Molecular structure, chemical and nutrient profiles, and metabolic characteristics of the proteins and energy in new cool-season corn varieties harvested as fresh forage for dairy cattle

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ABSTRACT

To our knowledge, no previous research exists concerning the molecular structure and metabolic characteristics of the proteins and energy that new cool-season corn varieties provide for dairy cattle. The objectives of this study were to identify the differences in the molecular structures of proteins among several new cool-season corn varieties [Pioneer P7443R, Pioneer P7213R, Pioneer P7535R (Pioneer Hi-Bred International Inc., Johnston, IA), Hyland Baxxos RR, Hyland SR22, and Hyland SR06 (Hyland Seeds, Blenheim, ON, Canada)] using Fourier transform infrared attenuated total reflectance (FT/IR-ATR) molecular spectroscopy, and to determine the nutrient profile and supply that each variety provided for dairy cattle. The protein molecular structure studies showed that the amide I to amide II ratio ranged from 1.09 to 1.66 and that the α-helix to β-sheet ratio ranged from 0.95 to 1.01 among the new cool-season corn varieties. Energy content was significantly different among the new varieties. We found significant differences in the protein and carbohydrate subfractions and in the ruminal degradation kinetics of the organic matter, crude protein, starch, and neutral detergent fiber of the new varieties. The new varieties had similar estimated intestinal digestibilities for rumen undegraded crude protein. However, the new varieties had significant differences in predicted total truly absorbable protein, ranging from 39 to 57 g/kg of dry matter, indicating that these newly developed varieties are satisfactory sources of truly absorbed protein for dairy cattle. Further study on the molecular structure profiles of cool-season corn in relation to its nutrient utilization and availability in dairy cattle is necessary. Key words: forage, protein molecular structure, metabolic characteristics, energy

INTRODUCTION

Corn is a crop with a long history as a foodstuff for both animals and humans (Lauer et al., 2001; Arturo, 2003), and 40% of current global corn production is used as animal feed (Gyori, 2010). Canada harvests over 190,000 ha of corn forage, with the highest production being in Ontario (63%) and the second highest (21%) in the province of Quebec (Coors and Lauer, 2001).

The corn grown in the Canadian prairies is different from the corn varieties grown in warmer climates (Las-siter et al., 1958). The main differences are due to the shorter growing season and lower growing temperatures in the Canadian prairies compared with the areas of warm-season corn production, such as the United States (Lauer et al., 2001). The differences among varieties include changes in chemical profile and the nutrient composition of silage (Mahanna, 2010).

In corn cultivation, crop heat units (CHU) are calculated from daytime temperatures above 10°C and nighttime temperatures above 4.4°C on a cumulative daily basis from seeding to harvest. Many corn varieties for western Canada require ≥2,000 CHU to reach the silage harvest stage, with a kernel maturity of 45% DM.

Recently, 6 cool-season corn varieties have been developed: Pioneer P7443R, Pioneer P7213R, Pioneer P7535R (Pioneer Hi-Bred International Inc., Johnston, IA), Hyland Baxxos RR, Hyland SR22, and Hyland SR06 (Hyland Seeds, Blenheim, ON, Canada). However, no systematic research has been conducted on the molecular structure, chemical and nutrient profiles, and metabolic characteristics of the protein and energy of these newly developed cool-season corn varieties.

Fourier transform infrared attenuated total reflectance (FT/IR-ATR) molecular spectroscopy is able to detect molecular structural features in biological materials, as well as processing-induced structural changes (Doiron et al., 2009; Liu and Yu, 2010). The hypotheses of this study were that (1) the differences in the molecular structures of the proteins among the new cool-season corn varieties could be detected by
molecular spectroscopy, (2) the magnitude of the differences among these new cool-season corn varieties was significant, and (3) the chemical and nutrient profiles of the newly developed cool-season corn varieties significantly differ from those of corn varieties grown in warm climates.

The objectives of this study were (1) to identify differences in the molecular structures of proteins among the new cool-season corn varieties using FT/IR-ATR molecular spectroscopy; (2) to investigate the differences in chemical profile, as well as the protein and carbohydrate subfractions, among the cool-season corn varieties; (3) to determine the ruminal degradation kinetics of various nutrients among the cool-season corn varieties; (4) to predict the intestinal availability of protein among the cool-season corn varieties; and (5) to reveal the metabolic characteristics of the proteins and model the amount of truly absorbable protein in the small intestine.

MATERIALS AND METHODS

Corn Cultivation, Experimental Design, and Sampling

Six new cool-season corn varieties were grown at the Canada-Saskatchewan Irrigation Diversification Centre (Outlook, SK, Canada) from May to September 2011. The varieties included Pioneer 7443R, Pioneer 7213R, Pioneer 7535R, Hyland Baxxos RR, Hyland SR22, and Hyland SR06. The experimental design was a randomized completed block design. The cultivation was designed with 4 replicates (4 fields or blocks) with a total of 24 plots for the 6 varieties. Seeding was performed on May 20, 2011, and harvesting (and sampling) was performed on September 29, 2011, after 2,160 CHU had been reached.

Molecular Spectroscopy

The molecular spectral data of the corn samples were collected and corrected for the background spectrum using FT/IR molecular spectroscopy (Jasco 4200, Jasco International Co. Ltd., Tokyo, Japan). The spectra were generated for the mid-infrared region (approximately 4,000–800 cm\(^{-1}\)) and the fingerprint region (approximately 1,800–800 cm\(^{-1}\)) with a spectral resolution of 4 cm\(^{-1}\). The FT/IR spectral data were processed using Omnic 7.3 (Spectra-Tech, Madison, WI). The regions of specific interest in this study included the protein amide I and II and the protein structure of the α-helix and β-sheet in the infrared regions of approximately 1,715–1,480 cm\(^{-1}\) (Samadi and Yu, 2011; Liu et al., 2012).

