Canadian Journal of Animal Science
Revue canadienne de science animale

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Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2016-0106.R2
Manuscript Type:	Article
Date Submitted by the Author:	01-Sep-2016
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Keywords:	barley silage, neutral detergent fiber digestibility, Fermentation, aerobic stability

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Ensiling barley cultivars selected for varied levels of *in vitro* neutral detergent fiber digestibility in mini- and bunker-silos to evaluate effects on fermentation.

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Running Head: Effect of NDFD on ensiled barley characteristics

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Preston, N. G., Nair, J., Yu, P., Christensen, D. A., McKinnon, J. J., and McAllister, T. A. 2016. **Ensiling barley cultivars selected for varied levels of *in vitro* neutral detergent fiber digestibility in mini- and bunker-silos to evaluate effects on fermentation.** Can. J. Anim. Sci. XX: YY-ZZ. Thirty hour *in vitro* NDF digestibility (NDFD) of three barley cultivars; Cowboy (High, H-NDFD), Copeland (Intermediate, I-NDFD), and Xena (Low, L-NDFD) was ranked from 80 commercial silage samples. Cultivars were seeded on the same day, harvested at mid-dough and ensiled in mini- or bunker-silos. Mini-silos were sequentially opened over 60 d and d 60 samples were exposed to air for 21 d. Bunker-silos were sampled after 60 d. Cultivars did not differ in NDFD, while terminal pH was lower ($P < 0.01$) for H-NDFD than other silages. The pH of H-NDFD was greater ($P < 0.01$), and pH of I- and L-NDFD lower ($P < 0.01$) in bunker- than mini-silos. Lactate and acetate were greater ($P < 0.05$) for H-NDFD in mini-silos, with lower ($P < 0.01$) acetate in mini- than bunker-silos. Intermediate NDFD mini-silos were greater ($P < 0.01$) in ADF and NDF as compared to other silages, traits which were also greater ($P < 0.01$) for H-NDFD and L-NDFD in bunker- vs mini-silos. High -NDFD silage was less aerobically stable than other silages. Using NDFD of field silage samples to select barley silage cultivars for improved NDFD is not a viable strategy based on the results of this study.

Key words: barley silage, neutral detergent fiber digestibility, fermentation, aerobic stability

Abbreviations: ADF, acid detergent fiber, ADIN, acid detergent insoluble nitrogen, CFU, colony forming units, CP, crude protein, DM, dry matter, H-NDFD, high neutral detergent fiber digestibility, I-NDFD, intermediate neutral detergent fiber digestibility, L-NDFD, low neutral detergent fiber digestibility, LAB, lactic acid bacteria, NDF, neutral detergent fiber, NDFD, neutral detergent fiber digestibility, OM, organic matter, VFA, volatile fatty acids, WSC, water soluble carbohydrates

INTRODUCTION

Ensiling is an effective method to store forages as feed for ruminants, particularly in areas of the world where the climate does not allow for year-round grazing or the production of sun-cured hay. Intensive farming operations like dairies and feedlots also require stored forages to formulate total mixed diets for cattle. With the cool, dry growing season of western Canada, barley (*Hordeum vulgare*) is more suited as a forage source for ensiling, than forages that have greater heat-unit requirements like corn and sorghum (Baron et al. 1992; Saskatchewan Ministry of Agriculture (SK AG) 2015; UNL Extension 2016). Acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin content of silage can dramatically influence its nutritional value for ruminants (Mertens 1997). Total tract NDF digestion varies considerably, ranging from 400 to 850 g kg⁻¹ NDF depending on forage type (Noziere et al. 2010). Total tract NDF digestion of barley silage has been reported to range from 450 to 520 g kg⁻¹ (McCartney and Vaage 1994; McAllister et al. 1995).

With advancing maturity, dry matter (DM) and fiber content increase and water-soluble carbohydrate (WSC) content of the forage decreases (Johnson et al. 1999; Rosser et al. 2013). As WSC is the principal substrate used by lactic acid bacteria (LAB) to produce lactic acid, maturity of the fresh forage can have a significant influence on acid production and the ensiling process (McEniry et al. 2013). Khorasani et al. (1997) tested the effect of ensiling on the chemical composition of different forage types (i.e., alfalfa, barley, triticale) and found that the time for adequate ensiling was inversely related to acid-detergent lignin content, a factor that can also influence fiber digestibility. Increased rumen digestibility of NDF in corn silage compared to fresh corn forage has been linked to breakdown of hemicellulose (Monteils and Jurjanz 2005). Thus, selecting for NDF digestibility (NDFD) of silage as opposed to NDFD of the fresh forage, may offer advantages as it would also consider the impact of the ensiling process on NDFD.

Studies to specifically ensile cereal silages on the basis of their differences in NDFD have not been conducted. Rather, most research targeted at improving fiber digestion in silage has investigated the impact of adding fibrolytic enzymes to the forage at the time of harvest (Addah et al. 2012; 2014; Lynch et al. 2014). This project is unique in that the barley cultivars selected arose from a survey of the *in vitro* NDFD of silage samples collected from commercial farms (Nair et al. 2016). However, it is not known if varietal differences in NDFD of field samples of barley silage reflect the NDFD of silage cultivars grown at a single site. The objectives of this study were to characterize the ensiling traits and *in vitro* NDFD of three barley silage cultivars, previously ranked based on NDFD of field silage samples, and to compare ensiling in mini- versus large scale bunker-silos on ensiling traits.

MATERIALS AND METHODS

Barley Selection

Barley silage was selected for *in vitro* NDFD by collecting silage samples from livestock producers across Alberta and Saskatchewan in 2012 and 2013. A total of 79 samples representing 16 cultivars and 56 samples representing 12 cultivars were collected in 2012 and 2013, respectively (Nair et al. 2016). Seven barley cultivars (AC Metcalfe, CDC Copeland, CDC Cowboy, Conlon, Falcon, Legacy, and Xena) with at least 3 separate samples collected for each cultivar per year ($n = 80$, total samples representing 7 cultivars over two years) were analyzed for *in vitro* NDF digestibility after 30 h of incubation using four ANKOM Daisy^{II} incubators (Ankom Technology Corporation, Fairport, NY) with sample replicates repeated in four runs (Nair et al. 2016). Samples were analyzed for residual NDF using an Ankom²⁰⁰ fiber analyzer (Ankom Technology Corporation, Fairport, NY) with sodium sulphite and heat-stable α -amylase. The cultivars with the highest, lowest and intermediate NDF digestibility (NDFD) after 30 h of incubation were selected for this experiment. All cultivars were of the two-row type with an NDFD ranking of CDC Cowboy (370 g kg^{-1} , high NDFD (H-NDFD); Ardell Seeds, Vanscoy, SK), CDC Copeland (283 g kg^{-1} intermediate NDFD (I-NDFD); Wylie Farms Ltd., Biggar, SK) and Xena (262 g kg^{-1} low NDFD (L-NDFD); Wylie Farms Ltd., Biggar, SK).

