Molecular basis of structural makeup of hulless barley in relation to rumen degradation kinetics and intestinal availability in dairy cattle: A novel approach

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ABSTRACT

To date, no study has been done of molecular structures in relation to nutrient degradation kinetics and intestinal availability in dairy cattle. The objectives of this study were to (1) reveal molecular structures of hulless barley affected by structural alteration using molecular spectroscopy (diffuse reflectance infrared Fourier transform) as a novel approach, and (2) quantify structure features on a molecular basis in relation to digestive kinetics and nutritive value in the rumen and intestine in cattle. The modeled feeds in this study were 4 types of hulless barley (HB) cultivars modified in starch traits: (a) normal starch cultivar, (b) zero-amylose waxy, (c) waxy, and (d) high-amylose. The molecular structural features were determined using diffuse reflectance infrared Fourier transform spectroscopy in the mid-infrared region (ca. 4,000–800 cm^{-1}) of the electromagnetic spectrum. The items assessed included infrared intensity attributed to protein amide I (ca. 1,715–1,575 cm^{-1}), amide II (ca. 1,575–1,490 cm^{-1}), α -helix (ca. 1,648–1,660 cm^{-1}), β -sheet (ca. 1,625–1,640 cm^{-1}), and their ratio, β -glucan (ca. 1,445–1,400 cm^{-1}), total carbohydrates (CHO; ca. 1,188–820 cm^{-1}) and their 3 major peaks, structural carbohydrates (ca. 1,277–1,190 cm^{-1}), and ratios of amide I to II and amide I to CHO. The results show that (1) the zero-amylose waxy was the greatest in amide I and II peak areas, as well as in the ratio of protein amide I to CHO among HB; (2) α -helix-to- β -sheet ratio differed among HB: the high-amylose was the greatest, the zero-amylose waxy and waxy were the intermediate, and the normal starch was the lowest; (3) HB were similar in β -glucan and CHO molecular structural makeup; (4) altered starch HB cultivars were similar to each other, but were different from the normal starch cultivar in protein molecular makeup; and (5) the rate and extent of rumen degradation of starch and protein were highly related

to the molecular structural makeup of HB. In conclusion, the molecular structural makeup on a molecular basis was related to rumen degradation kinetics and intestinal availability in dairy cattle. The alteration of starch structure in barley grain affects starch structure and the magnitude of protein and β -glucan contents, as well as the protein molecular structure of HB.

Key words: structural makeup, molecular spectroscopy, nutrient availability, hulless barley

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop produced in the world. The versatile composition of barley makes it suitable animal feed, malt, and food material. Worldwide, barley is predominantly used as feed (70%), with 20% used for malt, only 5% for food, and the remaining going toward miscellaneous uses (Wang, 2005). Canada is the third largest barley producer in the world, with an annual barley production close to 11 million tonnes (FAOSTAT, 2010). About 43% of the barley produced is exported, whereas 18% goes toward human consumption and industrial use, and the remainder (39%) is used as animal feed. Canada has locally adapted and registered 200 cultivars of barley (CFIA, 2009). Over 50 barley cultivars are produced in western Canada, including 8 hulless barley (HB) types, 13 malting types, and some others suitable for the livestock industry. Starch, the major storage compound in cereal endosperm, is composed of 2 distinct types of glucose polymers: amylose and amylopectin. Normal starch in grain consists of 750 to 850 g of amylopectin/kg and 150 to 250 g of amylose/kg (Ullrich et al., 1986). The concentration of amylopectin, amylose, and β -glucan influences the barley quality in both malting and the food industry (Hesselman and Thomke, 1982; Camm, 2008; Izydorczyk and Dexter, 2008). Amylopectin is a large branched polymer with α -1,4 linkages that serve as the backbone and α -1,6 bridges that serve as branching points (Zobel, 1988). Amylose is a mostly linear chain consisting of up to 3,000 glucose molecules interconnected primarily by

Received May 23, 2011.

Accepted June 20, 2011.

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α -1,4 glycosidic linkages (Takeda et al., 1993). Investigation of the effect of β -glucan on digestion and metabolism of barley in ruminants has been limited. Yet, β -glucan is positively related to barley grain hardness (Svihus et al., 2005) and this characteristic is assumed to benefit ruminants by slowing down starch degradation. Cattle in western Canadian feedlots are fed ad libitum with a ration containing up to 90% barley grain (John J. McKinnon, University of Saskatchewan, Saskatoon, SK, Canada, personal communication). Hulless barley was developed primarily for swine and poultry feeding (Bhatty, 1999) and characterized by the spontaneous loss of hulls during harvest (Yang et al., 1997). Hulless barley has a higher concentration of protein and starch, but lower fiber concentration than hulled cultivars (Edney et al., 1992; Ovenell-Roy et al., 1998; Ramsey et al., 2001). So far, very few studies have addressed the feed quality of HB (Zinn et al., 1996; Yang et al., 1997). The main concern with respect to feeding HB to ruminants is the rapid digestion in the rumen, resulting in increased incidences of digestive disorders such as acidosis, laminitis, and bloat (Zinn et al., 1996; Yang et al., 1997). Though, the hulless barley tested in these studies was all normal starch cultivar (Condor cultivar).