Univariate Spectral Analysis

The protein molecular structure spectral profile was determined from the 2 primary bands in the spectra; namely, the amide I and amide II regions (Yu, 2010; Khan and Yu, 2013). The amide I and amide II peak area absorption intensities and their ratios were calculated. Using the second-derivative functions in Omnic 7.3, the amide I peak was further resolved into several multi-component peaks in which α-helices (centered at ~1,655 cm\(^{-1}\)) and β-sheets (centered at ~1,630 cm\(^{-1}\)) were identified. The intensities of the peak heights for the α-helix and β-sheet were calculated.

Rumen Degradation Procedures

The rumen in situ degradation parameters were determined using the method described previously (Yu et al., 2002). Fresh forage samples were chopped to 1 cm in size, dried at 55°C for 72 h and ground through a Christy & Norris 10-inch feed mill (Christy Turner Ltd., Suffolk, UK) using a 2-mm screen. Approximately 7 to 9 g of each dried forage sample was weighed into a nylon bag (Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississauga, ON, Canada) with dimensions of 10 × 20 cm and a pore size of 41 μm. The ratio of the sample size to the bag surface area averaged ~17.5 mg/cm\(^{-2}\), similar to that used in previous work (Ørskov and McDonald, 1979; Nocek and Tamminga, 1991). A polyester mesh bag (45 × 45 cm with a 90-cm length of nylon rope for anchoring to the cannula) was used to hold the sample bags in the rumen. The sample bags were added to the polyester mesh bag according to a gradual addition–all out schedule and incubated for 72, 48, 24, 12, 6, 2, and 0 h. The number of bags incubated for each sample was determined according to the previous work (Bal et al., 2000; Jurjanz and Monteils, 2005; Zanton and Heinrichs, 2009). The maximum number of bags in the rumen at any one time was 30 based on previous effective degradability data (Huntington and Givens, 1995). All samples were incubated for 2 runs in 3 nonlactating Friesian cows fitted with rumen cannula and fed 570 g/kg of barley silage, 100 g/kg of alfalfa hay, 50 g/kg of dehydrated alfalfa pellets, and 280 g/kg of concentrates (containing barley, wheat, oats, canola meal, soybean meal, wheat distillers dried grains with solubles, corn gluten meal, molasses, golden flakes, canola oil, minerals, and vitamins). After incubation, the bags were removed from the rumen and, together with those samples representing 0 h, rinsed under cold water to remove excess ruminal contents. The samples were then washed with cool water and dried at 55°C for 48 h. The dry samples were stored at 4°C until further analysis.
Chemical Analyses

The dried forage samples and ruminal residue samples were further ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments Canada Ltd., Mississauga, ON, Canada) for use in chemical analyses. Dry matter (method 930.15), ash (method 942.05), crude fat (method 954.02), and CP (method 984.13) contents were analyzed according to the AOAC (1990) procedures. Starch content was analyzed using the Megazyme Total Starch Assay Kit (Megazyme, Co. Bray, Ireland) and the α-amylase/amyloglucosidase method (McCleary et al., 1997). The ADF, NDF, and ADL values were also analyzed (Van Soest et al., 1991). Amylase and sulfite were not used for ADF and ADL. For NDF, amylase was used, but sulfite was not, because the NDF residues were analyzed for neutral detergent insoluble CP (NDICP). The NPN content was analyzed by precipitating the true protein with tungstic acid (Licitra et al., 1996). 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 ADIN and NDIN values were also measured (Licitra et al., 1996). The NSC contents, including sugars, organic acids, and other reserve carbohydrates, were estimated using NFC and calculated (NRC, 2001).

Energy Estimate

The estimated energy for the total digestible CP (tdCP), fatty acids (tdFA), NDF (tdNDF), NFC (tdNFC), total digestible nutrients at 1× maintenance (TDN1×), digestible energy (DE) at the production level of intake (DE3×), ME at the production level of intake (ME3×) and NEL at the production level of intake (NEL3×) were determined using a summative approach (Weiss et al., 1992) from NRC (2001), whereas NEM and NEG were estimated using NRC (1996). Both the NRC (2001; dairy) and NRC (1996; beef) use the same formula to estimate NEM and NEG.

Cornell Net Carbohydrate and Protein System

The CP and carbohydrate (CHO) subfractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.1; Sniffen et al., 1992; Fox et al., 2004; Tylutki et al., 2008). The characterization of the carbohydrate fractions applied in this system is CA (CA1, CA2, CA3, and CA4), CB (CB1, CB2 and CB3), and CC (Fox et al., 2004; Tylutki et al., 2008).

The characterization of the CP fractions is as follows: fraction PA is NPN, fraction PB is true protein (TP), and fraction PC is unavailable protein. Fraction PB is further divided into 3 fractions (PB1, PB2, and PB3). The buffer-insoluble protein minus fraction PB3 is used to estimate fraction PB2. Fraction PB2 is insoluble in buffer but soluble in neutral detergent, whereas fraction PB3 is insoluble in both buffer and neutral detergent but is soluble in acid detergent solution. Fraction PB2 is fermented in the rumen at a lower rate than the buffer-soluble fraction, and some of PB2 fraction escapes to the lower gut. Fraction PB3 is believed to be more slowly degraded in the rumen than fractions PB1 and PB2 because of its association with the plant cell walls; a large proportion of PB3 is thus believed to escape the rumen. Fraction PC is ADIN, which is highly resistant to breakdown by microbial and mammalian enzymes; it is assumed to be unavailable to animal digestion (Fox et al., 2004; Tylutki et al., 2008).