¹; low NDFD (L-NDFD); Xena, Crop Production Services, Bow Island, AB). The three cultivars differed in maturity ranking with I-NDFD and L-NDFD ranked as medium maturing, and H-NDFD ranked as late maturing (Fedko 2015).

The three selected barley cultivars were grown in dark brown soil at the Lethbridge Research Centre (49.42°N, 112.45°W, 911 m elevation) in a dry land field divided into three 0.67 ha plots. Plots were seeded on 26 May 2014, using a John Deere 1560 no-till disc drill (John Deere Co., Moline, Ill.) at a seeding rate of 133 kg ha⁻¹. Fields were fertilized with 52 kg N ha⁻¹ pre-seeding, and 5.5 kg N ha⁻¹ and 23 kg P₂O₅ ha⁻¹ at seeding. The plots were sprayed for broadleaf weeds on July 4 with Axial Extreme (1.24 L ha⁻¹; Syngenta Canada Inc., Guelph, ON) and Refine SG (0.27g L⁻¹ ha⁻¹; DuPont Canada Inc., Mississauga, ON).

Harvesting and Ensiling

The three barley cultivars were harvested on 5 Aug. 2014 at mid-dough maturity, using a John Deere 6810 forage harvester at DM of 417 g kg⁻¹, 457 g kg⁻¹, and 436 g kg⁻¹ for H-NDFD, I-NDFD, and L-NDFD, respectively (Table 1). Fresh forage was randomly harvested from each plot, in four separate loads (n = 4). Approximately 2.3 kg forage was randomly selected from each truck and packed into pre-weighed 35 × 10 cm PVC pipe mini-silos to a density of ≈240 kg/m³ using a hydraulic press (Baah et al. 2011). For each cultivar, a total of three mini-silos (n = 3) were filled for each day that silos were opened (d 3, 7, 14 and 22), except for d 60 in which 6 silos per cultivar were prepared, with duplicate silos combined at opening to form one replicate. Silos were weighed during filling and prior to opening on designated days for estimation of DM loss. Mini-silos are used to mimic bunker-silos in order to have sufficient replication for statistical analysis of fermentation over time. There is also value in comparing these two ensiling approaches using the same forage at the same harvest time to determine the extent to which mini-silos represent field scale bunker-silos.

Barley cultivars were also packed into replicate $2.5 \times 2.5 \times 2.5$ m bunker-silos ($n = 2$) after lining the concrete floor and walls with an impermeable polythene film (AT Films Inc., Edmonton AB). Approximately 18.6 ± 0.5 tonnes of fresh forage was manually packed into each silo and two thermocouples (Thermochron iButtons, Embedded Data Systems, Lawrenceburg, KY) were placed in the centre of each bin to continuously record temperature during ensiling. Once silos were filled, they were sealed with plastic and tires were placed on top of each silo to hold the plastic in place.

Silage from each cultivar was fed to sheep first from one silo and then from the next, once the first silo was emptied. Samples were collected from the first silo 65 and 135 d after ensiling and from the second silo 177 and 209 d after ensiling. Duplicate samples were collected from five locations along the freshly exposed face of the silo after at least 25 cm of silage had been removed. The five locations on the face were combined for a representative sample and analysed for chemical composition and fermentation characteristics.

Aerobic stability

Aerobic stability of silages was tested after d 60. Silage from duplicate mini-silos was mixed and placed into insulated containers ($n = 3$), covered with two layers of cheese cloth and stored at 21°C . Two Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) were inserted in separate locations to record temperature hourly. Silage samples were removed after 3, 7, 14, and 21 d of aerobic exposure and analyzed for pH, VFA, $\text{NH}_3\text{-N}$, lactic acid, WSC, and microbial populations as described below.

Chemical Analysis and NDFD

Fresh and ensiled forage DM concentrations were determined by drying 20 g of forage in a forced air oven at 55°C for 48 h. Fresh and ensiled forage samples (15 g) were blended using a Waring commercial blender (Torrington, CT) with 135 mL double distilled water for 30 sec, strained through a double layer of cheesecloth and the filtrate collected. The pH of the filtrate was measured, and a 10 mL subsample was boiled for 15 min and stored at -20°C until analyzed for WSC. A second subsample of

filtrate (45 mL) was centrifuged at 4°C for 15 min at $10,000 \times g$ and a 1.5 mL aliquot was mixed with 0.3 mL of 25% metaphosphoric acid for analysis of VFA and lactic acid (Addah et al. 2012). A second aliquot (1.6 mL) was mixed with 0.15 mL of 65% trichloroacetic acid and stored at -20°C until analyzed for $\text{NH}_3\text{-N}$.

Volatile fatty acids and lactic acid were determined following the procedure of Kudo et al. (1987) using a Hewlett Packard model 5890A series Plus II gas-liquid chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a Zebron FFAP fused silica capillary, 30 m, 0.32 mm i.d. and 1.0 μm film thickness column (Phenomenex, Torrence, CA). Crotonic acid was used as an internal standard for VFA analysis. Malonic acid served as the internal standard for analysis of lactic acid, which was first methylated with boron trifluoride methanol. Ammonia-N was measured using the phenol-hypochlorite method of Broderick and Kang (1980) with a Technicon autoanalyzer (Tarrytown, NY) and color detection with an Astoria Pacific 305D Digital Detector with FASPac software (Astoria Pacific, Clackamas, OR).

Buffering capacity of fresh forage samples was measured following the procedure of Addah et al. (2011) by blending 15 g fresh forage with 200 mL of deionized water for 45 s, followed by placement on a magnetic stir plate for 2 min to reach equilibrium. The solution was titrated with 0.2 N HCl to a pH of 4, then back titrated using 0.2 N NaOH to a pH of 7. Buffering capacity was expressed as milliequivalents of NaOH required to raise the pH of 1 kg of forage from 4 to 6. The fermentation coefficient (FC) of forages was estimated using the equation of Weissbach and Honig (1996)

$$FC = DM\% + 8WSC/BC.$$

Fresh forage and d 60 silage samples were freeze-dried and ground through a 1-mm screen using a Retsch 2100 grinder (Verder Scientific Inc., Newton, PA). Analytical DM was measured by drying 1-g samples at 105°C overnight, followed by ashing in a muffle furnace at 550°C for 5 h to estimate organic matter. The procedure of Van Soest et al. (1991) was used to determine NDF and ADF with sodium sulfite and

heat stable α -amylase included in the NDF solution. Neutral detergent fiber and ADF were analyzed sequentially using the Ankom 200 fiber analyzer (Ankom Technology Corporation, Fairport, NY). The ADF residues were then ball ground on a Retsch MM400 (Verder Scientific Inc., Newton, PA) for estimation of acid detergent insoluble nitrogen (ADIN). Total N concentration in d 0 forage, silage, and ADF residue samples was determined using ball ground samples that were subject to elemental combustion in a NA1500 Nitrogen/Carbon analyzer (Carlos Erba Instruments, Milan, Italy). Crude protein of the samples was estimated as $N \times 6.25$. Starch concentration of fresh forage and silage samples was estimated by the enzymatic hydrolysis of alpha-linked glucose as described by Bach Knudsen (1997) after ball ground samples were gelatinized for 1 h at 90°C. Water-soluble carbohydrates were measured using the Nelson-Somogyi method (Nelson 1944) and expressed as glucose equivalents by reducing the cupric form of Cu to cuprous and reading absorbance at 620 nm on a microplate reader (Thermo Scientific, Hudson, NH). Day 60 silage samples were analyzed for NDFD using the procedure to rank and select field silage samples as described previously.

