of feeding silage with higher NDFD on total VFA concentrations. In a meta- analysis study, it has been found that the ruminal pH, total VFA, and acetate concentration are unaffected by corn silage hybrid type that differs in ivNDFD (Ferraretto and Shaver 2015). There were differences between HNDFD-TMR and LNDFD-TMR in the molar proportion of propionate and acetate to propionate ratio, where HNDFD-TMR had a higher molar proportion of propionate than LNDFD-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 production of VFAs in the dairy cow's rumen, in particular the concentration of propionic acid (Ramirez-Ramirez 2011).

There were significant differences between CS-TMR and the average of all barley silage-based TMR, where CS-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 (12.5 vs 15%). 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, while the lower butyrate can be related to the adverse effect of CS-TMR (CS based TMR) on fiber digestion. There was a significant difference in branched-chain volatile fatty acids between CS-TMR and the average of all barley silage-based TMR (2.4 vs 2.8; $P < 0.05$, Table 6). The branched-chain volatile fatty acid consists of isovalerate and isobutyrate, which are derived from microbial deamination of branched amino acids. In this study, CS-TMR had a higher branched-chain volatile fatty acid and no significant difference on ammonia compared with the average of all barley silage-based TMR was noted. This can happen only if efficiency of microbial protein synthesis increases (Goel et al. 2009).

Microbial N production for feed particle associated, feed particle-bound and effluent fraction of the barley silages with different levels of NDFD and of the corn silage in RUSITEC are given in Table 4. The results of this study showed that silage with higher digestible fiber content (HNDFD-TMR) did not increase microbial colonization of silage, 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 CS (CS-TMR) tended to be higher ($P = 0.06$) in total microbial protein production 52.74 (mg/d) compared with the average of HNDFD-TMR, INDFD-TMR and

LNDFD-TMR silage diets, suggesting a higher potential of corn silage to provide adequate amounts of energy for the synthesis of microbial N compared with barley silage. Although these findings were from an *in vitro* study which could not simulate adequately *in vivo* system, but it would give an indication about the higher nutritive value of corn silage compared to barley silage.

Nutrient Digestibility in RUSITEC

Nutrient disappearances in the TMR using RUSITEC are presented in Table 4. There was no significant effect of HNDFD-TMR ($P > 0.10$), which contained HNDFD silage, on digestibility of DM, OM and CP in the TMR when compared with INDFD-TMR (INDFD) and LNDFD-TMR (LNDFD). Furthermore, there was no significant effect of CS-TMR that contained CS on nutrient digestibility (DM, OM, and CP) of TMR compared to the barley silage treatments.

The nutrient disappearances of barley and corn silages were also evaluated in this study. The results showed that HNDFD had a higher NDFD compared to LNDFD (51 vs. 45% DM, $P = 0.02$; Table 5). However, the DM digestibility of HNDFD was not significantly different compared to LNDFD. This might be attributed to the presence of lower content of starch and a higher proportion of NDF compared to LNDFD. Furthermore, HNDFD exhibited a lower starch digestibility compared to INDFD and LNDFD.

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

to the barley silages. In the present study, CS showed a lower NDF disappearance than all barley silages varieties (41.5 vs. 48.1; $P < 0.01$).

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 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). Considering the estimation of the degradation kinetics (K_d /h) 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 HNDFD, intermediate in INDFD and lowest in LNDFD (Table 6). The in vitro K_d 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 HNDFD had the highest estimated ruminal NDFD (12.6 % DM) when compared with INDFD and LNDFD barley silage (9.7 % DM; $P < 0.05$). On the other hand, the predicted TRDC values was greater in INDFD (35% DM) than LNDFD and HNDFD (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 (1999), was from trials where diets had similar NDF content; hence

NDFD was the main variable among treatments. However, this is not the common when evaluating forages where both the NDF and NDFD can vary.

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 and microbial protein synthesis. In vitro NDFD assessment of forages could be used as an analytic tool for evaluating forage quality, when NDF concentrations are similar. The results of this study imply that feeding corn silage has a higher feeding value compared to barley silage. However, the results from the current study were from in vitro technique which could not mimic the in vivo system. Further work including animal trial is required to confirm these findings.