Rumen Degradation Model for In Situ Study

The first-order kinetic degradation model described by Ørskov and McDonald (1979), with several modifications (Robinson et al., 1986; Dhanoa, 1988), was applied to describe the rumen degradation characteristics of DM, OM, CP, starch, and NDF. The model was solved with the NLIN procedure of SAS (SAS Institute Inc., Cary, NC) via iterative least squares regression (Gauss-Newton method) using the following equation:

\[ R(t) = U + (100 - S - U) \times \exp^{-K_d \times (t - T_0)} \]

where \( R(t) \) = residue present (%) at t hours of incubation; \( U \) = undegradable fraction (%); \( S \) = soluble fraction (%); \( K_d \) = degradation rate (%/h); and \( T_0 \) = lag time (h).

Based on the parameters S, U, D, and \( K_d \), the rumen-undegraded feed CP and rumen-undegraded starch (RUST) contents were predicted by the following equations:

\[ RUP (%) = U + (D \times K_p)/(K_p + K_d) \]

and \[ RUST (%) = S \times 0.1 + (D \times K_p)/(K_p + K_d), \]

where \( D = 100 - S - U \) (%), and \( K_p \) is the estimated rate of digesta outflow from the rumen (%/h) and was assumed to be 6%/h, according to Tamminga et al. (1994). The value 0.1 is a compensation factor between the in situ and in vivo starch results, indicating that 10% of the S fraction of starch escapes rumen degrada-
tion (Nocek and Tamminga, 1991; Tamminga et al.,
1994; Yu et al., 2003).

Metabolic Characteristics of the Proteins
and Prediction of Nutrient Supply to Dairy Cattle

The PDI system was applied to detect the magnitude
of the differences among newly developed cool-season
corn varieties (INRA, 2007). The protein value of the
feeds and the animal requirements are both expressed
in terms of true protein truly digestible in the small
intestine, abbreviated as PDI. The PDI system was
first proposed by Verité and Geay (1987), and it was
used in this study to determine the PDI value for each
individual feed. The PDI system aims to balance the
nitrogen and energy available in the rumen for micro-
bial protein synthesis and to provide a value for the
feedstuffs reflective of the true protein absorbed in the
small intestine. The PDI system requires the accurate
measurement of the characteristics of feed protein
degradation in the rumen and feed protein intestinal
digestion.

The PDI content of a diet is the sum of 2 fractions:
PDIA, the dietary protein undegraded in the rumen
but truly digestible in the small intestine, and PDIM,
the microbial true protein that is truly digestible in the
small intestine. PDIMN indicates the amount of mi-
crobial protein that could be synthesized in the rumen
from degraded dietary N when energy and other nutri-
ents are not limiting. PDIME indicates the amount of
microbial protein that could be synthesized from the
energy available in the rumen when degraded N and
other nutrients are not limiting. The value of each feed
is given directly as the sum of PDIA and PDIM, and
each of the 2 following possible situations is considered
separately: PDIN = PDIA + PDIMN, and PDIE =
PDIA + PDIME.

The PDI values were obtained from 4 individual feed
characteristics: (1) CP content, (2) effective degradabil-
ity of CP (EDCP) as measured by the rumen incuba-
tion procedure, (3) fermentable OM content (FOM)
calculated from the total digestible OM (DOM) con-
tent after the subtraction of the contents of the ether
extract and the undegradable dietary protein in the
feed and fermentation products in the silage, and (4)
true intestinal digestibility of the rumen undegraded
dietary true protein (TId).

Prediction of Microbial Protein Synthesis
in the Rumen Based on Available Energy
or Ruminally Degraded Protein

Microbial protein synthesis was predicted from FOM,
or the amount of OM that could be fermented by the
bacteria in the rumen (Verité and Geay, 1987; Tam-
menga et al., 1994; NRC, 2001). The FOM content
corresponds to the product of the subtraction of the
digestible fractions that are of low or no value for mi-
crobial energy metabolism, such as ether extract (EE)
and those fractions previously unavailable in the rumen,
from the DOM content. This value was calculated using
the following formula: FOM (g/kg) = DOM – EE –
CP × (1 – EDCP). The amount of microbial protein
that could be synthesized from the energy available in
the rumen was calculated with the formula

\[
PDIME = FOM \times 0.145 \times 0.8 \times 0.8,
\]

where the factor 0.145 represents the yield of microbial
protein (assumed to be 145 g of CP/kg of FOM with
regard to energy substrates); the AA content of both
microbial protein and true digestible protein in the
small intestine are assumed to be constant and equal to
0.8 in the French PDI System (Verité and Geay, 1987).

The amount of microbial protein that could be syn-
thesized in the rumen from the degraded dietary N was
calculated using the following formula: PDIMN = CP 
\times [1 – 1.11 \times (1 – EDCP)] \times 0.9 \times 0.8 \times 0.8,
where 0.9 is the efficiency of the conversion of degraded N to
rumen microbial N.

Prediction of Feed Protein Rumen Effective
Degradability and Truly Absorbed Rumen
Undegraded Feed Protein in the Small Intestine

The effective degradability in the rumen of feed pro-
tein was estimated as follows:

\[
EDCP (%) = 51.2 + 0.14 \times CP – 0.00017 \times CP^2 + \Delta,
\]

where \(\Delta = -71\). The true digestibility in the intestine
of rumen undegraded feed protein was calculated as
follows:

\[
TId (%) = 100 \times [1.11 \times (1 – EDCP/100) \times CP – PANDI]/[1.11 \times (1 – EDCP/100) \times CP],
\]

where PANDI represents the quantity of rumen unde-
graded feed protein that was not digested in the small
intestine. This value was calculated using the following
formula:

\[
PANDI = 7.9 + 0.08 \times CP – 0.00033 \times CP^2 \\
+ \Delta_1 + \Delta_2 + \Delta_3,
\]
where $\Delta_1 = -1.9$, $\Delta_2 = -2.3$, and $\Delta_3 = -2.0$.