Microbial Analysis

Samples were enumerated for lactobacilli, yeasts and molds by agitating fresh forage or silage samples (10 g) in a Stomacher 400 laboratory blender (Seward Medical Limited, London, UK) with 90 mL of sterile 70 M potassium phosphate buffer (pH 7) for 1 min. An aliquot was then serially diluted (10^{-2} to 10^{-6}) and 100 μ L of three consecutive dilutions were plated in triplicate onto de Mann, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and Sabouraud's dextrose agar (SDA) containing 100 μ g mL⁻¹ each of tetracycline and chloramphenicol (Difco Laboratories Inc., Detroit, MI) for enumeration of lactic acid bacteria (LAB), or yeasts and molds, respectively. The MRS plates were incubated at 37°C and the SDA plates were incubated at 25°C for 72 h. Plates containing between 30 and 300 colonies were counted and the number of colony forming units (CFU) were expressed as log₁₀ CFU g⁻¹ of fresh or ensiled forage on a DM basis (Addah et al. 2012).

Statistical Analysis

Statistical analysis was completed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Fresh forage was analyzed as a nested design (CRD) with fixed effect of cultivar, and effect of replicate nested within cultivar. Microbial counts (CFU g⁻¹ of forage DM) were log₁₀ transformed before statistical analysis. Fermentation traits throughout ensiling were analyzed using the ante-dependence covariance structure based on lowest AIC and BIC values, with the replicate within cultivar repeated by day, cultivar as fixed effect, and replication within cultivar as a random effect. *In vitro* NDFD was analyzed as a completely randomized design with fixed effect of cultivar, and random effects of silo and run. The effect of Daisy^{II} machine was originally included in the model, but was removed as it proved insignificant.

Day 60 mini-silo silage and samples collected from bunker-silos were used to assess the impact of ensiling method on the fermentation characteristics and chemical composition of terminal silage as a nested design with ensiling method, barley cultivar and their interaction as fixed effects and silo nested within cultivar. For the aerobic stability of mini-silo silage, chemical and microbial measurements were analyzed using fixed effect of cultivar and the random effect of silo within cultivar on day of sampling. The effect of day of aerobic exposure was tested using day as a repeated measure. The optimal heterogeneous compound symmetry covariance structure based on lowest AIC and BIC was then selected. Temperature during aerobic exposure was averaged by day for each replicate and the differences between the ambient temperature and the temperature of all three cultivars were analyzed for each day of exposure using Dunnett's adjustment of LSMEANS. The PDIF option of LSMEANS was used to identify differences among barley silages that differed in *in vitro* NDFD, with means separated using pdmix800 (Saxton 1998). Results were declared significant when $P \leq 0.05$, and trends discussed when $0.5 < P < 0.1$.

RESULTS

Fresh Forage

Average daily temperature through the growing period was 16.4°C and the monthly precipitation for June and July was 193.8 mm and 21.2 mm, respectively. At harvest, the DM yield of H-NDFD, I-NDFD, and L-NDFD was 6446, 5385, and 5558 kg ha⁻¹, respectively (Table 1). The DM content of fresh forages was 416.8, 457.5, and 435.5 g kg⁻¹ for H-NDFD, I-NDFD, and L-NDFD, respectively (Table 1) and was assessed as being in the mid-dough stage of maturity at harvest. There was a trend ($P = 0.066$) in the fresh forage for L-NDFD to exhibit a lower pH than I-NDFD with the buffering capacity for L-NDFD being lower ($P = 0.016$) than the other two cultivars.

At the time of harvest, LAB ranged from 6.5 to 6.8 log₁₀ CFU g⁻¹ DM whereas yeast and molds ranged from 6 to 6.5 log₁₀ CFU g⁻¹ and 5.5 to 6 log₁₀ CFU g⁻¹ forage DM, respectively. The only difference in microbial populations associated with fresh forages was that yeasts were lower ($P = 0.004$) for H-NDFD as compared to I-NDFD and L-NDFD silage.

At harvest, CP was greater ($P = 0.001$) in H-NDFD forage than the other two cultivars, while L-NDFD was greater in starch ($P = 0.016$) than either H-NDFD or I-NDFD. The I-NDFD of fresh forage tended to have higher NDF ($P = 0.082$) and ADF ($P = 0.084$) content, and I-NDFD had greater ($P = 0.031$) WSC than H-NDFD and L-NDFD.

Silage *In Vitro* NDFD

The *in vitro* NDFD ranking of the silage cultivars after 60 d of ensiling did not align with the NDFD ranking derived from on-farm samples (Table 2). No difference ($P = 0.223$) was observed amongst cultivars with no impact ($P = 0.236$) of ensiling method on NDFD ranking.

Mini-silo Fermentation

The decrease in pH was similar between H-NDFD and L-NDFD silages during the first 7 d of ensiling, while the pH of I-NDFD was greater ($P < 0.01$) (Fig. 1A). After 14 d of ensiling, the pH of H-NDFD was lower ($P = 0.006$) than either I-NDFD or L-NDFD silage. Water-soluble carbohydrate

concentration increased throughout ensiling, with no differences among silages (Fig. 1B). In the first 14 d of ensiling, lactate concentrations were greatest ($P < 0.01$) in H-NDFD silage with L-NDFD having the lowest ($P < 0.01$) concentrations after 22 d (Fig. 1C).

Silage Composition

After 60 d of ensiling, the DM of the mini-silos declined to 399, 420 and 425 g kg⁻¹ for H-NDFD, I-NDFD and L-NDFD, respectively (Table 2). The DM of silage within bunker-silos was lower ($P = 0.021$) than in mini-silos, with no difference ($P > 0.1$) among cultivars regardless of ensiling method. The DM loss in mini-silos ranged from 20 to 30 g kg⁻¹, with terminal pH in mini-silos being the lowest ($P = 0.006$) for H-NDFD. The terminal pH was greater ($P < 0.001$) in bunker- as compared to mini-silos for H-NDFD silage, but lower for I- and L-NDFD silages. Crude protein tended to be greater ($P = 0.053$) for H-NDFD than I-NDFD and L-NDFD when forage was ensiled in mini-silos, with CP concentrations being generally greater ($P = 0.001$) when forage was ensiled in bunker-silos. Starch in mini-silos was greater ($P = 0.004$) in L-NDFD silage than the other silages, while starch concentrations of all silages were greater ($P = 0.029$) in bunker- than mini-silos.