Recently, several new HB with altered starch, including cultivars with reduced (waxy and with high amylopectin) and elevated levels of amylose have been developed (Rossnagel et al., 2005) by the University of Saskatchewan Crop Development Centre as a part of its food barley breeding program using a pedigree breeding system. Although these HB were developed for food purposes, that does not necessarily limit other possibilities for these cultivars. Fluctuations in the crop industry will allow some crops to be used for multiple purposes rather than for a single purpose. For instance, in case of (a) higher economic opportunity for barley and its market price going down, food barley may serve as a better alternative for livestock producers as animal feed, or (b) lower quality of the grain due to environmental, mechanical, storage, and other factors, preventing the barley from meeting the requirements of food standards, could result in it being better utilized as animal feed. Hence, these new HB should be investigated for their potential to be a feed source. The variations in the molecular makeup and characteristics of starches substantially affect functional properties; therefore, waxy and or high-amylose barley have enormous potential for unique food and industrial applications (Izydorczyk and Dexter, 2008). It was hypothesized that (1) hulless barley with altered starch may have different intrinsic molecular structural makeup than the normal starch counterpart and (2) these structural makeups could be related to the rate and the extent of rumen

degradation and nutrient supply to animals. Therefore, the objectives of this study were to (1) reveal molecular structures of hulless barley affected by structural alteration (zero-amylose waxy, waxy, and high-amylose vs. normal starch HB) using diffuse reflectance infrared Fourier transform (**DRIFT**) molecular spectroscopy and (2) quantify structure features on a molecular basis in relation to digestive kinetics and nutritive value in the rumen and intestine in cattle. The parameters assessed included (1) protein amide I and protein amide II; (2) protein α -helix and β -sheet structure; (3) structural carbohydrates (**StCHO**); (4) total carbohydrates (**CHO**); (5) β -glucan content; and (6) the ratios of protein amide I to amide II, amide I to CHO, and α -helix to β -sheet of HB.

MATERIALS AND METHODS

Sample Preparation

Four cultivars of HB with a range of amylopectin (and amylose) were selected for the study: CDC McGwire is a normal starch cultivar, feed-type HB, whereas CDC Fibar, CDC Rattan, and HB08302 are zero-amylose waxy, waxy, and high-amylose, food-type HB cultivars, respectively. Amylopectin contents were 75, 100 (zero-amylose waxy), 95, and 60% (high-amylose) for the normal starch, zero-amylose waxy, waxy, and high-amylose cultivars, respectively (Rossnagel et al., 2005). Cultivars CDC McGwire, CDC Fibar, and CDC Rattan are 2-row head types, and HB08302 is a 6-row head type cultivar. Cultivar CDC McGwire is a widely cultivated feed-type HB in western Canada and is used as a reference by barley breeders. All cultivars were grown at the University of Saskatchewan research station (Saskatoon, SK, Canada). Potential yield averaged about 78, 98 (Rossnagel et al., 2005), and 92% (B. G. Rossnagel, University of Saskatchewan, Saskatoon, SK, Canada, personal communication,) of normal starch HB yield for zero-amylose waxy, waxy, and high-amylose cultivars, respectively. Samples were ground 3 times using a Retsch mill (Retsch ZM-1; Brinkmann Instruments of Canada Ltd., Mississauga, ON, Canada) through a 250- μ m screen.

DRIFT Molecular Spectroscopy

Diffuse reflectance infrared Fourier transform molecular spectroscopy is capable of exploring the biopolymer conformation through molecular and functional group spectral analyses (Doiron et al., 2009; Liu and Yu, 2010). Using DRIFT, diffusely reflected energy can be collected and a spectrum is produced that is related to the sample chemical composition (Griffiths and De Haseth,

1986; Stuart, 2004). The diffuse reflectance technique is usually used on samples that are powdered, or with irregular surfaces, or on nontransparent samples (Stuart, 2004). Therefore, DRIFT spectroscopy is particularly suitable for the examination of ground plant-based food and feed samples to reveal structural and chemical differences. Hence, it was selected to determine molecular structural makeup of HB.

