Differentiation of mixtures of co-product blend with barley grain based on Fourier transform infrared attenuated total reflection molecular spectroscopy: Carbohydrate molecular spectral profiles and nutritive characteristics in dairy cattle

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ABSTRACT

The objectives of this study were to (1) differentiate mixtures of a co-product blend (70:30% mixture of wheat:corn before bioethanol fermentation) from bioethanol processing with barley grain based on Fourier transform infrared attenuated total reflection molecular spectroscopy with cluster analysis, (2) elucidate the effects of the co-product blend on the molecular structure spectral profile and nutritive characteristics of the hull-less barley carbohydrate (CHO) in dairy cattle, and (3) quantify the relationship between the CHO molecular structure spectral profiles and CHO nutritive characteristics of the mixtures in dairy cattle. The parameters assessed for CHO molecular structure spectral profiles included molecular spectral peak area and height intensities of lignin, structural CHO (STCHO), cellulosic compounds, and total CHO. The parameters assessed for CHO nutritive characteristics included the CHO nutrient profiles, CHO subfractions, energy values, and rumen-fermentable organic matter supply. Hull-less barley grains and the co-product blend were mixed according to the ratio of 100:0 (B100), 75:25, 50:50, 25:75, and 0:100 (BD100). The results showed that when the co-product blend was included at the different ratios, the predicted rumen-fermentable organic matter supply in the rumen was significantly decreased from 632 (B100) to 518 g/kg of dry matter (DM; BD100). The effective degradable dry matter in the rumen and the effective degradable starch in the rumen were also significantly decreased from 672 and 482 g/kg of DM (B100) to 475 and 43 g/kg of DM (BD100), respectively. By using Fourier transform infrared attenuated total reflection spectroscopy, we identified were significant changes in the spectral profiles of the CHO molecular structure in

terms of lignin, STCHO, cellulosic compounds, and total CHO spectral peak area and height and their ratios. Pearson analysis results suggested strong correlations between lignin peak area, cellulosic compound area, and total CHO peak area and CHO nutrient profiles except energy values (net energy for lactation at 3 times maintenance intake, net energy for maintenance, and net energy for gain). No correlation was observed between STCHO peak area and CHO nutrient profiles in the mixture samples.

Key words: carbohydrate molecular structure spectral profile, nutritive characteristic of carbohydrate, feed mixture

INTRODUCTION

Hull-less barley has been developed primarily for swine and poultry feeding (Bhatti, 1986, 1999). With alteration of the carbohydrate (CHO) molecular structure, such as the amylose-to-amylopectin ratio and β -glucan contents (Damiran and Yu, 2010, 2012), hull-less barley can be utilized more efficiently by dairy cattle. Hull-less barley is characterized by the spontaneous loss of hulls during harvest (Yang et al., 1997). It has greater concentrations of protein and starch but lower fiber concentration than hulled cultivars (Ramsey et al., 2001). Therefore, it has a very high degradation rate and extent, which may cause subacute rumen acidosis, laminitis, and bloats in the feedlot cattle (Zinn et al., 1996; Yang et al., 1997). However, with alterations of the CHO structure conformation, the nutrient availability of hull-less barley could be improved (Damiran and Yu, 2010, 2012).

The co-products from bioethanol processing usually contain high RUP and low degradation rate (Nuez-Ortín and Yu, 2009, 2010a,b). It is expected that when the bioethanol co-products are added to hull-less barley, the rumen degradation and nutritive characteristics of both protein and CHO in hull-less barley will be more favorable to the dairy cow. The hourly effective N-to-energy ratio will be more synchronized in dairy

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cows and provide close to an optimal degradation ratio (25 to 32 g of N/kg of CHO; Tamminga et al., 1994; Nuez-Ortín and Yu, 2010b). The favorable N-to-energy ratio will have the potential to decrease the incidence of rumen subacute acidosis in dairy cattle.

Recently studies showed that Fourier transform infrared attenuated total reflection (**FT/IR-ATR**) molecular spectroscopy is a powerful analytical tool to link feed chemistry information to molecular structure information and can be used to study feeds and nutrition on a molecular basis (Doiron et al., 2009; Yu, 2010; Yu et al., 2009, 2011; Jonker et al., 2012). Previous studies have shown correlations between the spectral profiles of feed molecular structure and their nutrient characteristics (Yu, 2012; Abeysekara et al., 2011). Zhang and Yu (2012) reported the spectral profile of the protein molecular structure in relation to protein availability in the co-product blend and barley. To date, no study has examined the effects of inclusion rate of the co-product blend on the spectral profiles of the CHO molecular structure in relation to CHO nutrient utilization of the hull-less barley in dairy cattle.