Ensiling method also had an effect on the NDF, ADF and ADIN content of silages, with these fractions generally being lower ($P < 0.001$) in mini- than bunker-silos. In mini-silos, concentrations of NDF and ADF were greatest ($P \leq 0.001$) in I-NDFD silage and similar between H-NDFD and L-NDFD silage (Table 2). In bunker-silos, the NDF concentration of L-NDFD silage was lower ($P = 0.008$) than other silages. Acid detergent insoluble N (ADIN) and WSC did not vary among silages in mini-silos, but WSC tended to be greater ($P = 0.069$) in I-NDFD silage ensiled in bunker-silos.

Populations of LAB increased from fresh forage levels to 8.19 log₁₀ CFU g⁻¹ silage DM for H-NDFD and L-NDFD, and 8.63 log₁₀ CFU g⁻¹ silage DM for I-NDFD after 60 d of ensiling in the mini-silos, whereas populations of LAB in silage ensiled in bunker-silos were almost 1 log₁₀ CFU g⁻¹ DM lower ($P = 0.009$; Table 2). In mini-silos, yeast averaged 3.49 log₁₀ CFU g⁻¹ forage DM, with molds being

detected intermittently at up to 2.48 to 2.85 log₁₀ CFU g⁻¹ forage DM. Both yeast and mold populations tended ($P < 0.1$) to be greater in silage ensiled in bunker as compared to mini-silos.

Lactate concentrations were greater ($P = 0.038$) for H-NDFD than L-NDFD silage in mini-silos, with no varietal difference ($P = 0.551$) among silages ensiled in bunker-silos. In mini-silos, acetate concentrations were greater in H-NDFD ($P = 0.004$) silage than other silages, with acetate concentration being greater ($P < 0.001$) in forage ensiled in bunker- as compared to mini-silos. Butyrate concentrations tended to be greater ($P = 0.056$) in I- and L-NDFD 60 d mini-silos than H-NDFD, with the bunker-silo silage having greater ($P = 0.035$) butyrate concentrations than mini-silos, but with no varietal effect ($P > 0.1$). Concentrations of total VFA in bunker-silos were greater ($P < 0.001$) than in mini-silos, with propionate and NH₃-N concentrations in H-NDFD silage being greater ($P < 0.01$) than other silages in bunker-silos.

Aerobic Exposure

Upon aerobic exposure, H-NDFD was the only silage to exhibit a substantial increase ($P < 0.01$) in temperature (Fig. 2a) and pH (Fig. 2b) ($P < 0.05$). The effect of day on fermentation parameters was always significant ($P < 0.05$) and consequently we did not report these individual parameters. These responses coincided with a decrease ($P < 0.05$) in the lactic acid concentration (Fig. 2c) in H-NDFD during aerobic exposure. The WSC concentration in H-NDFD declined ($P < 0.01$) with no change in the other silages during 21 d of exposure (Table 3). Concentration of NH₃-N increased ($P < 0.001$) in L-NDFD silage during aerobic exposure, while in H-NDFD silage it decreased. There was no difference ($P > 0.05$) in LAB or yeast populations among silages after 3 d of aerobic exposure (Table 3). However, after 7 d of aerobic exposure, yeasts were consistently greater ($P = 0.009$) in H-NDFD silage than other silages and by d 14 LAB were also greater ($P = 0.004$) in this silage. By d 21, molds were also substantially greater ($P = 0.003$) in H-NDFD silage than other silages.

DISCUSSION

In vitro NDFD

Our results indicate that prior selection of barley cultivars based on NDFD of field silage samples collected over two years did not result in a similar ranking of NDFD of silage produced from these cultivars at a single site in Lethbridge, Alberta. There is a paucity of research regarding approaches for selecting for improved fiber digestion in barley silage (Oba and Swift 2014). Past research focused on improving NDFD in silage has largely focused on breeding strategies to introduce the brown midrib-3 trait (bm3) into corn and sorghum (Grant et al. 1995; Oba and Allen 2000a). Oba and Allen (2000a) compared bm3 corn to nearly isogenic non-bm3 cultivars and noted that the NDFD of the bm3 cultivar was greater without any difference in NDF content. Oba and Swift (2014) compared the *in vitro* NDFD of Tyto vs Falcon barley cultivars based on anecdotal on-farm evidence that cows fed Tyto silage had reduced milk yield as compared to those fed Falcon silage. It was found that the Falcon silage had lower NDF and greater NDFD than Tyto, and cows fed Falcon silage exhibited greater feed efficiency as a result of a lower DMI, with no effect on milk production or composition (Oba and Swift 2014).

Similar to this experiment, Dado and Allen (1996) also selected forages based on the NDFD of the silage as opposed to the fresh forage. These researchers selected alfalfa silages with the same NDF content, but differing NDFD based on the time that the fresh forage was harvested. Barley cultivars grown in this experiment were ranked as having high, intermediate or low *in vitro* NDFD based on the analysis of farm samples of barley silage collected over two years (Nair et al. 2016). In an effort to reduce the impact of maturity at harvest on the NDFD of barley silage, only barley silages harvested at mid-dough were included in the selection. On-farm silage samples exhibited an 11% difference in NDFD between the L-NDFD and H-NDFD cultivar (Nair et al. 2016).

In the present experiment, all three cultivars were grown under identical conditions, while the field samples that were used to rank cultivars on the basis of NDFD (Nair et al. 2016) were collected over

two growing seasons at differing locations and growing conditions across two Prairie Provinces. Chow et al. (2008) compared barley planted in May to that planted in June. The harvest date was also staggered by one month to test the theory that exposure of the June seeded barley to greater temperature would increase lignin deposition in plant cell walls and decrease the NDFD of barley silage. They found that due to unseasonable weather, temperatures in June were actually lower and the barley planted at this time had a greater NDFD than that planted in May. The lower temperatures caused the June-planted barley to spend more time in the vegetative stage than the May-planted barley. Subsequently, kernel development from heading to harvest took longer for the June-planted than May-planted barley (Chow et al. 2008).

The present study accounted for growing days and differences in weather by planting and harvesting all three cultivars on the same day. However, H-NDFD (CDC Cowboy) was considered a late maturing cultivar; whereas I-NDFD (CDC Copeland) and L-NDFD (Xena) were moderate maturing (Fedko 2015). Any differences in rate of maturation that existed among cultivars did not seem to be sufficient to alter the NDFD or chemical composition of the silages. This is probably due to the variation in growing climates experienced within western Canada making it difficult to establish maturity differences, where a couple days difference in southern Alberta is closer to a week-long difference in time to maturity in northern Alberta (Fedko 2015).

This trial harvested the barley at mid-dough maturity, although the DM was greater than what is typical of barley silage harvested at this stage (350 g kg^{-1}). This suggests that while the kernels were still soft, the leaves and stems had started to dry down. At the mid-dough stage, DM yields are greater than at earlier maturities, with sufficient WSC to ensure easy ensiling (Bergen et al. 1991). The NDF and ADF concentrations are greater at mid-dough than earlier maturities, but the increased DM yield still results in more digestible DM, despite a slight reduction in the digestibility of leaves and stems. As barley forage reaches full maturity, digestibility of whole plant silage can decline as the increased deposition of starch is insufficient to offset the decline in plant cell wall digestibility that occurs as a result of lignification (Fisher and Fowler 1975).