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Table 1. Ingredients and chemical composition of the total mixed ration (TMR) in the four silage diets fed to RUSITEC

Items	Diets ^a			
	CS-TMR	HNDFD-TMR	INDFD-TMR	LNDFD-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, 75% PI	20.7	24.3	24.3	24.3
Corn grain, ground Medium	6.7	6.4	6.4	6.4
Canola meal solvent	11.3	10.2	10.2	10.2
Soybean meal solvent	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 ^b	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
Fermentation profile of silage, (n= 2)				
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

^aCS-TMR= TMR with corn silage P7213R; HNDFD-TMR = TMR with CDC Cowboy barley silage; INDFD-TMR = TMR with CDC Copeland barley silage; LNDFD-TMR = TMR with Xena barley silage.

^bFormulated 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.

Table 2. Protein and carbohydrate chemical profiles and in situ indigestible NDF at 288-h incubation (mean $\pm$ standard deviation) for Cowboy (High, HNDFD), Copeland (Intermediate, INDFD), Xena (Low, LNDFD) barley silage and corn silage P7213R (CS)

Item	Silages ^a			
	CS (n=2)	HNDFD (n=2)	INDFD (n=2)	LNDFD (n=2)
Basic chemical profile of silage				
DM (%)	33.80 $\pm$ 0.66	27.51 $\pm$ 1.21	30.95 $\pm$ 0.98	30.50 $\pm$ 0.17
Ash (%DM)	5.71 $\pm$ 0.49	8.39 $\pm$ 0.11	7.43 $\pm$ 0.30	8.24 $\pm$ 0.93
EE (%DM)	2.66 $\pm$ 0.23	2.88 $\pm$ 0.06	2.82 $\pm$ 0.16	2.61 $\pm$ 0.04
Protein profile ^b				
CP (% DM)	9.05 $\pm$ 0.21	11.70 $\pm$ 0.85	11.20 $\pm$ 0.14	10.65 $\pm$ 0.78
SCP (% CP)	51.85 $\pm$ 3.47	65.40 $\pm$ 0.70	64.35 $\pm$ 4.60	59.45 $\pm$ 4.29
ADITP (%CP)	9.95 $\pm$ 1.48	9.25 $\pm$ 0.49	10.05 $\pm$ 1.48	11.80 $\pm$ 1.98
NDITP (%CP)	12.00 $\pm$ 1.31	11.55 $\pm$ 2.47	11.65 $\pm$ 0.35	12.40 $\pm$ 1.41
Carbohydrates profile (%DM) ^c				
CHO	82.59 $\pm$ 0.51	77.03 $\pm$ 0.91	78.56 $\pm$ 0.61	78.51 $\pm$ 1.68
NFC	44.03 $\pm$ 0.92	27.83 $\pm$ 3.28	35.43 $\pm$ 0.26	32.89 $\pm$ 1.12
NSC	27.45 $\pm$ 2.33	11.10 $\pm$ 0.71	15.80 $\pm$ 0.99	17.22 $\pm$ 0.97
Starch	26.35 $\pm$ 2.76	8.65 $\pm$ 0.35	13.25 $\pm$ 1.77	15.25 $\pm$ 1.99
NDF	38.56 $\pm$ 0.41	49.20 $\pm$ 2.47	43.13 $\pm$ 0.34	45.63 $\pm$ 2.80
ADF	26.25 $\pm$ 0.92	35.95 $\pm$ 2.62	33.95 $\pm$ 0.35	34.15 $\pm$ 2.19
ADL	2.85 $\pm$ 0.08	5.20 $\pm$ 0.18	4.85 $\pm$ 0.16	4.50 $\pm$ 0.32
Hemicellulose	12.31 $\pm$ 1.31	13.25 $\pm$ 0.15	9.18 $\pm$ 0.70	11.48 $\pm$ 0.60
Cellulose	23.39 $\pm$ 0.83	30.74 $\pm$ 2.43	29.10 $\pm$ 0.19	29.70 $\pm$ 1.87
30 h ivNDFD	32.4 $\pm$ 7.84	39.0 $\pm$ 7.91	38.0 $\pm$ 0.83	29.7 $\pm$ 0.83
In situ indigestible NDF at 288-h ^d				
iNDF (%DM)	15.50 $\pm$ 1.51	25.80 $\pm$ 0.30	20.87 $\pm$ 3.45	17.80 $\pm$ 2.13