The truly absorbed rumen undegraded feed protein in the small intestine (PDIA) was calculated using the following formula:

$$\text{PDIA} = \text{CP} \times [1.11 \times (1 - \text{EDCP})] \times \text{TId},$$

where the effective rumen bypass of protein is assumed to be $1.11 \times (1 - \text{EDCP})$ (French PDI system).

### Statistical Analysis

The data from the chemical analyses, in situ assays, and model estimations were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The model used for the analysis was as follows:

$$Y_{ij} = \mu + F_i + e_{ij},$$

where $Y_{ij}$ was an observation of the dependent variable $i$; $\mu$ was the population mean for the variable; $F_i$ was the fixed effect of variety; fields (block) and in situ cow and runs were random effects; and $e_{ij}$ was the random error associated with the observation $ij$.

To solve the first-order kinetic degradation model, the NLIN procedure of SAS with iterative least squares regression (Gauss-Newton method) was applied. For all statistical analyses, significance was declared at $P < 0.05$ and significance trending at $P \leq 0.10$. The treatment means were compared using the Tukey-Kramer honestly significant difference (HSD) procedure.

### RESULTS AND DISCUSSION

### Protein Structure Spectral Profiles

**Amide I and Amide II.** Table 1 gives the protein molecular structure parameters for the cool-season corn forage varieties. Amide I and amide II intensity did not differ among the varieties. However, the amide I to amide II ratio exhibited significant differences, ranging from 1.09 to 1.66 (Table 1). These differences in the amide I to amide II ratio indicated different molecular structure compositions or conformations among the cool-season corn forage varieties, which may be related to nutrient availability.

**α-Helix and β-Sheet.** Table 1 shows the protein molecular structure characteristics in terms of the α-helix and β-sheet peaks. The intensities of the α-helix and β sheet peak heights were not significantly different among the cool-season corn forage varieties. The α-helix to β sheet ratio ranged from 0.95 to 1.01 (Table 1). Again, the differences in the α-helix to β sheet ratios may indicate different protein molecular structure conformations among the cool-season corn forage varieties, which may be related to nutrient digestion. No previous work in corn could be found with which to compare our present results.

### Chemical Profiles of Fresh Forage

The corn fresh forage chemical profiles are presented in Table 2. Hyland SR 22 had the lowest DM content (424 g/kg) and Pioneer 7213R had the highest (603 g/kg). The DM content of whole corn plants has previously been reported as 320 to 400 g/kg of DM (Bal et al., 2000; Jurjanz and Monteils, 2005), a value lower than our present findings. These differences may be attributable to the dry weather conditions of the prairies in which the experiment was conducted (Bal et al., 2000; Mahanna, 2010), lower than our present findings. These differences may be attributable to the dry weather conditions of the prairies in which the experiment was conducted (Bal et al., 2000; Mahanna, 2010) or to the presampling time and procedure. The chemical profiles of the varieties were different on a DM basis. However, higher temperatures and greater light intensity have been shown to increase the DM content of whole corn plants (Struik et al., 1985). The Hyland varieties had significantly different values for NDF (~500 g/kg of DM), starch (~211 g/kg of DM) and CP (~74 g/kg of DM) compared with the Pioneer varieties (NDF 489, starch 260, and CP 69 g/kg of DM). Both the NDF and ADF values were significantly lower ($P < 0.05$) in Hyland SR 22 compared...
with the other varieties. Starch was higher in the Pioneer varieties, with the highest starch content (275 g/kg of DM) observed for P7213R. In previous research, starch levels were >300 g/kg of DM in corn fresh forage (Johnson et al., 2003; Hallada et al., 2008; Mahanna, 2010), a value slightly higher than our present findings.

Crude protein compositions varied across the varieties, with the highest value (90 g/kg of DM) observed in Hyland SR06 (Table 2). Additionally, Hyland Baxxos RR had the highest SCP (511 g/kg of CP) and NPN (697 g/kg of CP) values. These CP values were in agreement with those of previous work (Sniffen et al., 1992; Jurjanz and Monteils, 2005). The lowest lignin levels (31 g/kg of DM) were found in Pioneer 7535R and 7213R (Table 1); however, these levels were higher than previously reported values (Jurjanz and Monteils, 2005). The presence of lignin may be reflected by a physical shielding against fiber digestion (Hatfield, 1989). Both the neutral and acid detergent insoluble crude protein (NDICP and ADICP) values were in agreement with previous results reported for corn forage (Sniffen et al., 1992). Nutrient values for whole corn plants (NDF 445, lignin 30, and starch 310 g/kg of DM) have been reported previously (Hunt et al., 1989; Coors and Lauer, 2001) and are in close agreement with our present results. Therefore, the nutrient concentrations of corn grown in cooler prairie climates were not inferior to those of warm-season corn. In the NRC (2001) report, corn fresh forage is not listed; however, the nutrient compositions (NDF 45, CP 8.8, and EE 3.2% of DM) reported for corn silage (NRC, 2001) were in agreement with our findings (NDF 49, CP 7.1, and EE 1.7% of DM) for fresh forage. No starch data were listed for corn silage by the NRC (2001).