Silage produced in this trial had greater NDF concentration as compared to the commercial silage samples (Nair et al. 2016), especially I-NDFD, where NDF was 12% greater (DM basis). It appears as if this greater NDF concentration arose mainly because of higher levels of hemicellulose as the ADF concentration of the commercial silage samples (Nair et al. 2016) was similar to that of silage ensiled in the mini- and bunker-silos in our study. A higher hemicellulose component, with I-NDFD silage could explain the higher NDFD as hemicellulose is often more readily broken down than cellulose within plant cell walls. Nair et al. (2016) did not find any difference in lignin concentration of the three cultivars collected on farm, as all were harvested at mid-dough.

Effect of NDFD on Fermentation

A terminal pH of 4.2 to 4.5 (Aasen 2014) is sufficient to preserve barley silage until feeding, but frequently the terminal pH of barley silage is below 4.0 (Zahiroddini et al. 2006). Barley ensiled in bunker-silos had a pH that was on the high end of Aasen's range (2014) with the pH being even greater for I- and L-NDFD cultivars ensiled in mini-silos. This value is similar to that reported by Khorasani and Kennelly (1997) for three barley cultivars ensiled in bag silos. All three cultivars were ensiled above the targeted optimal DM of 350 g kg⁻¹, and the terminal pH was similar to other studies that ensiled barley at similar DM (Addah et al. 2011; Baah et al. 2011). Despite the greater DM at ensiling, DM losses were low (20 to 40 g kg⁻¹) and consistent with high-quality silage (McDonald et al. 1991). High-NDFD silage had the lowest pH and highest lactate concentrations after 60 d ensiling in mini-silos, despite the fact that it had the highest buffering capacity and the lowest WSC concentration of the barley cultivars. The H-NDFD fresh forage had the lowest DM content at harvest, a characteristic that is often associated with a more extensive fermentation (McDonald et al. 1991).

Lactate concentration in H-NDFD mini-silo silages increased the most during ensiling explaining the lower pH. Lactic acid bacteria populations were lower and total VFA concentrations greater in bunker- vs. mini-silos. Enumerated LAB populations in both silo types were comparable to those in the

literature (Zahiroddini et al. 2006; Baah et al. 2011; Jin et al. 2015). Butyrate concentrations, particularly in I-NDFD and H-NDFD, were greater than that typically reported for barley silage in bunker- and mini-silos (Baah et al. 2011; Amanullah et al. 2014), but were not at levels associated with a clostridial fermentation (McDonald et al. 1991). Furthermore, the low DM loss in mini-silos also suggests that clostridial fermentation was not a factor in these high DM silages (Charmley and Veira 1991; McDonald et al. 1991).

The H-NDFD silage had the greatest increase in ADIN during the ensiling process in both mini- and bunker-silos. Concentrations of ADIN were comparable to other studies with barley silage (Zahiroddini et al. 2004, 2006; Baah et al. 2011; Jin et al. 2015). The other two cultivars of silage exhibited minimal change in ADIN, indicating that while pH did not drop below 4.5, heating of the silage did not occur and Maillard products were not formed.

It is generally accepted that mini-silos are an easily replicated method to assess ensiling practices and are representative of higher capacity ensiling systems such as bunker-silos. The similar pH of the silage in mini- and bunker-silos suggests that both ensiling methods produced silage of similar quality, but differences in ensiling traits have been identified between silo types. Zahiroddini et al. (2004) found that a more gradual fermentation occurred in bag silos as the rate of pH decline was slower than in mini-silos, enabling cellulases to remain active longer and lower the ADF content of bagged silage as compared to that ensiled in mini-silos. A slower decline in pH in bunker-silos may account for the greater $\text{NH}_3\text{-N}$ concentrations in barley ensiled in this manner as compared to the mini-silo silage, as a slow decline in pH can lead to more proteolysis and deamination during the ensiling process (McDonald et al. 1991). Concentrations of $\text{NH}_3\text{-N}$ in our study ranged from $1.68 \text{ g kg}^{-1} \text{ DM}$ in mini-silos up to $2.11 \text{ g kg}^{-1} \text{ DM}$ in bunker-silos. Ammonia-N concentrations in barley silage in other trials have been reported to be as low as $0.23 \text{ g kg}^{-1} \text{ DM}$ (Addah et al. 2011; Baah et al. 2011). However, others have measured greater $\text{NH}_3\text{-N}$ concentrations: $1.7 \text{ g kg}^{-1} \text{ DM}$ (Mills and Kung 2002) and $2.48 \text{ g kg}^{-1} \text{ DM}$ (Jin et al. 2015), suggesting that concentrations of $\text{NH}_3\text{-N}$ in barley silage in our study were not excessive with either

ensiling method. High $\text{NH}_3\text{-N}$ concentrations can be negatively correlated with DMI (Charmley 2001) if they exceed 150 g kg^{-1} of total N, a level indicative of excessive proteolysis (Bagg 2016). Exposing fresh forage to air before ensiling can also increase $\text{NH}_3\text{-N}$ concentrations (Mills and Kung 2002). This is another factor that may account for differences in $\text{NH}_3\text{-N}$ concentrations between mini- and bunker-silos. Mini-silos were filled, packed and sealed immediately upon delivery of the fresh forage, while bunker-silos were gradually filled over a 2 h period.

Nutrient Composition

The composition of silages was not consistent between ensiling methods with CP, ADF, NDF and starch concentrations being different. Zahiroddini et al. (2004) also found that the fiber composition of barley silage ensiled in mini- vs large bag silos differed. The NDF and ADF fractions of both mini- and bunker-silo silages were greater than in fresh forage. More often fiber concentrations in barley silage decrease (Zahiroddini et al. 2004) or remain similar to fresh forage (Baah et al. 2011). Charmley and Veira (1991) observed a slight increase in NDF and ADF content in alfalfa silage as compared to fresh forage, due to DM losses as CO_2 and other volatile compounds are released during fermentation. The observed decline in starch during ensiling could account for the greater levels of fiber in terminal silage.

The CP concentration in barley silage is often around 120 to 140 g kg^{-1} of DM (McCartney and Vaage 1994; Hristov and McAllister 2002; Baah et al. 2011). All three barley cultivar silages in the current trial had low CP content 75 to 85 g kg^{-1} DM; but others have also reported similar CP levels in barley silage (Burgess et al. 1973; McKenzie et al. 2004). The same barley cultivars grown in Saskatoon had CP levels ranging from 110 to 130 g kg^{-1} of DM (Nair, unpublished data). This suggests that the low CP was a result of the growing conditions at Lethbridge. McKenzie et al. (2004) tested the fertilizer response of barley silage and found CP levels of silage grown on dryland in southern AB ranged between 80 to 100 g kg^{-1} DM, when barley was fertilized with 120 kg N ha^{-1} . These barley silage CP concentrations are only slightly greater than those of the current study, despite N application rates being

half those of McKenzie et al (2004). Buffering capacity of the barley silages was low compared to those measured by Addah et al. (2011), an observation that reflects the lower CP levels of the barley silage in the present experiment.