The hypothesis of this study was that by integrating hull-less barley grain with bioethanol co-products, the CHO utilization [in terms of fermentable OM (**FOM**) and energy supply in the rumen] of the barley could be improved. The N-to-energy synchronization ratio could be more optimal. The nutritive characteristics of CHO were associated with CHO molecular spectral profiles in the mixtures. The objectives of this study were to (1) elucidate the effects of inclusion rate of the co-product blend on the spectral profiles of the molecular structural and nutritive characteristics of the CHO in hull-less barley for dairy cattle; (2) differentiate mixtures of the co-product blend with barley grain based on FT/IT-ATR molecular spectroscopy with cluster analysis; and (3) quantify the relationship between spectral profiles of the CHO molecular structure and CHO nutritive characteristics of the mixtures in dairy cattle.

MATERIALS AND METHODS

Hull-less Barley and Co-product Blend Mixtures

In this study, CDC McGwire hull-less barley with normal starch traits (Damiran and Yu, 2010) and a co-product blend from bioethanol processing (a blend of distillers dried grains with solubles: 70:30% mixture of wheat:corn before bioethanol fermentation) were mixed at 5 different ratios: 100:0 (**B100**), 75:25 (**B75BD25**), 50:50 (**B50BD50**), 25:75 (**B25BD75**), and 0:100 (**BD100**), respectively. Before FT/IR-ATR spectral determination and chemical analysis, all samples were ground to pass through a 1-mm screen (Retsch ZM-

1; Brinkmann Instruments Ltd., Mississauga, ON, Canada).

FT/IR-ATR Spectroscopic Investigations

The FT/IR-ATR spectroscopic experiments were conducted at the University of Saskatchewan. The molecular spectral data were collected from the feed mixtures samples by using a Jasco FT/IR-ATR-4200 instrument (Jasco Corporation, Tokyo, Japan). Thirty-two scans per sample were collected in the midinfrared range from 4,000 to 700 cm^{-1} in transmission mode at a spectral resolution of 4 cm^{-1} . The collected spectra were corrected against air as the background (Jonker et al., 2012).

Univariate CHO Molecular Spectral Analysis

The software OMNIC 7.2 (Spectra Tech, Madison, WI) was used for spectral data analysis. The spectral parameters of the CHO molecular structure, including the lignin, structural CHO (**STCHO**), cellulosic compounds, and total CHO peak area and height, as well as their ratios, were studied (Figure 1). Chemical function groups were identified according to published reports (Himmelsbach et al., 1998; Wetzel et al., 1998; Wetzel, 2001; Marinkovic and Chance, 2006; Yu et al., 2011). The spectral region of analysis in this study was approximately 1,539 to 946 cm^{-1} . The region and baseline of the lignin peak area and height were approximately 1,539 to 1,504 cm^{-1} . For the STCHO, which is mainly associated with hemicellulosic and cellulosic compounds, the peak area and height region and baseline were approximately 1,485 to 1,186 cm^{-1} , and 3 subpeaks were found in this region, whose heights were 1,415, 1,370, and 1,240 cm^{-1} , respectively. The peak area region mainly associated with cellulosic compounds was at approximately 1,272 to 1,186 cm^{-1} (height centered at approximately 1,240 cm^{-1}). The peaks area region mainly associated with CHO was at approximately 1,186 to 946 cm^{-1} . Again, 3 subpeaks were found in this region, with peak area regions being approximately 1,186 to 1,132, 1,132 to 1,066, and 1,066 to 946 cm^{-1} and the peak centers at 1,152, 1,080, and 1,025 cm^{-1} , respectively. Different ratios were calculated according to the respective absorbance intensity values.

Multivariate CHO Molecular Spectral Analysis

The FT/IR-ATR CHO fingerprint spectral data of hull-less barley and the co-product blend mixtures were analyzed by agglomerative hierarchical cluster analysis and principal components analysis using Statistica 8.0 software (StatSoft Inc., Tulsa, OK). These results were

Figure 1. Fourier transform infrared attenuated total reflection molecular spectra of carbohydrate (CHO) molecular structures of the mixtures of a bioethanol processing co-product blend with barley grain, obtained using a Jasco FT/IR-ATR 4200 instrument (Jasco Corporation, Tokyo, Japan; spectral region: $\sim 1,539\text{--}946\text{ cm}^{-1}$). Mixtures: B100 = 100% barley; B75BD25 = 75% barley + 25% distillers dried grains with solubles (DDGS) blend; B50BD50 = 50% barley + 50% DDGS blend; B25BD75 = 25% barley + 75% DDGS blend; BD100 = 100% DDGS blend. STCHO = structural CHO. Color version available in the online PDF.

able to classify and distinguish inherent CHO structural differences among the different mixtures, and then identify the main sources of variation within the lignin, STCHO, and CHO fingerprint spectra ($\sim 1,539$ to 946 cm^{-1} ; Yu, 2005).