Digestible Nutrients and Estimated Energy Values

Total digestible nutrients (TDN1×), DE, ME for production, and NE (for lactation, maintenance, and weight gain) estimated using the NRC (2001) methods are presented in Table 3. These values ranged from 65.5 to 68.4% of DM for TDN1×, 2.79 to 2.90 Mcal/kg of DM for DE3×, 2.14 to 2.24 Mcal/kg of DM for ME3×, and 1.31 to 1.74 Mcal/kg of DM for NEL3×. Pioneer 7213R contained the highest energy values of the studied varieties, whereas Hyland SR22 had the lowest values (NRC, 2001). Truly digestible nutrients, such as NFC, NDF, CP, and FA, were used to estimate TDN and energy (Yu et al., 2003). The energy estimates were relatively similar between the Pioneer (2.9, 2.2, and 1.4 Mcal/kg of DM for DE1×, ME1×, and NEL1×, respectively) and Hyland (2.8, 2.2, and 1.5 Mcal/kg of DM for DE1×, ME1×, and NEL1×, respectively) varieties. These findings were in agreement with published values for regular corn silage (68.8%, 2.9, 2.3, and 1.5 Mcal/kg for TDN, DE1×, ME1×, and NEL1×, respectively; NRC, 2001).
Therefore, these cooler climate varieties provided adequate energy and digestible nutrients for ensiling or for the direct feeding of ruminants as fresh forage.

**Estimated Carbohydrate and Protein Fractions**

Total carbohydrate levels in the corn fresh forage were found to be >800 g/kg of DM (Table 2). The carbohydrate pool of the Pioneer varieties was higher (868 g/kg of DM; \( P = 0.02 \)) than that of the Hyland varieties (857 g/kg of DM). The carbohydrate fractions among the corn fresh forages were different (Table 3); however, the CHO factions (CB, CC) of the Pioneer varieties were similar to those of the Hyland varieties (Table 4). These variations in the concentration and digestion characteristics of the carbohydrates may affect energy intake and animal performance. The CNCPS carbohydrate fractions reveal energy availability to the animal (Lanzas et al., 2007; Tylutki et al., 2008). Hyland Baxxos RR had the highest values for the NSC and CA fractions (C4, 234 g/kg of CHO). Hyland SR22 had the highest values for the CB2 (511 g/kg of CHO) and CB3 (309 g/kg of CHO) fractions (Table 4). The lowest value for the CC fraction (80 g/kg of CHO) was found in Pioneer 7535R.

The variation in the protein factions among the varieties was high (Table 4). The highest values for the PA, PB3, and PC factions were observed in Hyland Baxxos RR, which had the lowest true protein and PB2 values. The values of the protein fractions did not differ between the Pioneer (PA: 449, PB2: 375, PB3: 115, and PC: 63 g/kg of CP) and Hyland (PA: 460, PB2: 351, PB3: 130, and PC: 61 g/kg of CP) varieties (\( P = 0.45 \)). The values for true protein and NPN (50 and 30 g/kg) found in this study were higher than those previously reported in the literature (Hunt et al., 1989; Johnson et al., 1999). The values of the CNCPS fractions reported in corn fresh forage were different from the values of these fractions in corn silage (Lanzas et al., 2007; Tylutki et al., 2008), suggesting that ensiling may increase CP (Lanzas et al., 2007; Tylutki et al., 2008; González et al., 2010).

**Rumen Degradation Kinetics of DM and OM**

As presented in Table 5, the highest in situ DM degradation rate (\( K_{d} \); 4.5%/h) was observed in Pioneer 7213R and the lowest rate in Hyland Baxxos RR (3.0%/h; \( P < 0.05 \)). The soluble fractions (S) of DM were significantly different between Hyland SR22 and Pioneer 7213R. Hyland Baxxos RR had the highest value for the rumen degradable DM fraction (52%), and Pioneer 7535R had the lowest value (44%). The effectively degraded feed DM was found to be high in Pioneer 7535R and low in Hyland Baxxos RR. On average, the Pioneer varieties had higher values than the Hyland varieties for degradation rate (4.03%/h), \( \text{U} \) fraction (30.9%), and effectively degraded DM (405 g/kg). González et al. (2010) reported similar \( K_{d} \) values (3 to 4%/h) to those of the present study; however, the previous work also observed a higher effective degradability value (60%) and a lower undegraded fraction.
value (15%; González et al., 2010). The degradability values of fresh corn and silage from whole corn plants, as previously reported by rumen DM degradability kinetics, were similar to our values for the new corn varieties (Jurjanz and Monteils, 2005). Rumen OM was degraded similarly to DM (Table 4); however, the descending order of the varieties for the soluble fraction value was Hyland SR22, Pioneer 7443R, Pioneer 77535R, Hyland Baxxos RR, and Hyland SR06 (Table 4; *P* < 0.05). Additionally, Pioneer 7535R had the highest effectively degraded feed OM value (385 g/kg of DM; *P* < 0.05). Furthermore, Pioneer 7535R exhibited significantly higher trending values for the soluble (22%) and degradable (46%) fractions of OM (*P* < 0.1; Table 4). In comparison, the Pioneer and Hyland varieties had the following OM degradability kinetics: K$_d$: 4.3 versus 3.3%/h; S: 20.5 versus 21.5%; D: 50.4 versus 51.1%; U: 29.8 versus 27.4%; and effectively degraded OM: 380 versus 369 g/kg of DM. These values indicate that the Pioneer varieties were faster in degradation. The OM degradability of corn fresh forage is similar to that of grass and other forages, such as oat and barley (Van Vuuren et al., 1991; Abeysekara, 2004; Jančík et al., 2009).