Water-soluble carbohydrates in silage were greater than those in fresh forage (23 to 31 g kg⁻¹ forage DM), which nearly doubled to 69 to 86 g kg⁻¹ silage DM in terminal silage. Fresh forage WSC concentrations were similar to those reported in the literature (Baah et al. 2011; Jin et al. 2015). Increases in WSC during ensiling have been observed in both barley and wheat silage (Weinberg et al. 1993; Addah et al. 2011; Baah et al. 2011) as well as in grass silage where WSC nearly doubled after 70 d of ensiling (Charmley and Veira 1991). Baah et al. (2011) observed an increase in starch concentrations throughout ensiling from 230 g kg⁻¹ DM to 260 g kg⁻¹ DM, with an increase in WSC from 54 g kg⁻¹ DM to 63 g kg⁻¹ DM. Increases in WSC during this trial were observed for both ensiling methods. The I-NDFD silage had similar WSC concentrations in the first bunker-silo that was fed out as compared to the d 60 mini-silos. However, the second I-NDFD bunker silage had a much greater WSC concentration increasing the variation in this parameter. The increase in NDF content during ensiling suggests that plant cell walls were not degraded to WSC.

A more likely explanation for the increase in WSC between fresh and ensiled forage would be that starch was broken down into WSC during fermentation, as there was a decrease in starch concentration between d 0 and d 60. Other researchers (Mills and Kung 2002; Zahiroddini et al. 2004) have observed a decrease in starch during ensiling, but this observation was also accompanied by a decrease in WSC. One possibility is that the fresh barley forage in these other trials (Mills and Kung 2002; Zahiroddini et al. 2004) had limited WSC so as the starch was hydrolyzed by plant enzymes, and the released WSC were utilized by LAB. While none of the cultivars exhibited a sufficient enough decrease in starch to account for the increased WSC, a decline in starch was observed in all silages, except in I-NDFD bunker-silo silage. Kung and Muck (2015) observed an increasing availability of starch for digestion throughout the ensiling period, an observation they attributed to continual proteolysis during

ensiling (Hoffman et al. 2011). We also observed a continuous decrease in the concentration of starch in barley ensiled in mini-silos, a factor that could account for the increase in WSC.

Aerobic Stability

The temperature profiles of I-NDFD and L-NDFD silage did not deviate from ambient temperature over 21 d of aerobic exposure. However, both the temperature and pH of H-NDFD silage increased substantially while lactic acid concentration decreased after 3 d of exposure. This period corresponded with an increase in the yeast population, an observation consistent with their ability to metabolize lactic acid and accelerate the process of aerobic spoilage (Ashbell et al. 2002). Aerobic stability is typically defined as the length of time silage temperature remains stable before it increases a defined number of degrees above ambient temperature (Driehuis et al. 1999; Kung and Ranjit 2001). Using the procedure of Kung and Ranjit (2001) of 2°C above ambient temperature, one replicate of H-NDFD became unstable after 105 h, whereas the other two replicates did not become unstable until after 229±2 h of aerobic exposure. This observation may reflect the uneven distribution of spoilage organisms within barley silage. Baah et al. (2011) and Addah et al. (2011) recorded temperature change over 7 d of aerobic exposure, and did not observe any increase in barley silage temperature, a result similar to our observations for I- and L-NDFD silages.

Prolonged aerobic stability has come to be associated with acetic acid produced by heterofermentative LAB (Addah et al. 2012; Jin et al. 2015) with acetic acid levels reaching as high as 50 g kg⁻¹. High-NDFD had the highest acetic acid concentration at opening, but it was far below the levels associated with improved aerobic stability. Although I-NDFD and L-NDFD silages had greater levels of WSC, which can provide a readily available source of energy for spoilage microorganisms (Holzer et al., 2003) all three silages had WSC contents greater than anticipated for terminal silage. As opposed to acetate, butyrate levels tended to be numerically greater in I-NDFD and L-NDFD a factor that could have extended the aerobic stability of these silages. Danner et al. (2003) showed that addition of butyric acid to

silage was more effective at preventing silage spoilage than acetic acid. All three cultivars when ensiled in bunker-silos had similar butyrate levels throughout the feeding period, so the aerobic stability of silages ensiled in this manner may have been more consistent. Numerically greater yeast and mold counts in bunker- as compared to the mini-silos could reflect the greater aerobic exposure that occurs during the feed out of bunker-silos (Ashbell et al. 2002). However, lactic acid concentrations in barley silage did not differ between ensiling methods. This observation suggests that the face management of the bunker-silos did not contribute to aerobic deterioration as reflected by the lack of assimilation of lactic acid by spoilage yeasts.

CONCLUSION

Selecting barley cultivars for high, intermediate and low *in vitro* NDFD based on collection of field silage samples did not identify differences in NDFD among cultivars grown at the same location and harvested at the same stage of maturity. Chemical composition differences such as lower NDF in L-NDFD (Xena) did not alter the extent of 30 h *in vitro* NDFD compared to the other two cultivars. Differences in fermentation traits were observed between ensiling methods and differences in fermentation products such as the lower concentration of butyrate may have reduced the aerobic stability of H-NDFD silage. A continuation of research in this field would need to take into account the differences in growing seasons. As well barley breeders should consider selecting for increased hemicellulose content as opposed to cellulose and lignin, in attempts to increase NDFD through increasing the digestible portion of NDF. However, first and foremost, producers must ensure they employ the best ensiling practices possible, attempting to improve silage digestibility is pointless if conditions conducive for optimal ensiling are not assured.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Beef Cattle Research Council for funding this research program. As well thank you for the technical support of B. Baker, C. Barkley, B. Hill, Z. Matic, D. Michael, L. Schneider, T. Silva, F. Van Herk, D. Veders.

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For Review Only

TABLES

Table 1. Composition of fresh barley forage (n = 4) selected on the basis of differences in *in vitro* NDFD of silage samples collected on farm^a.