Animals and Diets

The experiment protocol was reviewed and approved by the Animal Care Committee of Saskatchewan and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993). Three nonlactating Holstein cows with flexible rumen cannulas (10 cm i.d., Bar Diamond Inc., Parma, ID) were used in this study to determine the in situ degradation kinetics of the mixtures of hull-less barley and co-product blend. The cows were fed 20.5 kg/d of a TMR (barley silage:standard dairy concentrate was 78:22%) according to NRC (2001) recommendations. The concentrate consisted of 63.9% rolled barley,

35.0% standard dairy supplement (containing barley, wheat, oats, dairy supplement pellets, and molasses), 1.0% fresh dairy cow supplement [containing barley, oats, canola meal, soybean meal, wheat distillers dried grains with solubles, corn gluten meal, molasses, dried fat supplement (golden flakes), canola oil, minerals, and vitamins], and 0.1% mold control. The cows were housed in the animal nutritive experimental station, University of Saskatchewan, and fed half of the ration twice daily at 0800 and 1600 h. Fresh, clean water was supplied ad libitum.

Rumen In Situ Incubation

According to the in situ method (Yu et al., 2003), approximately 7 g of each mixture sample (ground to pass through a 0.5-mm screen) was placed into each numbered nylon bag (Nitex 03-41/31 monofilament open mesh fabric; Screentec Crop., Mississauga, ON, Canada) measuring $10 \times 20\text{ cm}$ with a pore size of

approximately 40 μm . The bags were added according to the gradual addition-all out schedule. The multiple bags for each treatment at each incubation time in each experimental run were 2, 2, 2, 3, 3, and 4 bags for incubation times 0, 4, 8, 12, 24, and 48 h, respectively. Treatments were randomly assigned to 3 nonlactating cows in 2 experimental runs. After incubation, the bags were taken out of the rumen and, including those samples for 0 h, washed with cold water several times, and then dried at 55°C for 48 h. The dried samples were stored in a refrigerated room (4°C) until chemical analysis.

Rumen degradation characteristics of starch were determined using the NLIN procedure of SAS v9.2 (SAS Institute, 2005) based on the first-order kinetics equations (Ørskov and McDonald, 1979; Tamminga et al., 1994):

$$R(t) = D \times \exp^{(-K_d \times t)},$$

where $R(t)$ is the residue of the incubated material after t h of rumen incubation (%); D (in %) is the undegradable and potentially degradable fractions, respectively; and degradation rate (K_d) is the percentage rate per hour.

Chemical Analysis

Dry matter, ash, ether extract (**EE**), and CP were measured according to the method of the Association of Official Analytical Chemists (AOAC, 1990). For starch determination, samples were ground to pass through a 0.5-mm screen and analyzed using the method of the Megazyme Total Starch Assay Kit (Megazyme International, Bray, Co. Wicklow, Ireland) and by the α -amylase/amyloglucosidase method (McCleary et al. 1997). Neutral detergent fiber, ADF, and ADL were analyzed according to the filtration method (Van Soest et al., 1991). Total CHO were calculated as $\text{CHO} = 100 - \text{EE} - \text{CP} - \text{ash}$. Similarly, other CHO were calculated as $\text{NFC} = 100 - (\text{NDF} - \text{NDICP}) - \text{EE} - \text{CP} - \text{ash}$ (where NDICP is neutral detergent-insoluble CP), hemicellulose = $\text{NDF} - \text{ADF}$, and cellulose = $\text{ADF} - \text{ADL}$ (NRC, 2001).

CHO Subfraction Partitioning

Carbohydrate fractions of the mixtures were partitioned into CA, CB₁, CB₂, and CC (Sniffen et al., 1992; Fox et al., 2007). Fraction CA was composed of sugars and organic acids, which had a rapid degradation rate of 300%/h, and was calculated as NFC minus CB₁ ($\text{CA} = \text{NFC} - \text{CB}_1$); CB₁ included starch and pectin, which

had an intermediate degradation rate of 20 to 50%/h ($\text{CB}_1 = \text{starch} + \text{pectin}$); CB₂ was digestible cell walls, which had a slow degradation rate of 2 to 10%/h and equaled $\text{NDF}_{\text{n-free}}$ minus CC [$\text{CB}_2 = (\text{NDF} - \text{NDICP}) - \text{CC}$]; CC was unavailable cell walls, which were unavailable CHO in the rumen and were calculated by lignin [$\text{CC} = (\text{lignin} \times 2.4)/100$].

Estimation of Truly Digestible Nutrients and Energy Values

Truly digestible NDF (**tdNDF**), truly digestible NFC (**tdNFC**), and TDN at 1 $\times$ maintenance (**TDN_{1x}**) as well as energy values (digestible energy, ME, net energy) were determined using the NRC summary approach (NRC, 1996, 2001).

Statistical Analysis

CHO Molecular Structure Spectral Profiles of FT/IR-ATR, Rumen Degradation Kinetics, and Predicted Nutrient Supply. Statistical analysis was performed using the MIXED procedure of SAS 9.2 (SAS Institute, 2005) using a complete randomized design. The model used for analysis was $Y_{ij} = \mu + a_i + \varepsilon_{ij}$, where Y_{ij} is an observation of the dependent variable ij ; μ is the population mean for the variable; a_i is the effect of the treatments (5 mixtures) as a fixed effect; and ε_{ij} is the random error associated with observation ij . The polynomial contrasts of linear, quadratic, and cubic effects were carried out using the mixed-methods procedure (PROC MIXED) with the contrast statement in SAS.