### Rumen Degradation Kinetics of NDF

We found no significant differences (*P* > 0.05) in rumen in situ degradation kinetics of the new corn fresh forages, with the exception of rumen undegraded NDF (Table 5). On average, many of the NDF degradability kinetic parameters (S 5.6%, D 54.8%, and U 39.5%) were similar between the Pioneer and Hyland varieties. However, the K$_d$ and effectively degraded NDF values were significantly different between the varieties (4.6 vs. 4.2%/h and 124 vs. 118 g/kg, respectively, *P* < 0.05). Jurjanz and Monteils (2005) reported faster rumen degradability kinetics for similar forages (2 vs. 5%/h); however, the S, D, and U fraction values were similar across both studies. The typical rumen degradability kinetics reported by Zanton and Heinrichs (2009) were similar to our present values (K$_d$ = 3, S = 8, and D = 60).

### Rumen Degradation Kinetics of Starch

The starch in situ rumen degradability kinetics differed significantly (*P* < 0.05) among the corn fresh...
Table 5. Differences in in situ rumen degradation kinetics of DM, OM, NDF, and starch: Comparison among the newly developed cool-season corn forage varieties

<table>
<thead>
<tr>
<th>Item</th>
<th>Pioneer</th>
<th>Hyland</th>
<th>Contrast (P-value)</th>
<th>Pioneer vs. Hyland</th>
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<tr>
<td><strong>In situ DM rumen degradation kinetics</strong></td>
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<tr>
<td>Kₙ (%/h)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
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<tr>
<td>Tₙ (h)</td>
<td>3.40</td>
<td>4.54</td>
<td>4.19</td>
<td></td>
</tr>
<tr>
<td>S (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>D (%)</td>
<td>23.58ₐ</td>
<td>18.86ₐ</td>
<td>22.83ₐ</td>
<td></td>
</tr>
<tr>
<td>U (%)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td></td>
</tr>
<tr>
<td>Rumen undegraded DM (g/kg of DM)</td>
<td>597.68</td>
<td>597.18</td>
<td>589.62</td>
<td>611.23</td>
</tr>
<tr>
<td>Effectively degraded DM (g/kg of DM)</td>
<td>402.32</td>
<td>402.83</td>
<td>410.38</td>
<td>388.77</td>
</tr>
<tr>
<td><strong>In situ OM rumen degradation kinetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₙ (%/h)</td>
<td>3.41</td>
<td>4.57</td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td>Tₙ (h)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>S (%)</td>
<td>22.51ₐ</td>
<td>17.26ₐ</td>
<td>21.74ₐ</td>
<td></td>
</tr>
<tr>
<td>D (%)</td>
<td>48.29</td>
<td>52.41</td>
<td>45.88ₐ</td>
<td></td>
</tr>
<tr>
<td>U (%)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td></td>
</tr>
<tr>
<td>Rumen undegraded OM (g/kg of DM)</td>
<td>638.48</td>
<td>640.50</td>
<td>632.18</td>
<td>653.20</td>
</tr>
<tr>
<td>Effectively degraded OM (g/kg of DM)</td>
<td>377.58ₐ</td>
<td>376.77ₐ</td>
<td>385.28ₐ</td>
<td>359.76ₐ</td>
</tr>
<tr>
<td><strong>In situ NDF rumen degradation kinetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₙ (%/h)</td>
<td>4.03</td>
<td>3.00</td>
<td>6.76</td>
<td></td>
</tr>
<tr>
<td>Tₙ (h)</td>
<td>6.38</td>
<td>4.56</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>S (washable, %)</td>
<td>8.09</td>
<td>4.07</td>
<td>4.73</td>
<td></td>
</tr>
<tr>
<td>D (%)</td>
<td>52.96</td>
<td>63.01</td>
<td>48.60</td>
<td></td>
</tr>
<tr>
<td>U (%)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td></td>
</tr>
<tr>
<td>Rumen undegraded NDF (g/kg of DM)</td>
<td>414.53ₐ</td>
<td>414.61ₐ</td>
<td>388.53ₐ</td>
<td>390.39ₐ</td>
</tr>
<tr>
<td>Effectively degraded NDF (g/kg of DM)</td>
<td>124.30</td>
<td>98.57</td>
<td>147.66</td>
<td>137.42</td>
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<tr>
<td><strong>In situ starch rumen degradation kinetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₙ (%/h)</td>
<td>28.34ₐ</td>
<td>45.24ₐ</td>
<td>41.20ₐ</td>
<td></td>
</tr>
<tr>
<td>Tₙ (h)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>S (%)</td>
<td>45.88ₐ</td>
<td>37.70ₐ</td>
<td>42.58ₐ</td>
<td></td>
</tr>
<tr>
<td>D (%)</td>
<td>49.88ₐ</td>
<td>57.85ₐ</td>
<td>52.76ₐ</td>
<td></td>
</tr>
<tr>
<td>U (%)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td></td>
</tr>
<tr>
<td>Rumen undegraded starch (g/kg of DM)</td>
<td>39.31ₐ</td>
<td>33.83ₐ</td>
<td>33.56ₐ</td>
<td>42.38ₐ</td>
</tr>
<tr>
<td>Effectively degraded starch (g/kg of DM)</td>
<td>217.02ₐ</td>
<td>207.33ₐ</td>
<td>229.77ₐ</td>
<td>167.70ₐ</td>
</tr>
<tr>
<td><strong>In situ CP rumen degradation kinetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₙ (%/h)</td>
<td>2.98</td>
<td>4.30</td>
<td>4.05</td>
<td></td>
</tr>
<tr>
<td>Tₙ (h)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>S (%)</td>
<td>18.17ₐ</td>
<td>10.74ₐ</td>
<td>9.10ₐ</td>
<td></td>
</tr>
<tr>
<td>D (%)</td>
<td>56.80ₐ</td>
<td>63.46ₐ</td>
<td>53.94ₐ</td>
<td></td>
</tr>
<tr>
<td>U (%)</td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td></td>
</tr>
<tr>
<td>Rumen undegraded CP (g/kg of DM)</td>
<td>51.35ₐ</td>
<td>50.94ₐ</td>
<td>50.12ₐ</td>
<td>54.09ₐ</td>
</tr>
<tr>
<td>Effectively degraded CP (g/kg of DM)</td>
<td>26.22ₐ</td>
<td>23.09ₐ</td>
<td>18.7ₐ</td>
<td>22.8ₐ</td>
</tr>
</tbody>
</table>