Spectra Collections Using DRIFT Spectroscopy

Ground samples were mixed with KBr in a ratio of 1 part sample to 4 parts KBr in a 2-mL centrifuge tube and mixed by vortex for 10 s. Spectra from samples were then obtained using an FTS-40 Fourier transform vibrational infrared (FTIR) spectrometer (Bio-Rad Inc., Cambridge, MA) with diffuse-reflectance accessory, equipped with a water-cooled Mercury-Cadmium-Telluride detector and KBr beam splitter at the Saskatchewan Structural Sciences Centre, University of Saskatchewan. The spectra were recorded using the WinIR 3.6.0.5 software (Bio-Rad Inc.). Spectra were generated from the mid-infrared (IR; ca. 4,000–800 cm^{-1}) portion of the electromagnetic spectrum with 256 co-added scans and a spectral resolution of 4 cm^{-1} . Each sample spectrum was corrected by subtracting the corresponding background spectrum and displayed using the Kubelka-Munk (KM) function. Automatic baseline correction was applied. Poor-quality spectra with high noise or high absorbance (above 14 KM units), indicating detector saturation, were removed from the data set before statistical analysis and analysis was repeated until 8 spectra with acceptable quality were produced ($n = 8$).

Molecular Spectral Analysis

Spectral analysis was done with OMNIC software (Spectra Tech Inc., Oak Ridge, TN) 7.2. Protein amides I and II, CHO, StCHO, and β -glucan were identified according to published reports (Wetzel et al., 1998, 2003; Yu et al., 2010). The relative contribution of α -helix and β -sheet protein secondary structure to the protein amide I reflectance band was determined by means of the second derivative spectrum to locate the α -helix (ca. 1,655 cm^{-1}) and β -sheet (ca. 1,630 cm^{-1}) component peaks (Yu, 2006). Spectral data were analyzed using univariate molecular spectral analysis, described by Yu (2008), to clarify differences between HB in molecular structural makeup.

Univariate Molecular Spectral Analysis

The spectral data were analyzed based on IR reflectance peak area intensities attributed to (1) protein

amide I (IR region: ca. 1,715–1,575 cm^{-1} ; C = O and C-N stretching vibration) and molecular structural differences in the secondary structure profile of protein α -helix and β -sheet, (2) protein amide II (ca. 1,575–1,490 cm^{-1} , N-H bending and C-N stretching vibration), (3) CHO (ca. 1,188–820 cm^{-1} ; C-O stretching vibration) and its main peak areas, (4) StCHO (ca. 1,277–1,190 cm^{-1}), and (5) total β -glucan (ca. 1,445–1,400 cm^{-1} ; carboxylate anion's symmetric stretching vibration), to determine differences among the HB cultivars. The IR reflectance intensity peak area baselines were ca. 1,715 to 1,490 cm^{-1} ; ca. 1,180 to 820 cm^{-1} ; ca. 1,277 to 1,190 cm^{-1} ; and ca. 1,445 to 1,400 cm^{-1} for the protein amide I, amide II, α -helix, and β -sheet; CHO and their major peaks; StCHO; and β -glucan, respectively. The vibrational frequency of the protein amide I band is particularly sensitive to protein secondary structure (Jackson and Mantsch, 1995, 1996; Yu, 2007) and can be used to predict protein secondary structure. Typically, for α -helix, the protein amide I is in the range of ca. 1,648 to 1,660 cm^{-1} , whereas for β -sheet, the peak can be found within the range of ca. 1,625 to 1,640 cm^{-1} .

To estimate the peak intensity of α -helix and β -sheet of the protein molecular structures, 2 steps were applied. Briefly, the first step used Fourier self-deconvolution (FSD: a method for resolving intrinsically overlapped bands) and the second derivative function in OMNIC 7.2 to obtain the FSD and second derivative spectra in protein amide I region at ca. 1,720 to 1,575 cm^{-1} and then identified protein amide I component peak frequencies. The detailed concepts and algorithm of FSD were described by Kauppinen et al. (1981) and Griffiths and Pariente (1986). The second step was to quantify the intensity of peak height of α -helix and β -sheet and their ratio (Yu, 2006, 2007). Ratios between functional groups (components) were obtained by dividing the area under the one functional group reflectance peak by another compound's functional group reflectance peak area. Assessed ratios between functional groups included (1) amide I-to-II (ca. 1,715–1,575 cm^{-1} /ca. 1,575–1,490 cm^{-1}), (2) α -helix-to- β -sheet height ratio (ca. $\sim$ 1,655 cm^{-1} /ca. $\sim$ 1,630 cm^{-1}), (3) ratio of CHO 3 major peaks, (4) amide I-to-CHO (ca. 1,715–1,575 cm^{-1} /ca. 1,188–820 cm^{-1}), and (5) amide I-to-CHO third major peak.