Correlation Analysis. The relationship between changes in the CHO molecular structure spectral profile and their ratios and CHO chemical and nutrient profiles, Cornell Net Carbohydrate and Protein System CHO fractions (Sniffen et al., 1992), in situ degradation kinetics, and predicted FOM supply to dairy cattle among the 5 mixtures were analyzed using PROC CORR of SAS v9.2 (Liu and Yu, 2010).

For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Treatment means were compared using Tukey's test.

RESULTS AND DISCUSSION

Differences in CHO Molecular Spectral Profiles Among the Mixtures

Univariate CHO Molecular Spectral Analysis. The differences in CHO spectral makeup among the hull-less barley and co-product blend mixtures were revealed by Jasco FT/IR-ATR spectroscopy and OM-

NIC 7.2 software (Table 1). The results showed that the lignin peak centered at approximately $1,510\text{ cm}^{-1}$ and the peak area and height intensities of lignin were increased significantly ($P < 0.01$) from 0.057, 0.003 (B100) to 0.141, and 0.006 infrared absorbance units (BD100), respectively (Table 1). The lignin peak at approximately $1,510\text{ cm}^{-1}$ is mainly due to aromatic ring stretching in the lignin biopolymer (Himmelsbach et al., 1998). The peak area intensity of STCHO was decreased significantly in a quadratic fashion ($P < 0.05$), from 5.185 to 4.524 infrared absorbance units (Table 1). Normally, 3 relatively unique subpeaks were located in the STCHO region, with the peak I, II, and III centers at approximately 1,415, 1,370, and $1,240\text{ cm}^{-1}$, respectively (Wetzel et al., 1998). The intensities of STCHO peaks I and III were relatively increased in a linear or quadratic way, whereas the intensity of STCHO peak II was relatively decreased in a linear way ($P < 0.01$) with an increasing level of co-product blend. The peak area and height intensities of cellulosic compounds were linearly increased ($P < 0.01$), whereas all the subpeak area and height intensities in the total CHO region were linearly decreased ($P < 0.01$). In addition, the ratios of functional groups were linearly changed ($P < 0.01$) among the mixtures (Table 1). In the literature, reports are limited on the CHO molecular spectral features in feed mixtures (Yu, 2012), so no comparison could be made. The results from this study indicated that molecular spectroscopy is sensitive to picking up the CHO molecular structural changes in the feed mixtures. Whether these CHO molecular structural changes were associated with nutritive characteristics of CHO in dairy cattle needs to be investigated in a future study.

Multivariate CHO Molecular Spectral Analysis. Figures 2 and 3 showed results of the cluster analysis and the PCA (Yu, 2005) of CHO molecular spectra in the broadband fingerprint region of approximately $1,539$ to 946 cm^{-1} that was obtained from the feed mixtures. Results indicated that with the proportion of co-product blend increasing in barley, the CHO structural makeup could gradually be distinguished and grouped into a separate ellipse. The first and second principal components explained 71.11 and 26.49% of the total variance, respectively. These results indicate that the treatments were related each other, although the structures of some of the treatments were different.

Differences in CHO Chemical Profiles, CHO Fractionations, Truly Digestible Nutrients, and Energy Values Among the Mixtures

Results of chemical analysis of the 5 mixtures of co-product blends with barley grain are presented in Table 2. The results showed that distinctive changes occurred

in chemical compositions among the mixtures. With an increasing inclusion of co-product blend in the hull-less barley, DM content showed a linear decrease, with a mean of 930 g/kg and a range of 14 g/kg. Ash and EE were increased by about 3 times, from 17 and 20 g/kg of DM (B100) to 42 and 61 g/kg of DM (BD100). The concentrations of CHO, starch, and NFC were decreased linearly from 816 to 524 g/kg, 613 to 58 g/kg, and 730 to 233 g/kg of DM (from B100 to BD100), respectively. However, the contents of NDF, ADF, and ADL were increased linearly from 100, 14, and 3 (B100) to 454, 93, and 20 g/kg of DM (BD100). Similarly, the contents of hemicellulose and cellulose were increased linearly by about 4 and 7 times. These results are in agreement with a previous study on the predicted nutrient supply of corn with wheat distillers dried grains with solubles (Damiran et al., 2012).

The results (Table 2) indicated that CA and CB₂ were increased linearly and CC was increased quadratically, whereas CB₁ was decreased linearly from B100 to BD100. The results implied that the co-product blend added extra soluble sugars and available fiber and lignin but that it reduced the starch content in the mixtures to a great degree. The CHO fractions for the co-product blend and hull-less barley were similar to those reported by Nuez-Ortín and Yu (2009) and Damiran and Yu (2010). This result indicates that through different types of feed mixtures, the CHO subfraction profiles could be formulated to an optimal level, which would affect the degradation behaviors (Sniffen et al., 1992), available rumen-undegradable CHO (Cornell Net Carbohydrate and Protein System, v6.1), and hourly effective N-to-energy synchronization ratio (Tammenga et al., 1994; Nuez-Ortín and Yu, 2010b).