Item	Barley Cultivar			SEM ^b	P - value
	H-NDFD	I-NDFD	L-NDFD		
DM yield (kg ha ⁻¹)	6446	5385	5558		
DM (g kg ⁻¹ fresh matter)	416.8	457.5	435.5	11.35	0.088
Buffering capacity ^c	257.33a	250.10a	226.95b	5.606	0.016
Fermentation coefficient ^d	42.41	46.75	44.47	1.151	0.078
pH	6.42	6.53	6.39	0.039	0.066
<i>Chemical analysis (g kg⁻¹ DM basis)</i>					
OM	930.3c	939.5a	935.2b	0.88	<0.001
CP	83.6a	68.0b	70.9b	2.03	0.001
Starch	185.9b	189.7b	230.1a	9.37	0.016
NDF	501.6	516.3	490.7	7.03	0.082
ADF	264.7	270.8	252.3	4.89	0.084
WSC ^e	23.6b	31.2a	26.1b	1.71	0.004
<i>Microbial Counts (log₁₀ CFU g⁻¹ forage DM)</i>					
LAB ^f	6.54	6.68	6.78	0.136	0.394
Yeasts	6.01b	6.29a	6.52a	0.077	0.004
Molds	5.60	6.04	6.02	0.130	0.069

Note: In the same row means with different letters indicate significant difference ($P < 0.05$) between cultivars

^aBarley cultivars were: CDC Cowboy (H-NDFD, high NDF digestibility), CDC Copeland (I-NDFD, intermediate NDF digestibility), and Xena (L-NDFD, low NDF digestibility). All three were grown in Lethbridge, AB and were harvested on the same day at mid-dough.

^bStandard error of mean.

^cBuffering capacity expressed as mEq of 0.1 M NaOH required to raise pH of 1 kg (DM) of fresh forage from 4 to 6.

^dFermentation coefficient = DM% + 8WSC/BC.

^eWater soluble carbohydrates (WSC) expressed as g of glucose equivalent kg⁻¹ silage on a DM basis

^fLactic acid bacteria

Table 2. Composition of barley silage cultivars (n = 3) previously ranked on the basis of on-farm *in vitro* NDFD of silage samples^a.

	Barley Cultivar				P-value	
Item	H-NDFD	I-NDFD	L-NDFD	SEM ^b	Cultivar	Impact of Ensiling Method ^c
Mini-silos						
DM (g kg ⁻¹ fresh matter)	398.6	420.1	415.3	9.00	0.112	0.021
DM loss (% initial DM weight)	2.26	2.85	3.01	0.255	0.096	
pH	4.35 ^b	4.79 ^a	4.79 ^a	0.068	0.006	<0.001
On-farm NDFD (g kg ⁻¹ NDF) ^d	370 ^a	311 ^b	288 ^c	25.5	<0.01	
NDFD (g kg ⁻¹ NDF)	323.9	350.9	343.6	16.42	0.223	0.236
<i>Chemical Analysis (g kg⁻¹ DM)</i>						
OM	922.0	913.0	927.0	4.54	0.170	0.467
CP	84.9	76.7	76.3	2.18	0.053	0.001
Starch	162.7 ^b	139.5 ^b	195.7 ^a	7.11	0.004	0.029
NDF	515.4 ^b	561.2 ^a	496.9 ^b	6.74	0.001	<0.001
ADF	288.0 ^b	324.9 ^a	278.8 ^b	3.54	<0.001	<0.001
ADIN (g kg ⁻¹ Total N)	40.7	49.6	45.9	2.39	0.100	<0.001
WSC ^e	69.27	86.18	80.05	5.121	0.139	0.416
<i>Microbial Counts (log₁₀ CFU g⁻¹ silage DM)</i>						
LAB ^f	8.19 ^b	8.63 ^a	8.18 ^b	0.107	0.027	0.009
Yeasts	3.49	3.40	3.55	0.252	0.923	0.095
Molds	2.85	2.48	2.61	0.156	0.325	0.067
<i>Fermentation Products (g kg⁻¹ silage DM)</i>						
Lactate	24.54 ^a	18.68 ^{ab}	14.17 ^b	2.802	0.038	0.111
Acetate	5.15 ^a	2.31 ^b	2.56 ^b	0.392	0.004	<0.001
Propionate	0.18	0.39	0.29	0.074	0.218	<0.001
Butyrate	1.23	5.61	8.04	1.566	0.056	0.035
Total VFA	6.69	8.47	11.05	1.497	0.198	<0.001
NH ₃ -N	1.87	1.82	1.68	0.063	0.175	<0.001
Bunker-silos						
DM (g kg ⁻¹ fresh matter)	384.9	401.8	403.2	6.38	0.158	-
pH	4.53	4.50	4.39	0.046	0.146	-
NDFD (g kg ⁻¹ NDF)	345.0	353.3	334.4	16.66	0.124	-
<i>Chemical Analysis (g kg⁻¹ DM basis)</i>						
OM	916.6 ^b	926.5 ^a	924.3 ^a	1.32	0.004	-
CP	86.7	86.8	85.2	1.65	0.761	-
Starch	191.7	220.9	195.9	16.08	0.440	-
NDF	586.7 ^a	566.5 ^a	545.6 ^b	5.89	0.008	-
ADF	339.6	327.5	311.1	6.77	0.065	-
ADIN (g kg ⁻¹ Total N)	65.3	61.3	69.1	5.46	0.620	-
WSC	54.65	131.21	78.28	18.86	0.069	-
<i>Microbial Counts (log₁₀ CFU g⁻¹ silage DM)</i>						
LAB	7.32	7.44	7.25	0.331	0.919	-

Yeasts	4.84	4.75	4.37	0.967	0.937	-
Molds	3.88	4.14	3.40	0.845	0.882	-
<i>Fermentation Products (g kg⁻¹ silage DM)</i>						
Lactate	30.76	28.17	22.62	5.125	0.551	-
Acetate	8.08	7.70	5.74	1.651	0.728	-
Propionate	2.37 ^a	1.64 ^b	1.26 ^b	0.156	0.007	-
Butyrate	7.38	7.13	8.95	1.256	0.569	-
Total VFA	18.49	17.67	22.48	3.518	0.611	-
NH ₃ -N	2.11 ^a	1.96 ^b	1.92 ^b	0.023	0.003	-

Note: In the same row means with different letters indicate significant difference ($P < 0.05$) between cultivars

^aBarley cultivars were: CDC Cowboy (H-NDFD, high NDF digestibility), CDC Copeland (I-NDFD, intermediate NDF digestibility), and Xena (L-NDFD, low NDF digestibility). All three were grown in Lethbridge, AB and were harvested on the same day at mid-dough and ensiled in mini- or bunker silos.

^bStandard error of mean.

^cImpact of ensiling method ie., bunker silo vs mini silo.

^dBasis for cultivar selection as H-, I-, and L-NDFD (Nair et al. 2016, in press).

^eWater-soluble carbohydrates (g of glucose equivalent kg⁻¹ silage DM basis).

^fLactic acid bacteria

Table 3. Changes in barley silages (n = 3) previously ranked on the basis of on-farm *in vitro* NDF digestibility of silage samples during 21 d aerobic exposure^a.