*Values in each row having different letters are statistically different at P < 0.05.
1Corn varieties were from Pioneer Hi-Bred International Inc. (Johnston, IA) and Hyland Seeds (Blenheim, ON, Canada).
2Kₙ = fractional degradation rate (%/h); Tₙ = lag time (h); S = fractional portion of nutrient washed or solubilized at initiation of incubation (washable soluble fraction at time 0); D = fraction of nutrient potentially degradable in the rumen; U = undegradable fraction.
forage varieties (Table 4). The highest $K_d$ was observed in Hyland SR22, and the lowest rate was observed in Hyland Baxxos RR. The highest S fraction (70.9%) was found in Hyland SR22, whereas the lowest S (30.3%) was found in Hyland SR06. In general, the undegraded fraction (U) was small, but statistically significant differences were observed among the varieties. The average values of the Pioneer and Hyland varieties were similar for $K_d$ (38.5%/h) and D (53.1%). However, the values of S (42.1 vs. 45.2%) and effectively degraded starch (218 vs. 198 g/kg of DM) were significantly different. Two of the Hyland varieties (Baxxos RR and SR22) had rumen degradability kinetics similar to the values (S = 66 and D = 32) previously reported by Jurjanz and Monteils (2005); however, the values of the other varieties were not similar. The disappearance of starch in several previous studies may help to explain the differences among these varieties because starch content and quality are strongly influenced by plant genetic composition (Bal et al., 2000; Mahanna, 2009; Der Bedrosian et al., 2012) and plant maturity.

### Rumen Degradation Kinetics of CP

With the exceptions of $K_d$ and U, the protein degradability characteristics were significantly different ($P < 0.05$) among the varieties (Table 5). Pioneer 7443R possessed the highest soluble protein fraction among the varieties (S, 18.2%), whereas Hyland Baxxos RR had the lowest S fraction (1.3%). However, Hyland Baxxos RR had the highest degradable fraction (D, 74.4%), whereas Pioneer 7535R had the lowest (53.9%). Hyland SR06 had the highest effectively degraded CP value (30.5 g/kg of DM), and Hyland Baxxos RR had the lowest value (12.9 g/kg of DM). The CP degradability kinetics differed between the Pioneer and Hyland varieties ($K_d$: 3.8 vs. 3.1%/h; S: 12.7 vs. 9.3%; D: 58.1 vs. 63.5%; U: 29.3 vs. 27.2%; and EDCP: 22.7 vs. 22.2 g/kg). These CP degradation kinetics were not in agreement with those of previous recent work ($K_d = 5.8%/h$, S = 57%, D = 28%, U = 13%, and EDCP = 60 g/kg of DM) conducted in Europe (González et al., 2010). These differences may be attributable to the variety (Clarica) and climate (Spain) of the previous study (Lauer et al., 2001; Bernard et al., 2004; González et al., 2010).

### Metabolic Characteristics of the Proteins and Potential Protein Supply to Dairy Cattle

The predicted values of the potential protein supply to dairy cattle from the corn fresh forage varieties using the PDI system are shown in Table 6. The absorbable microbial protein synthesis in the rumen was different among the varieties ($P < 0.05$). The highest and lowest DOM values were found in the Hyland varieties SR06 (666 g/kg of DM) and SR22 (634 g/kg of DM), respectively. A similar pattern was found for FOM and the amount of microbial protein synthesized from the available energy in the rumen. However, the Pioneer and Hyland varieties were not significantly different in terms of synthesized microbial proteins. The Pioneer varieties had an average PDIME value of 57.0 g/kg of DM, and the Hyland varieties had an average value of 56.7 g/kg of DM. The average value for PDIMN in the Pioneer varieties was 25.8 g/kg of DM, and the average value in the Hyland varieties was 28.2 g/kg of DM.

The EDCP in the rumen, as predicted with the PDI system, was determined to be highest ($P < 0.05$) in Hyland SR06, in accordance with its higher CP value. However, TId was not affected by variety. The values obtained in this study for PDIA, PDIN, and PDIE were again highest ($P < 0.05$) for Hyland SR06 (Table 6). The PDIA and PDIN values obtained in this study, except those for Hyland SR06, are in agreement with previous findings for corn fresh forage (INRA, 2007). The net energy values for milk production (UFL) and for meat production (UFV) were found to be highest for Pioneer 7213R forage (Table 6). The UFV and UFL values obtained in our study for the corn fresh forage were lower than those reported by CIHEAM (1981). The predicted values of the potential truly absorbable protein supply to dairy cattle from the corn fresh forage for the Pioneer and Hyland varieties confirmed the lack of major differences in CP between these 2 groups (Coors and Lauer, 2001). Intestinally digestible ruminally undegraded feed protein was not different between these varieties (Table 7). Total intestinally digestible feed proteins (TDP) were highest (95.35% CP) in Hyland SR22 and lowest (65.83% CP) in Pioneer 7535R. On average, the Hyland varieties were superior to the Pioneer varieties in both aspects (dRUP: 60.9 vs. 56.2% RUP and TDP: 78.9 vs. 83.1% CP). The values for the intestinal digestibility of RUP (52%) reported by González et al. (2010) for corn green feed were similar to ours. In González et al. (2010), the TDP values (average 81% CP) were closer to the effective degradability of CP (86% CP).