Truly digestible nutrients reflect the energy value of feedstuffs (NRC, 2001). With an increasing co-product blend inclusion rate in the mixtures with the hull-less barley, truly digestible NFC and TDN_{1x} were linearly decreased, whereas tdNDF was linearly increased. The values of ME_{p3x} (ME at production level at 3 times maintenance intake) and NE_{L3x} (NE_L at 3 times maintenance intake) for dairy cows showed a linear increase. However, no difference was observed in the values of DE_{1x}, (digestible energy at maintenance level) NE_M, and NE_G among the mixtures.

Differences In Situ Rumen Degradation Kinetics Among the Mixtures

The results of in situ rumen degradation analysis from the 5 co-product blend mixtures with hull-less barley are presented in Table 3. In the characteristics of in situ starch rumen degradability, BST and effectively degradable starch in the rumen (**EDST**) were decreased

Table 1. Molecular structure spectral characteristics (in terms of peak area and height intensity) of structural and nonstructural carbohydrates (CHO) in mixtures¹ of a co-product blend with barley

CHO molecular structure profiles ² (infrared absorbance intensity)	Region, baseline or peak center (cm ⁻¹)	B100	B75BD25	B50BD50	B25BD75	BD100	SEM	P-value		
								Linear	Quadratic	Cubic
Lignin peak area	~1,539–1,504	0.057 ^d	0.067 ^d	0.094 ^c	0.110 ^b	0.141 ^a	0.0031	<0.001	0.460	0.221
Lignin peak height	~1,510	0.003 ^c	0.003 ^c	0.004 ^b	0.005 ^b	0.006 ^a	0.0002	0.211	0.378	0.257
Structural CHO area (STCHO)	~1,485–1,186	5.185 ^a	4.595 ^{ab}	4.805 ^{ab}	4.543 ^b	4.524 ^b	0.1510	0.014	0.033	0.064
STCHO peak I height	~1,415	0.026 ^{ab}	0.023 ^b	0.025 ^b	0.025 ^b	0.028 ^a	0.0008	0.009	0.323	0.209
STCHO peak II height	~1,370	0.026 ^a	0.022 ^b	0.022 ^b	0.020 ^b	0.019 ^b	0.0008	0.005	0.137	0.145
STCHO peak III height	~1,240	0.016 ^b	0.015 ^b	0.016 ^{ab}	0.016 ^{ab}	0.018 ^a	0.0005	<0.001	0.002	0.158
Cellulosic peak area	~1,272–1,213	0.187 ^{cd}	0.183 ^d	0.216 ^{bc}	0.234 ^b	0.323 ^a	0.0074	<0.001	0.774	0.264
Cellulosic peak height	~1,240	0.007 ^b	0.006 ^b	0.007 ^{ab}	0.007 ^{ab}	0.008 ^a	0.0002	<0.001	0.259	0.220
CHO area	~1,186–946	20.436 ^a	16.840 ^b	16.790 ^b	14.741 ^{bc}	14.069 ^c	0.5983	<0.0001	0.977	0.429
CHO peak I area	~1,186–1,132	1.723 ^a	1.377 ^b	1.324 ^b	1.007 ^c	0.665 ^d	0.0511	0.509	0.032	0.063
CHO peak II area	~1,132–1,066	4.279 ^a	3.587 ^b	3.696 ^b	3.631 ^b	3.929 ^{ab}	0.1297	<0.001	0.307	0.257
CHO peak III area	~1,066–946	14.440 ^a	11.872 ^b	11.770 ^{bc}	10.104 ^{cd}	9.477 ^d	0.4211	<0.0001	0.776	0.425
CHO peak I height	~1,152	0.060 ^a	0.048 ^b	0.045 ^b	0.035 ^c	0.025 ^d	0.0018	0.004	0.214	0.208
CHO peak II height	~1,080	0.104 ^a	0.087 ^b	0.087 ^b	0.079 ^b	0.078 ^b	0.0030	<0.001	0.232	0.326
CHO peak III height	~1,025	0.197 ^a	0.161 ^b	0.157 ^b	0.131 ^c	0.126 ^c	0.0058	<0.001	0.060	0.337
Area ratio of CHO peak I:II		0.402 ^a	0.384 ^b	0.358 ^c	0.276 ^d	0.169 ^e	0.0033	<0.001	0.023	0.602
Area ratio of CHO peak I:III		0.119 ^a	0.116 ^a	0.112 ^b	0.099 ^c	0.070 ^d	0.0009	<0.001	0.026	0.261
Area ratio of CHO peak II:III		0.296 ^d	0.302 ^d	0.314 ^c	0.360 ^b	0.415 ^a	0.0016	<0.001	0.087	0.510
Height ratio of CHO peak I:II		0.572 ^a	0.552 ^b	0.521 ^c	0.443 ^d	0.323 ^e	0.0048	<0.001	0.016	0.691
Height ratio of CHO peak I:III		0.302 ^a	0.298 ^a	0.289 ^b	0.267 ^c	0.199 ^d	0.0019	<0.001	0.945	0.276
Height ratio of CHO peak II:III		0.528 ^c	0.539 ^d	0.556 ^c	0.602 ^b	0.616 ^a	0.0025	<0.001	0.689	0.933
Area ratio of lignin:STCHO		0.003 ^e	0.004 ^d	0.006 ^c	0.007 ^b	0.010 ^a	0.0002	<0.001	<0.001	0.006
Area ratio of lignin:CHO		0.254 ^c	0.273 ^d	0.286 ^c	0.308 ^b	0.322 ^a	0.0017	<0.001	0.511	0.684
Area ratio of STCHO:CHO		0.011 ^e	0.015 ^d	0.020 ^c	0.024 ^b	0.031 ^a	0.0005	<0.001	0.549	0.786
Protein amide I peak area	~1,721–1,580	6.916 ^c	56.916 ^d	66.916 ^c	76.916 ^b	86.916 ^a	0.9574	<0.001	0.039	0.149
Area ratio of amide I:CHO		0.352 ^d	3.390 ^c	4.012 ^c	5.323 ^b	6.211 ^a	0.1645	<0.001	0.062	0.186