	Barley Cultivar				
Item	H-NDFD	I-NDFD	L-NDFD	SEM ^b	P - Value
Day 0					
Dry matter (%)	39.86	42.01	41.53	0.900	0.112
WSC ^c (g kg ⁻¹ DM)	69.27	86.18	80.05	5.121	0.139
NH ₃ -N (g kg ⁻¹ total N)	1.87	1.82	1.68	0.063	0.175
<i>Microbiology (log₁₀ CFU g⁻¹ silage DM)</i>					
LAB ^d	8.19 ^b	8.63 ^a	8.18 ^b	0.107	0.027
Yeasts	3.49	3.40	3.55	0.252	0.923
Molds	2.85	2.48	2.61	0.156	0.325
Day 3					
Dry matter	40.09	42.42	41.88	1.014	0.110
WSC (g kg ⁻¹ DM)	79.23	85.10	89.42	4.837	0.167
NH ₃ -N (g kg ⁻¹ total N)	1.98 ^a	1.89 ^a	1.72 ^b	0.048	0.020
<i>Microbiology (log₁₀ CFU g⁻¹ silage DM)</i>					
LAB	7.82	8.20	7.97	0.180	0.137
Yeasts	4.49	2.77	2.53	0.894	0.430
Molds	NF ^e	NF	NF		
Day 7					
Dry matter	42.21	45.54	44.48	1.168	0.071
WSC (g kg ⁻¹ DM)	63.11	87.10	86.58	11.119	0.293
NH ₃ -N (g kg ⁻¹ total N)	1.65	1.93	1.79	0.188	0.614
<i>Microbiology (log₁₀ CFU g⁻¹ silage DM)</i>					
LAB	8.07	7.84	8.03	0.282	0.832
Yeasts	7.25 ^a	3.07 ^b	2.76 ^b	0.578	0.009
Molds	NF	NF	NF		
Day 14					
Dry matter	41.74	46.70	46.23	0.989	0.022
WSC (g kg ⁻¹ DM)	24.26 ^b	91.01 ^a	81.31 ^a	7.456	0.002
NH ₃ -N (g kg ⁻¹ total N)	0.98 ^b	1.98 ^a	1.78 ^a	0.124	0.006
<i>Microbiology (log₁₀ CFU g⁻¹ silage DM)</i>					
LAB	9.44 ^a	8.07 ^b	7.66 ^b	0.235	0.004
Yeasts	9.40 ^a	3.20 ^b	3.20 ^b	0.274	<0.001
Molds	NF	NF	NF		
Day 21					
Dry matter	45.15	49.69	49.63	1.312	0.081
WSC (g kg ⁻¹ DM)	9.99 ^b	90.06 ^a	79.26 ^a	6.092	<0.001
NH ₃ -N (g kg ⁻¹ total N)	0.46 ^b	1.92 ^a	1.82 ^a	0.078	<0.001
<i>Microbiology (log₁₀ CFU g⁻¹ silage DM)</i>					

LAB	9.46 ^a	7.63 ^c	8.52 ^b	0.146	0.001
Yeasts	9.42 ^a	4.11 ^b	3.35 ^b	0.249	<0.001
Molds	6.16 ^a	2.67 ^b	2.44 ^b	0.433	0.003

Note: In the same row means with different letters indicate significant difference ($P < 0.05$) between cultivars

^aBarley cultivars were: CDC Cowboy (H-NDFD, high NDF digestibility), CDC Copeland (I-NDFD, intermediate NDF digestibility), and Xena (L-NDFD, low NDF digestibility). All three were grown in Lethbridge, AB and were harvested on the same day at mid-dough and ensiled in mini-silos.

^bStandard error of mean.

^cWater soluble carbohydrates (g glucose kg⁻¹ silage DM).

^dLactic acid bacteria

^eNot found.

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Figure Captions

Figure 1. Effect of barley cultivars selected for high (H-NDFD), intermediate (I-NDFD), and low (L-NDFD) NDF digestibility (NDFD) on fermentation traits during 60 d ensiling. Where visible, bars indicate standard error of LSmeans, effect of day ($P < 0.001$) for all parameters.

A. pH decline. * indicates I-NDFD pH is greater ($P = 0.004$) than the other two cultivars. ** indicates H-NDFD is lower ($P = 0.004$) than the other two cultivars. *** indicates I-NDFD is greater than L-NDFD ($P = 0.004$).

B. Water-soluble carbohydrates, expressed as g of glucose equivalent kg^{-1} silage on a DM basis.

C. Lactate concentration. * indicates H-NDFD is greater than L-NDFD ($P = 0.001$). ** indicates H-NDFD is greater than I-NDFD ($P = 0.001$). *** indicates L-NDFD is lower than the other two cultivars ($P = 0.001$).

D. Change in lactic acid bacteria (LAB) populations, expressed as $\log_{10}$ CFU g^{-1} silage (DM basis). * indicates H-NDFD is greater than L-NDFD ($P = 0.028$). ** indicates I-NDFD is greater than the other two cultivars ($P = 0.028$).

Figure 2. Effect of barley cultivars selected for high (H-NDFD), intermediate (I-NDFD), and low (L-NDFD) NDF digestibility (NDFD) on the aerobic stability of silage over 21 d. Where visible, bars indicate standard error of LSmeans.

A. Temperature change. * indicates significant difference ($P < 0.01$) between H-NDFD average daily temperature and ambient temperature.

B. pH change. * indicates difference ($P < 0.05$) between H-NDFD and the other cultivars.

C. Lactate concentration. * indicates difference ($P < 0.05$) between H-NDFD and L-NDFD on day of sampling. ** indicated difference ($P < 0.05$) between all cultivars.

Figure 1. Effect of barley varieties selected for high (H-NDFD), intermediate (I-NDFD), and low (L-NDFD) NDF digestibility (NDFD) on fermentation traits during 60 d ensiling. Where visible, bars indicate standard error of LSmeans, effect of day ($P < 0.001$) for all parameters.

A. pH decline. * indicates I-NDFD pH is greater ($P = 0.004$) than the other two varieties. ** indicates H-NDFD is lower ($P = 0.004$) than the other two varieties. *** indicates I-NDFD is greater than L-NDFD ($P = 0.004$).

B. Water-soluble carbohydrates, expressed as g of glucose equivalent kg⁻¹ silage on a DM basis.

C. Lactate concentration. * indicates H-NDFD is greater than L-NDFD ($P = 0.001$). ** indicates H-NDFD is greater than I-NDFD ($P = 0.001$). *** indicates L-NDFD is lower than the other two varieties ($P = 0.001$).

D. Change in lactic acid bacteria (LAB) populations, expressed as log₁₀ CFU g⁻¹ silage (DM basis). * indicates H-NDFD is greater than L-NDFD ($P = 0.028$). ** indicates I-NDFD is greater than the other two varieties ($P = 0.028$).

190x254mm (96 x 96 DPI)

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Figure 2. Effect of barley varieties selected for high (H-NDFD), intermediate (I-NDFD), and low (L-NDFD) NDF digestibility (NDFD) on the aerobic stability of silage over 21 d. Where visible, bars indicate standard error of LSmeans.

A. Temperature change. * indicates significant difference ($P < 0.01$) between H-NDFD average daily temperature and ambient temperature.

B. pH change. * indicates difference ($P < 0.05$) between H-NDFD and the other varieties.

C. Lactate concentration. * indicates difference ($P < 0.05$) between H-NDFD and L-NDFD on day of sampling. ** indicated difference ($P < 0.05$) between all varieties.

190x254mm (96 x 96 DPI)