Chemical Analysis

Dry matter, ash, crude fat, and CP contents were analyzed according to the procedure of AOAC (1990). Neutral detergent fiber content was determined according to Van Soest et al. (1991) with sodium sulfite and heat-stable α -amylase and expressed including residual ash. Acid detergent lignin (ADL) was determined ac-

cording to Van Soest et al. (1991). Acid detergent fiber content was determined according to the AOAC (AOAC, 1990; method 973.18) and expressed with the inclusion of residual ash. The NDF, ADL, and ADF procedures were adapted for use in an Ankom²⁰⁰ Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). The starch was analyzed using the Megazyme Total Starch Assay Kit (McCleary et al., 1997; Megazyme International Ltd., Wicklow, Ireland).

In Situ Rumen Degradation Kinetics and Prediction of the Protein Supply

Rumen degradation kinetics of CP were determined according to Ørskov and McDonald (1979) model. Rumen degradation kinetics of starch were determined following Tamminga et al. (1994). The potential nutrient supply from the HB was determined according to the Dutch protein evaluation system (DVE/OEB system; Tamminga et al., 1994). For each feed ingredient of diet, the DVE/OEB system (Tamminga et al., 1994) predicts 2 major outputs: (1) the truly digested and absorbed protein in the small intestine (DVE) and (2) the degraded protein balance (OEB). The first output includes (1) the truly absorbable rumen synthesized microbial protein in the small intestine, (2) truly absorbed rumen undegraded feed protein in the small intestine, and (3) endogenous protein losses. The second output reflects the balance between available N and energy in the rumen. This balance is critical to achieve efficient synthesis of microbial protein, which ultimately contributes to the post-ruminal pool of true protein. Chemical profile and in situ rumen degradation kinetics of HB are discussed in detail by Damiran and Yu (2010).

Statistical Analysis

Statistical analysis was performed using the MIXED procedure of SAS 9.2 (SAS Institute, 2003). Data were analyzed with a CRD model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij ; μ is the population mean for the variable; T_i is the effect of the HB cultivars, as a fixed effect; and e_{ij} is the random error associated with the observation ij . When a significant difference was detected ($P < 0.05$), means were separated using the Tukey-Kramer post hoc test. If data were unbalanced, the pooled standard error was calculated and reported. The Pearson correlations between structural makeup and (1) starch, (2) protein biodegradation kinetics, and (3) protein metabolic characteristics were analyzed using the PROC CORR of SAS.

RESULTS

Table 1 shows that the zero-amylose waxy had the largest ($P < 0.05$) protein amide I and II peak area and peak height (IR reflectance intensity, KM) among the cultivars, indicating higher concentration of protein in the zero-amylose waxy, revealed by the DRIFT spectroscopy. Although the protein amide I and II peak area ratio for the altered starch HB cultivars were similar ($P > 0.05$) to each other, these values were dramatically lower (5.3, 5.5, and 4.9 KM) than the HB with normal starch structure (11.7 KM). This indicates that altered starch HB were similar to each other, but different than normal starch cultivars in protein structural makeup. All 4 HB contained both α -helix and β -sheet. As shown in Table 2, zero-amylose waxy HB was the greatest (4.0 vs. 2.6, 2.6, 2.0 KM; $P < 0.05$) among

Table 1. Comparison of 4 hullless barley cultivars in the structural characteristics of protein amide I, amide II, and their ratios using diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy

Item ¹	Cultivar				SEM ²	P-value
	Normal starch (control)	Zero-amylose waxy	Waxy	High-amylose		
Protein amide I						
Peak area	160.8 ^a	256.8 ^b	121.8 ^a	109.8 ^a	15.49	<0.001
Peak height	2.5 ^a	3.9 ^b	2.5 ^a	1.9 ^a	0.31	0.001
Protein amide II						
Peak area	14.1 ^a	49.2 ^b	21.6 ^a	22.4 ^a	2.73	<0.001
Peak height	0.4 ^a	1.1 ^b	0.7 ^a	0.6 ^a	0.07	<0.001
Protein amide I and II area						
Peak area	174.9 ^a	306.0 ^b	143.4 ^a	132.2 ^a	18.03	<0.001
Ratio of protein amide I and II						
Peak area	11.7 ^a	5.3 ^b	5.5 ^b	4.9 ^b	0.44	<0.001

^{a,b}Means (n = 8) within a row with different superscript letters differ ($P < 0.05$).