¹B100 = 100% barley; B75BD25 = 75% barley + 25% blend distillers dried grains with solubles (DDGS); B50BD50 = 50% barley + 50% DDGS blend; B25BD75 = 25% barley + 75% DDGS blend; BD100 = 100% DDGS blend.

²Analyses were conducted using a Jasco Fourier transform infrared attenuated total reflection molecular spectroscope (Jasco Corporation, Tokyo, Japan).

Figure 2. Agglomerative hierarchical cluster of Fourier transform infrared attenuated total reflection carbohydrate (CHO) spectra obtained from 5 mixtures of a bioethanol processing co-product blend with barley grain. Agglomerative hierarchical cluster analysis: (1) spectral region: $\sim 1,539\text{--}946\text{ cm}^{-1}$; (2) distant method: Euclidean; (3) cluster method: Ward's algorithm (Yu, 2005). 1 = B100 = 100% barley; 2 = B75BD25 = 75% barley + 25% distillers dried grains with solubles (DDGS) blend; 3 = B50BD50 = 50% barley + 50% DDGS blend; 4 = B25BD75 = 25% barley + 75% DDGS blend; 5 = BD100 = 100% DDGS blend. Color version available in the online PDF.

significantly from 130 and 482 (B100) to 15 and 43 g/kg of DM (BD100; $P < 0.01$). The results showed that the hull-less barley had a significantly greater EDST content [482 g/kg of FOM was decreased significantly from B100 (632 g/kg of DM) to BD100 (518 g/kg of DM)] and may explain why the CHO fractions CA, CB₂, and CC were increased but CB₁ decreased linearly. The in situ results from the co-product blend and hull-less barley were similar to those previously reported by Nuez-Ortín and Yu (2009, 2010a,b) and Damiran and Yu (2012). The implication from these in situ results is that the rumen N-to-energy ratio could be manipulated through the feed mixtures of hull-less barley (greater energy and a greater degradation rate and extent) with the co-product blend (greater truly absorbable protein; lower degradation rate and extent) to an optimal degradation ratio of 25 to 32 g of N/kg of CHO (Tamminga et al., 1994; Nuez-Ortín and Yu, 2010a,b).

Relationship Between Spectral Characteristics of the CHO Molecular Structure and CHO Nutrient Profiles

Pearson correlations of the CHO molecular structural characteristics determined by using FT/IR-ATR spectroscopy to determine CHO chemical composition, CHO fractionations, truly digestible nutrients, in situ degradation kinetics, and predicted FOM supply in the mixtures are presented in Table 4. The results showed that the spectra of functional groups of lignin, cellulosic compounds, and total CHO were very sensitive to the feed mixtures.

First, the spectral infrared absorbance intensity of lignin and cellulosic compounds had a strongly negative correlation ($P < 0.01$) with CHO, starch, NFC, CB₁, tdNFC, TDN_{1x}, and FOM but had a strongly positive correlation ($P < 0.01$) with NDF, ADF, ADL, hemicellulose, cellulose, tdNDF, CB₂, and CC. A significant

Figure 3. Principal components analysis of Fourier transform infrared attenuated total reflection carbohydrate (CHO) molecular spectrum in the fingerprint region of $\sim 1,539\text{--}946\text{ cm}^{-1}$ obtained from 5 mixtures of a bioethanol processing co-product blend with barley grain (Yu, 2005). 1 = B100, 100% barley; 2 = B75BD25, 75% barley + 25% distillers dried grains with solubles (DDGS) blend; 3 = B50BD50, 50% barley + 50% DDGS blend; 4 = B25BD75, 25% barley + 75% DDGS blend; 5 = BD100, 100% DDGS blend. Color version available in the online PDF.

correlation ($P < 0.10$) between lignin and CA tended to appear. No correlations ($P > 0.05$) were found between lignin and cellulosic spectral feature and energy values. The results indicate an association between spectral features of the chemical functional groups and the chemical and nutrient composition.