The current results indicate that newly bred cool-season corn varieties grown under Canadian prairie weather conditions have nutrient compositions similar to those of varieties grown in warm weather (Hallada et al., 2008; González et al., 2010; Mahanna, 2011). However, our results confirmed that low CHU access leads to relatively low CP and SCP values despite the lack of effect on NDF contents. Other corn growth factors, such as soil quality, irrigation, and fertilization (even with barn manure), would improve the nutritional quality of
Table 6. Metabolic characteristics of protein and potential protein supply to dairy cattle from corn fresh forage using the PDI system: Comparison among the cool-season corn forage varieties

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pioneer</th>
<th>Hyland</th>
<th>SEM</th>
<th>Contrast (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7443R</td>
<td>7213R</td>
<td>7535R</td>
<td>Baxxos RR</td>
</tr>
<tr>
<td>Absorbable microbial protein synthesis in the rumen (g/kg of DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDIME</td>
<td>56.7&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>57.5&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>56.8&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>55.9&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>PDIMN</td>
<td>27.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Truly absorbable rumen undegraded protein in small intestine (g/kg of DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDIA</td>
<td>19.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PDIN (PDIA + PDIMN)</td>
<td>46.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>40.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PDIE (PDIA + PDIME)</td>
<td>73.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Degraded protein balance</td>
<td>−29.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−31.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−33.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−33.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Forage unit for net energy in production (g/kg of DM)</td>
<td>0.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>UFL</td>
<td>0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a–d</sup>Values in each row having different letters are statistically different at P < 0.05 by Tukey mean comparison.

<sup>1</sup>Corn varieties were from Pioneer Hi-Bred International Inc. (Johnston, IA) and Hyland Seeds (Blenheim, ON, Canada).

<sup>2</sup>PDIME = amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded N is not limiting; PDIMN = amount of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; PDIA = dietary protein undegraded in the rumen, but truly digestible in the small intestine; PDIN = digestible proteins in the small intestine where N is the limiting factor for rumen microbial activity; PDIE = digestible proteins in the small intestine where energy is the limiting factor for rumen microbial activity; DPB = balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen; UFL = forage unit for the net energy value for milk production; UFV = forage unit for the net energy value for meat production.


<table>
<thead>
<tr>
<th>Variable</th>
<th>Pioneer</th>
<th>Hyland</th>
<th>SEM (n = 4)</th>
<th>Contrast (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dRUP (g/kg of RUP)</td>
<td>611.80</td>
<td>689.23</td>
<td>953.45</td>
<td>0.54</td>
</tr>
<tr>
<td>TDP (g/kg of CP)</td>
<td>873.95</td>
<td>817.90</td>
<td>689.23</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Values in each row having different letters are statistically different at P < 0.05 by Tukey mean comparison.

Corn varieties were from Pioneer Hi-Bred International Inc. (Johnston, IA) and Hyland Seeds (Blenheim, ON, Canada).

dRUP = estimated intestinal digestibility of RUP; TDP = total intestinally digestible feed protein.

Table 7. Differences in estimated intestinal digestibility and total available protein from fresh forage: Comparison of cool-season corn forage varieties

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pioneer</th>
<th>Hyland</th>
<th>SEM (n = 4)</th>
<th>Contrast (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dRUP (g/kg of RUP)</td>
<td>611.80</td>
<td>689.23</td>
<td>953.45</td>
<td>0.54</td>
</tr>
<tr>
<td>TDP (g/kg of CP)</td>
<td>873.95</td>
<td>817.90</td>
<td>689.23</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Values in each row having different letters are statistically different at P < 0.05 by Tukey mean comparison.

Corn varieties were from Pioneer Hi-Bred International Inc. (Johnston, IA) and Hyland Seeds (Blenheim, ON, Canada).

dRUP = estimated intestinal digestibility of RUP; TDP = total intestinally digestible feed protein.

silage by enhancing plant growth and maturation (Anderegg and Lichtenstein, 1981; Mahanna, 2010). The nutrient composition of this corn fresh forage contains adequate levels of carbohydrate and protein (Weiss et al., 1992; Taylor and Allen, 2005) for ensiling. The present findings demonstrate that cool corn fresh forage is comparable to other conventional forages in nutrient content and availability to animals (NRC, 2001).

CONCLUSIONS

Protein molecular structure studies showed that the amide I to amide II ratio ranged from 1.09 to 1.66 and that the α-helix to β-sheet ratio ranged from 0.95 to 1.01 in the new cool-season corn varieties, differences that may be related to nutrient digestion and availability. Energy content significantly differed among the new varieties. We found significant differences in the protein and carbohydrate subfraction profiles and in the ruminal degradation kinetics of OM, CP, starch, and NDF among the new varieties. The varieties had similar estimated intestinal digestibilities for rumen undegraded CP. The new cool-season corn varieties had different total MP values, ranging from 39 to 57 g/kg of DM. A variety effect on the nutritive and digestive properties of corn forage was found in new corn varieties grown in cooler weather. However, the nutrient content, degradability, and digestive characteristics of this corn fresh forage indicated that these varieties are nutritionally adequate as ruminant feed in their fresh forms and are likely also adequate after ensiling. Further study of the molecular structure profiles of cool-season corn in relation to nutrient utilization and availability in dairy cattle is necessary.

ACKNOWLEDGMENTS

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