^aCS = P7213R corn silage; HNDFD = CDC Cowboy high NDFD barley silage; INDFD = CDC Copeland intermediate NDFD barley silage; LNDFD = Xena low NDFD barley silage.

^bCP = crude protein ; SP= soluble protein ; ADTCP= acid detergent insoluble true protein ; NDITP= neutral detergent insoluble true protein ; 30 h ivNDFD = In vitro NDF digestibility after 30 hour of incubation.

^cCHO = carbohydrate ; CHO = 100–EE–CP–ash; NFC = non-fiber CHO; NFC = 100–(NDF–NDICP) – EE – CP – ash; ADF = acid detergent fiber; ADL= acid detergent lignin; Hemicellulose = NDF – ADF; cellulose = ADF – ADL.

^diNDF = indigestible NDF (288h in situ incubation).

Table 3. Ruminal fermentation characteristics determined using RUSITEC for diet treatments containing Cowboy (High, HNDFD), Copeland (Intermediate, INDFD), Xena (Low, LNDFD) barley silage and corn silage P7213R (CS)

Items	Diets ^a				SEM ^b	Contrasts <i>P</i> -value			
	Corn	Barley				Corn vs. Barley	HNDFD	HNDFD	INDFD
	CS-TMR	HNDFD- TMR	INDFD- TMR	LNDFD- TMR			vs. INDFD	vs. LNDFD	vs. LNDFD
pH	6.66	6.72	6.74	6.70	0.029	0.01	0.65	0.15	0.31
NH ₃ -N (mmol /d)	8.89	8.70	8.57	8.60	0.532	0.63	0.86	0.90	0.90
Total VFA (mmol)	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 ^c	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

^aCS-TMR= TMR with CS P7213R corn silage; HNDFD-TMR = TMR with HNDFD CDC Cowboy high NDFD barley silage; INDFD-TMR = TMR with INDFD CDC Copeland intermediate NDFD barley silage; LNDFD-TMR = TMR with LNDFD Xena low NDFD barley silage.

^bSEM = standard error of mean.

^cBCVFA = branched-chain volatile fatty acids (isobutyrate + isovalerate).

Table 4. Gas production, bacterial nitrogen production and nutrient disappearance in RUSITEC for diet treatments containing Cowboy (High, HNDFD), Copeland (Intermediate, INDFD), Xena (Low, LNDFD) barley silage and corn silage P7213R (CS)

Items	Diets ^a				SEM ^b	Contrasts <i>P</i> -value			
	Corn	Barley				Corn vs. Barley	HNDFD vs. INDFD	HNDFD vs. LNDFD	INDFD vs. LNDFD
	CS-TMR	HNDFD-TMR	INDFD-TMR	LNDFD-TMR					
Gas production									
Total, mL/d	1845	1712	1637	1629	138.3	0.27	0.71	0.67	0.95
Gas, ml/g OM fermented	270	248	238	240	19.9	0.25	0.72	0.77	0.94
Bacterial nitrogen production, mg/d									
Total	52.7	49.6	49.5	48.6	1.51	0.06	0.92	0.63	0.62
Feed particle-bound (TMR)	16.8	17.9	18.7	17.4	1.29	0.44	0.66	0.76	0.48
Feed particle-bound (silage)	10.4	8.9	10.6	10.3	1.02	0.77	0.26	0.36	0.81
Feed particle-associated (TMR)	6.3	5.6	4.8	5.9	0.74	0.32	0.44	0.72	0.27
Effluent	29.7	26.1	26.0	25.3	1.44	0.07	0.96	0.68	0.71
Nutrient disappearance of TMR, %									
DM	73.0	74.3	73.4	72.0	1.17	0.61	0.85	0.40	0.62
OM	74.1	74.7	73.8	72.2	1.19	0.53	0.69	0.39	0.60
CP	79.9	80.4	78.9	76.6	2.06	0.54	0.59	0.40	0.61