Second, no correlation ($P > 0.05$) was found between STCHO and all the conventional parameters in terms of chemical profiles, CHO partitions, and truly nutrient digestibility, as well as in situ degradation characteristics. The results indicate that STCHO cannot be used as an indicator to predict nutrient profiles in these feed mixtures.

Third, the spectra of CHO had a strongly negative correlation ($P < 0.01$) with NDF, ADF, ADL, hemicellulose, cellulose, CA, CB₂, CC, tdNDF, and the energy value of NE_{L3x} (NE_L at 3 times maintenance intake; $P = 0.06$) but had a strong positive correlation ($P < 0.01$) with total CHO, starch, NFC, CB₁, tdNFC, TDN_{1x}, and FOM. No correlation ($P > 0.05$) was found between

all spectral intensities of CHO and energy values. These results indicate that CHO molecular spectral features in the mixtures were associated with CHO-related chemical and nutrient profiles and could be used as potential predictors.

CONCLUSIONS

It was concluded that the increasing inclusion of the co-product blend altered the chemical constituents, nutritive characteristics, and spectral features of the intrinsic molecular structure of hull-less barley. Integration with the co-product blend significantly decreased CHO, starch, and NFC, whereas it increased NDF, ADF, ADL, hemicellulose, and cellulose in the mixture. Subsequently, these changes resulted in a significant decrease in CB₁, tdNFC, effective degradable content of DM in the rumen, EDST, and FOM and a significant increase in CA, CB₂, CC, tdNDF, and bypass DM,

Table 2. Chemical composition ($\pm$ SD), carbohydrate (CHO) fractions ($\pm$ SD), and TDN ($\pm$ SD) of mixtures¹ of the co-product blend with barley

Item ²	B100	B75BD25	B50BD50	B25BD75	BD100	Mean $\pm$ SD (n = 5)	Range from all data	P-value		
								Linear	Quadratic	Cubic
Basic chemical composition (g/kg of DM)										
DM (g/kg)	937 $\pm$ 0.8	932 $\pm$ 0.2	929 $\pm$ 0.3	927 $\pm$ 0.5	923 $\pm$ 0.4	930 $\pm$ 5.3	14	0.015	0.185	0.119
Ash	17 $\pm$ 0.6	23 $\pm$ 0.1	28 $\pm$ 0.3	35 $\pm$ 0.5	42 $\pm$ 0.1	29 $\pm$ 9.8	25	0.019	0.325	0.690
Ether extract	20 $\pm$ 0.1	30 $\pm$ 0.1	41 $\pm$ 0.2	49 $\pm$ 0.4	61 $\pm$ 2.1	40 $\pm$ 16.0	41	0.026	0.872	0.602
Structural and nonstructural CHO profiles (g/kg of DM)										
CHO	816 $\pm$ 0.6	741 $\pm$ 1.3	672 $\pm$ 5.6	603 $\pm$ 1.9	524 $\pm$ 2.4	671 $\pm$ 114.2	292	0.001	0.140	0.060
Starch	613 $\pm$ 10.3	514 $\pm$ 7.7	353 $\pm$ 3.4	216 $\pm$ 7.8	58 $\pm$ 0.1	351 $\pm$ 223.2	555	0.022	0.355	0.560
NDF	100 $\pm$ 2.1	179 $\pm$ 3.1	259 $\pm$ 0.9	341 $\pm$ 0.7	454 $\pm$ 0.8	267 $\pm$ 138.0	354	0.008	0.113	0.216
ADF	14 $\pm$ 0.4	33 $\pm$ 1.6	50 $\pm$ 4.4	66 $\pm$ 3.4	93 $\pm$ 2.1	51 $\pm$ 30.3	79	0.014	0.202	0.197
ADL	3 $\pm$ 0.1	6.6 $\pm$ 0.7	10 $\pm$ 0.7	15 $\pm$ 0.0	20 $\pm$ 0.3	11 $\pm$ 6.7	17	0.019	0.212	0.902
NFC	730 $\pm$ 2.4	612 $\pm$ 5.1	498 $\pm$ 7.9	383 $\pm$ 2.2	233 $\pm$ 4.6	491 $\pm$ 193.7	497	0.006	0.125	0.174
Hemicellulose	87 $\pm$ 1.7	146 $\pm$ 1.4	208 $\pm$ 3.5	275 $\pm$ 2.8	362 $\pm$ 1.3	216 $\pm$ 107.7	275	0.005	0.061	0.179
Cellulose	11 $\pm$ 0.3	26 $\pm$ 1.0	40 $\pm$ 3.7	51 $\pm$ 3.4	73 $\pm$ 1.8	40 $\pm$ 23.8	62	0.026	0.367	0.297
Fractions of carbohydrates (CNCPS, g/kg of CHO)										
CA	282 $\pm$ 12.5	312 $\pm$ 16.0	387 $\pm$ 2.2	418 $\pm$ 9.5	386 $\pm$ 6.6	357 $\pm$ 57.3	104	0.054	0.117	0.153
CB ₁	613 $\pm$ 10.3	514 $\pm$ 7.7	353 $\pm$ 3.4	216 $\pm$ 7.8	58 $\pm$ 0.1	351 $\pm$ 223.2	555	0.022	0.355	0.560
CB ₂	96 $\pm$ 2.0	153 $\pm$ 6.2	223 $\pm$ 2.8	307 $\pm$ 1.6	466 $\pm$ 5.0	249 $\pm$ 144.7	370	0.016	0.078	0.223
CC	9 $\pm$ 0.3	21 $\pm$ 2.1	36 $\pm$ 2.8	59 $\pm$ 0.1	90 $\pm$ 1.7	43 $\pm$ 2.2	81	0.006	0.031	0.230
Truly digestible nutrients and TDN (g/kg of DM)										
tdNFC	715 $\pm$ 0.2	600 $\pm$ 0.5	488 $\pm$ 0.8	375 $\pm$ 0.2	228 $\pm$ 0.5	481 $\pm$ 189.9	487	0.006	0.125	0.183
tdNDF	55 $\pm$ 0.1	79 $\pm$ 0.3	105 $\pm$ 0.0	129 $\pm$ 0.0	170 $\pm$ 0.1	108 $\pm$ 44.6	115	0.020	0.198	0.334
TDN _{1x}	870 $\pm$ 1.6	857 $\pm$ 3.4	844 $\pm$ 1.1	823 $\pm$ 0.5	800 $\pm$ 2.2	839 $\pm$ 27.8	70	0.019	0.140	0.770
Predicted energy values ³ (Mcal/kg of DM)										
DE _{1x} -Dairy	3.85 $\pm$ 0.007	3.88 $\pm$ 0.017	3.89 $\pm$ 0.005	3.87 $\pm$ 0.001	3.85 $\pm$ 0.011	3.87 $\pm$ 0.018	0.04	0.628	0.087	0.412
ME _{P3x} -Dairy	2.72 $\pm$ 0.003	2.76 $\pm$ 0.008	2.80 $\pm$ 0.006	2.82 $\pm$ 0.002	2.86 $\pm$ 0.006	2.79 $\pm$ 0.054	0.14	0.042	0.592	0.540
NE _{L3x} -Dairy	1.72 $\pm$ 0.002	1.75 $\pm$ 0.006	1.78 $\pm$ 0.004	1.80 $\pm$ 0.001	1.83 $\pm$ 0.005	1.78 $\pm$ 0.043	0.11	0.027	0.592	0.540
NE _M -Beef	2.39 $\pm$ 0.006	2.41 $\pm$ 0.013	2.42 $\pm$ 0.004	2.41 $\pm$ 0.001	2.40 $\pm$ 0.009	2.41 $\pm$ 0.011	0.03	0.328	0.106	0.540
NE _G -Beef	1.67 $\pm$ 0.005	1.69 $\pm$ 0.011	1.70 $\pm$ 0.003	1.69 $\pm$ 0.001	1.68 $\pm$ 0.008	1.69 $\pm$ 0.011	0.03	0.328	0.106	0.540