¹Protein amide data are given in infrared reflectance Kubelka-Munk units; the protein peak baseline is ca. 1,715 to 1,490 cm^{-1} ; the protein amide I area region is ca. 1,715 to 1,575 cm^{-1} ; the protein amide II area region is ca. 1,575 to 1,490 cm^{-1} .

²SEM = pooled SEM.

Table 2. Characteristics of protein secondary structure α -helix, β -sheet, and their ratios from 4 hullless barley cultivars, revealed using diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy

Protein molecular structure ¹	Cultivar				SEM ²	P-value
	Normal starch (control)	Zero-amylose waxy	Waxy	High-amylose		
α -helix structure	2.6 ^a	4.0 ^b	2.6 ^a	2.0 ^a	0.32	0.001
β -sheet structure	1.9 ^{ab}	2.7 ^a	1.7 ^b	1.2 ^b	0.23	0.001
α -helix-to- β -sheet ratio	1.4 ^a	1.5 ^b	1.6 ^c	1.7 ^d	0.03	<0.001

^{a-d}Means (n = 8) within a row with different superscript letters differ ($P < 0.05$).

¹The data units are infrared reflectance Kubelka-Munk units; the peak baseline is ca. 1,715 to 1,575 cm^{-1} ; the α -helix peak height center is $\sim 1,655 \text{ cm}^{-1}$; the β -sheet peak height is $\sim 1,630 \text{ cm}^{-1}$.

²SEM = pooled SEM.

the HB in α -helix structure. Furthermore, zero-amylose waxy (2.7) was similar ($P > 0.05$) to normal starch cultivar (1.9), but was greater ($P < 0.05$) than waxy (1.7) and high-amylose (1.2) in β -sheet peak height. However, normal starch, waxy, and high-amylose were not different ($P > 0.05$) from each other in β -sheet IR peak height. Differences in the ratio of α -helix to β -sheet were observed ($P < 0.05$) among HB cultivars; high-amylose was the greatest (1.7), zero-amylose waxy (1.5) and waxy (1.6) were intermediate, and normal starch was the lowest (1.4).

Infrared reflectance intensity peak area values of CHO were 1,307.0; 1,518.8; 1,179.6; and 1,408.8 KM, for the normal starch, zero-amylose waxy, waxy, and high-amylose HB, respectively (Table 3); although the ranges were large among HB, they were not significantly different ($P = 0.197$). Accordingly, the component heights of all 3 CHO major peaks and IR areas of the

second and third major peak components were similar ($P > 0.05$) among HB. Yet, the peak area of the first major peak component of CHO was larger ($P < 0.05$) in zero-amylose waxy compared with that of waxy cultivar. Furthermore, zero-amylose waxy, normal starch, and high-amylose cultivars were similar ($P > 0.05$); likewise normal starch, waxy, and high-amylose were similar ($P > 0.05$) to each other in the IR reflectance peak area size of the first peak of the CHO component.

As Table 4 shows, a difference was detected ($P = 0.050$) among HB in IR reflectance peak area, attributed to structural carbohydrates: waxy was lower ($P < 0.05$) than high-amylose HB, although no difference was detected ($P = 0.813$) in structural carbohydrate IR intensity peak height among HB cultivars. Conversely, both IR reflectance peak area and peak height attributed to β -glucan differed ($P < 0.05$) among HB cultivars. Waxy and high-amylose cultivars were the greatest (P

Table 3. Comparison of 4 hullless barley cultivars in the molecular structural characteristics of total carbohydrates (CHO) in terms of the component peak area and height and their ratios using diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy

Item ¹	Cultivar				SEM ²	P-value
	Normal starch (control)	Zero-amylose waxy	Waxy	High-amylose		
CHO						
Peak area	1,307.0	1,518.8	1,179.6	1,408.8	113.59	0.197
Peak height	8.2	10.2	9.2	8.6	1.04	0.560
Carbohydrate component						
First peak						
Peak area	235.1 ^{ab}	287.9 ^a	212.3 ^b	262.1 ^{ab}	17.92	0.031
Peak height	6.3	7.9	7.1	7.0	0.75	0.496
Second peak						
Peak area	463.1	579.5	438.9	545.6	37.68	0.040
Peak height	8.2	10.2	9.2	8.6	1.04	0.553
Third peak						
Peak area	559.0	632.8	496.9	575.4	57.01	0.410
Peak height	7.9	9.4	8.6	8.0	1.02	0.708

^{a,b}Means (n = 8) within a row with different superscript letters differ ($P < 0.05$).