^aCS-TMR= TMR with CS P7213R corn silage; HNDFD-TMR = TMR with HNDFD CDC Cowboy high NDFD barley silage; INDFD-TMR = TMR with INDFD CDC Copeland intermediate NDFD barley silage; LNDFD-TMR = TMR with LNDFD Xena low NDFD barley silage.

^bSEM = standard error of mean.

Table 5. Nutrient disappearance for Cowboy (High, HNDFD), Copeland (Intermediate, INDFD), Xena (Low, LNDFD) barley silage and corn silage P7213R (CS) in RUSITEC

Items	Silages ^a				SEM ^b	Contrasts <i>P</i> -value			
	Corn	Barley variety				Corn	HNDFD	HNDF	INDFD
	CS	HNDFD	INDFD	LNDFD		vs.	vs.	D vs.	vs.
						Barley	INDFD	LNDFD	LNDFD
Nutrient disappearance of silage, %									
DM	69.1	64.6	65.6	64.2	1.44	0.02	0.65	0.85	0.53
OM	69.7	65.5	66.4	65.1	1.29	0.02	0.63	0.82	0.50
aNDF	41.5	51.2	48.4	45.0	1.70	<0.01	0.26	0.02	0.19
ADF	37.4	46.7	43.8	37.6	2.16	0.05	0.36	0.01	0.06
Hemicellulose ^c	48.7	59.5	56.9	56.7	1.47	<0.01	0.24	0.21	0.90
CP	80.0	77.1	80.7	78.4	2.12	0.63	0.24	0.87	0.45
Starch	98.8	95.4	98.3	98.7	0.93	0.20	0.04	0.02	0.72

^aCS = P7213R corn silage; HNDFD = CDC Cowboy high NDFD barley silage; INDFD = CDC Copeland intermediate NDFD barley silage; LNDFD = Xena low NDFD barley silage.

^bSEM = standard error of mean.

^cHemicellulose = NDF – ADF

Table 6. Total carbohydrates and fiber digestibility predictions based on in vitro results for Cowboy (High, HNDFD), Copeland (Intermediate, INDFD), Xena (Low, LNDFD) barley silage and corn silage P7213R (CS)

Items	Silages ^a				SEM ^b	Contrasts <i>P</i> -value			
	Corn	Barley				Corn vs. Barley	HNDF D vs. INDFD	HNDF D vs. LNDF D	INDFD vs. LNDF D
	CS	HNDF D	INDF D	LNDF D					
Predicted ruminal fiber digestibility ^c									
RDCB 3, % DM	6.71	12.63	10.54	8.93	0.390	<0.01	<0.01	<0.01	<0.01
Degradation rate of available NDF ^d									
K _d , /h	2.24	5.74	4.26	2.65	0.312	<0.01	0.01	<0.001	0.04
Predicted ruminal carbohydrates digestibility ^e									
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 ^f									
Total-tract RDCB 3, %DM	10.14	16.25	14.10	13.01	1.164	<0.01	<0.01	<0.01	0.10

^aCS = P7213R corn silage; HNDFD = CDC Cowboy high NDFD barley silage; INDFD = CDC Copeland intermediate NDFD barley silage; LNDFD = Xena low NDFD barley silage.

^bSEM = standard error of mean.

^cRDCB3 = rumen degraded CB3 fraction.

^dDegradation rate for of available NDF fraction (RDCB3) using 48-h NDFD, a fixed lag of 3-h and iNDF at 288-h following the method of Van Amburgh et al. (2003).

^eTRDC (total degraded CHO fraction); TRUC (total escaped CHO fraction).

^fTotal-tract RDCB3 = [RDCB3 (rumen degraded CB3 fraction) + IntDigCB3(B3 carbohydrate intestinal digestibility; default = 0.20)].