¹The data units are infrared reflectance Kubelka-Munk units; the CHO peak baseline and region is ca. 1,188 to 820 cm^{-1} ; the barley CHO has 3 major component peaks with the first, second, and third peak centered at ca. $\sim 1,150$, $\sim 1,080$, and $\sim 1,025 \text{ cm}^{-1}$, respectively. The first, second, and third peak regions are ca. $\sim 1,188$ to 1,130, $\sim 1,130$ to 1,063, and $\sim 1,063$ to 954 cm^{-1} , respectively.

²SEM = pooled SEM.

Table 4. Comparison of 4 hulless barley cultivars in the molecular structural characteristics of structural carbohydrates and β -glucan using diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy

Item ¹	Cultivar				SEM ²	<i>P</i> -value
	Normal starch (control)	Zero-amylose waxy	Waxy	High-amylose		
Structural carbohydrate						
Peak area	21.3 ^{ab}	21.6 ^{ab}	17.1 ^b	24.3 ^a	1.76	0.050
Peak height	0.6	0.7	0.6	0.7	0.06	0.813
β -glucan						
Peak area	10.6 ^a	10.8 ^a	14.1 ^{ab}	15.8 ^b	1.01	0.009
Peak height	0.4 ^a	0.4 ^{ab}	0.5 ^{bc}	0.6 ^c	0.04	0.001

^{a-c}Means ($n = 8$) within a row with different superscript letters differ ($P < 0.05$).

¹The data units are infrared reflectance Kubelka-Munk units; the structural carbohydrate peak baseline and region is ca. 1,277 to 1,190 cm^{-1} ; the β -glucan peak baseline and region is ca. 1,445 to 1,400 cm^{-1} .

²SEM = pooled SEM.

< 0.05), but zero-amylose waxy was similar ($P > 0.05$) to normal starch in IR reflectance peak height associated with β -glucan. The same trend with the latter was observed on β -glucan peak area; the only exception was that waxy HB was numerically greater (14.1 vs. 10.6 KM, $P > 0.05$) than normal starch cultivar.

Table 5 shows ratios of major peaks of CHO and the ratio of amide I to CHO peak area. The peaks and ratios associated with the CHO regions of the three major components did not differ ($P > 0.05$) among HB cultivars, indicating similarity in CHO molecular structural makeup between these HB cultivars. With regard to the amide I-to-CHO ratio, zero-amylose waxy showed the highest (0.17 vs. 0.12, 0.10, 0.08; $P < 0.05$) value, which was probably due to the greater protein amide I peak area of zero-amylose waxy HB. The ratio of protein amide I to the third major peak of CHO (starch) is considered one of the important parameters in evaluating the nutritive value of grains. Differences were detected among HB in the amide I-to-CHO third

peak ratio: zero-amylose waxy was the greatest ($P < 0.05$) and high-amylose was the lowest ($P < 0.05$), but waxy was similar ($P > 0.05$) as compared with normal starch HB.

DISCUSSION

Typically, for the spectral analysis, differences of protein amide I and amide II infrared intensity in terms of peak area or height indicate quantitative differences of functional groups (or contents), whereas their ratios demonstrate differences in protein molecular structural makeup between biological tissues (Yu et al., 2010). Consequently, the univariate analysis in the current study suggested that all 3 of the altered starch HB are similar to each other, but differ from normal starch HB in protein molecular structural makeup. As reported previously, the highest protein amide I to amide II ratio was found in normal starch HB. Furthermore, the correlation analysis showed (Table 6) the decreas-

Table 5. Comparison of 4 hulless barley cultivars in the structural characteristics of protein, total carbohydrates (CHO), and their ratios using diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy

Item ¹	Cultivar				SEM ²	<i>P</i> -value
	Normal starch (control)	Zero-amylose waxy	Waxy	High-amylose		
Ratio of CHO peak 1 to peak 2 (~1,188–1,130/~1,130–1,063 cm^{-1})	0.51	0.50	0.48	0.48	0.013	0.3957
Ratio of CHO peak 1 to peak 3 (~1,188–1,130/~1,063–954 cm^{-1})	0.43	0.47	0.44	0.46	0.022	0.5602
Ratio of CHO peak 2 to peak 3 (~1,130–1,063/~1,063–954 cm^{-1})	0.84	0.94	0.91	0.95	0.036	0.1694
Ratio of amide I to CHO (~1,715–1,575/~1,188–818 cm^{-1})	0.12 ^a	0.17 ^b	0.10 ^{ac}	0.08 ^c	0.007	< 0.0001
Ratio of amide I to CHO peak 3 (~1,715–1,575/~1,063–954 cm^{-1})	0.29 ^a	0.42 ^b	0.25 ^{ac}	0.19 ^c	0.020	< 0.0001

^{a-c}Means ($n = 8$) within a row with different superscript letters differ ($P < 0.05$).

¹The protein peak baseline is ca. 1,715 to 1,490 cm^{-1} ; the carbohydrate peak baseline is 1,188 to 820 cm^{-1} .

²SEM = pooled SEM.

ing protein amide I-to-amide II ratio with decreasing rate of starch degradability ($r = 0.92$, $P = 0.01$) in the rumen and decreasing degraded protein balance ($r = -0.72$, $P = 0.045$; this means decreased N limitation to animals). Usually, barley starch sources that are less rapidly degraded in the rumen are preferable for ruminant feeding because they are less likely to cause an accumulation of acidic fermentation products, which are detrimental to the rumen function (Yang et al., 1997). Concurrently, the results of the present study indicate that altered starch HB seem more favorable to ruminants by the magnitude of the amide I-to-amide II ratio than normal starch HB.

In addition, the magnitudes of the ratio of α -helix to β -sheet of HB determined in the current study were in agreement with Yu (2007), who found the ratio of α -helix to β -sheet ranging from 1.4 to 2.0 for hulled barley. As noted by others (Yu, 2007), the differences of the protein α -helix and β -sheet structures affecting access to gastrointestinal digestive enzymes may result in differences in protein value and protein availability. Interestingly, results of the current study revealed that these HB differed in protein secondary structure conformation in terms of the α -helix and β -sheet and their ratio, indicating that the alteration of starch of barley through breeding using a pedigree breeding system does not only change the grain protein amide I-to-amide II ratio, but it further affects the ratio of α -helix to β -sheet within protein amide I of the molecular structural makeup. In regard to the ratio of α -helix to β -sheet, our results were dissimilar to those of Walker et al. (2009), who found no differences in the ratio of α -helix to β -sheet between the hulled normal starch barley cultivars. The different findings obtained by these 2 studies could be explained partly by the differences in starch structures of barley cultivars. As Table 6 shows, the ratio of α -helix to β -sheet was correlated negatively with the rate of starch degradation ($r = -0.75$, $P = 0.032$) and truly absorbed protein in the small intestine ($r = -0.76$, $P = 0.03$), suggesting that protein secondary structure differences among barley cultivars may affect protein utilization and availability in the rumen and the intestine in dairy cows.

As reported previously, both multivariate analyses indicated that very little difference was detected in carbohydrate molecular structural makeup between normal starch HB and altered starch HB using DRIFT spectroscopy, even though these cultivars differ in amylopectin-to-amylose ratio in the starch content. Furthermore, we found that the amylopectin-to-amylose ratio of HB had (data not shown) very strong correlation with IR intensity peak height attributed to protein amide I ($r = 0.92$; $P = 0.001$), protein amide I-to-CHO ($r = 0.87$; $P = 0.005$), and protein amide I-to-CHO

Table 6. Pearson correlation analysis between the structural characteristics of protein amide I and II, protein secondary structure, total carbohydrates (CHO), and their ratios and rumen degradation kinetics and predicted protein supply for dairy cows of 4 hullless barley cultivars¹

Item	K _d ST		EDST		K _d CP		EDCP		DVE		OEB	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Protein amide I												
Peak area	0.12	0.774	-0.36	0.384	0.14	0.740	0.90	0.002	0.83	0.010	0.67	0.068
Peak height	-0.04	0.927	-0.57	0.140	0.06	0.884	0.93	0.001	0.87	0.005	0.72	0.045
Protein secondary structure profile												
α -Helix	-0.05	0.908	-0.58	0.134	0.06	0.883	0.93	0.001	0.87	0.005	0.72	0.042
β -Sheet	0.16	0.698	-0.46	0.257	0.02	0.963	0.85	0.008	0.93	0.001	0.55	0.161
Ratio of α -helix to β -sheet	-0.75	0.032	-0.11	0.799	0.12	0.776	-0.23	0.576	-0.76	0.030	0.23	0.588
Carbohydrate												
Peak area	-0.01	0.981	0.08	0.853	0.40	0.322	0.65	0.079	0.22	0.599	0.71	0.049
Peak height	-0.54	0.169	-0.82	0.013	0.07	0.865	0.90	0.002	0.58	0.135	0.92	0.001
Ratio of protein amide I to amide II												
Peak area	0.92	0.001	0.60	0.115	-0.12	0.776	-0.35	0.395	0.24	0.570	-0.72	0.045
Ratio of protein amide I to CHO component peak area	0.20	0.644	-0.40	0.331	0.05	0.900	0.85	0.007	0.92	0.001	0.56	0.153
β -Glucan												
Peak area	-0.62	0.101	0.03	0.936	0.04	0.928	-0.49	0.213	-0.87	0.005	-0.06	0.884
Peak height	-0.65	0.080	0.01	0.981	0.06	0.881	-0.44	0.280	-0.85	0.008	0.01	0.986

¹The structural characteristics include protein amide I and II, carbohydrates, and their ratios in the hullless barley, revealed using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy; K_dST = degradation rate of starch; EDST = effective degradability of starch; K_dCP = degradation rate of protein; EDCP = effective degradability of protein; DVE = truly absorbed protein in the small intestine; OEB = degraded protein balance in the DVE/OEB system.

third peak (starch; $r = 0.91$; $P = 0.002$). These results suggest that the increasing amylopectin-to-amylose ratio in the starch granule corresponds to the increasing protein-to-starch ratio of the grain granule.

Typically, as a previous study (Izydorczyk and Dexter, 2008) found, barley cultivars with altered starch contain a higher content of β -glucan than normal starch barley. The same trend was observed for β -glucan content in the current study using the DRIFT technique. Nonetheless, as multivariate analyses revealed, these 4 HB cultivars were similar in β -glucan molecular intrinsic structure. Published information on β -glucan content and its effects on feed degradation and nutrient supply in ruminants are rare. Still, as others found (Bhatti, 1999; Camm, 2008), HB with greater β -glucan content tended to remain in larger pieces after mechanical processing. Simultaneously, several studies have shown that the larger particle of grain facilitates slower degradation of feed in the rumen (McAllister et al., 1993; Surber et al., 2000; Zheng et al., 2000). This means HB with greater β -glucan content is expected to degrade more slowly in the rumen. Our results were partly in agreement with the latter; as Table 6 showed, the β -glucan tended to be correlated negatively with the rate of starch degradability ($r = -0.65$, $P = 0.080$).

Protein within the endosperm tissue of the cereal grain is arranged in a matrix that surrounds and protects the starch granules, making them less available for digestion by rumen bacteria (McAllister et al., 1993). Likewise, a greater protein-to-starch ratio (i.e., more protein per unit of starch) may be indicative of a greater degree of protection of starch granules within the protein matrix, resulting in a lower rate and extent of rumen degradation (Yu et al., 2004). Hence, based on the amide I-to-CHO ratio value obtained in the current study, more protein would have surrounded CHO in zero-amylose waxy, but less in waxy and high-amylose than in normal starch HB, which would directly affect starch degradation. However, a weaker correlation between protein amide I-to-CHO ratio and the effective degradability of starch (Table 6: $r = -0.40$, $P = 0.331$) was observed, which is probably explained by the barley degradation characteristics and nutrient supply likely dictated by not only the protein-to-starch ratio, but other interrelated factors such as effects of the protein matrix, anti-nutritional factors (Yu, 2006, 2007), starch structure, β -glucan, lipid-starch complexes (Svihus et al., 2005), and processing technique (Bhatti, 1999; Camm, 2008) on the barley feed quality, fermentation, and degradation behavior.

CONCLUSIONS

In conclusion, (1) significant differences between the 4 HB in protein molecular structural makeup were

identified; (2) no significant difference in total and structural carbohydrate molecular structural makeup was detected; (3) zero-amylose waxy HB had the greatest IR intensity peak area and height for protein amide I and II, as well as the highest ratio of protein amide I to CHO among the HB; (4) cultivars with altered starch were lower than their normal starch counterpart in protein amide I-to-II ratio; (5) all starch-altered HB cultivars were greater in α -helix to β -sheet height ratio than the normal starch cultivar; and (6) altered starch cultivars were greater in β -glucan than the normal starch cultivar. Still, further studies are needed to quantify the relationships and fully understand differences between the molecular structural makeup of HB cultivars and correlate these findings with the nutritive value and degradation or digestive behavior of various cultivars of feeds.

ACKNOWLEDGMENTS

We are grateful to Brian Rossnagel and Aaron Beattie, Crop Development Centre, the University of Saskatchewan (Saskatoon, SK, Canada) for providing us with barley samples. The authors also thank Ken Thoms (Saskatchewan Structural Sciences Centre, University of Saskatchewan) and Zhi Yuan Niu (Department of Animal and Poultry Science, University of Saskatchewan) for their support during the laboratory analysis. The authors thank the ADF-SRP Feed Research Chair Program, Natural Sciences and Engineering Research Council of Canada (NSERC), and Agriculture Development Fund (ADF)-Saskatchewan for financial support of feed research programs.

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