¹B100 = 100% barley; B75BD25 = 75% barley + 25% blend distillers dried grains with solubles (DDGS); B50BD50 = 50% barley + 50% DDGS blend; B25BD75 = 25% barley + 75% DDGS blend; BD100 = 100% DDGS blend.

²CNCPS = Cornell Net Carbohydrate and Protein System; CA = fraction composed of sugars and organic acids, which had a rapid degradation rate of 300%/h (calculated as CA = NFC - CB₁); CB₁ = fraction including starch and pectin, which had an intermediate degradation rate of 20 to 50%/h (CB₁ = starch + pectin); CB₂ = digestible cell wall fraction, which had a slow degradation rate of 2 to 10%/h and equaled NDF_{n-free} minus CC [CB₂ = (NDF - NDICP) - CC], where NDICP is neutral detergent-insoluble CP; CC = unavailable cell wall fraction, which were unavailable CHO in the rumen and were calculated by lignin [CC = (lignin $\times$ 2.4)/100]; tdNFC = truly digestible NFC; tdNDF = truly digestible NDF; TDN_{1x} = total digestible nutrients at a maintenance level (TDN data were reported in Zhang and Yu, 2012); DE_{1x} = digestible energy at maintenance level (NRC, 2001-Dairy); ME_{P3x} = ME at production level at 3 $\times$ maintenance intake (NRC, 2001-Dairy); NE_{L3x} = NE_L at 3 times maintenance intake (NRC, 2001-Dairy); NE_M = NE_M in the growing animal (NRC, 1996-Beef); NE_G = NE_G or net energy for retention (NRC, 1996-Beef).

³NRC (2001)-Dairy and NRC (1996)-Beef.

Table 3. Characteristics of in situ starch rumen degradability of the mixtures¹ of co-product blend with barley

Characteristics of in situ starch rumen degradation ²	B100	B75BD25	B50BD50	B25BD75	BD100	SEM	<i>P</i> -value		
							Linear	Quadratic	Cubic
K _d (%/h)	17.43	18.50	16.55	15.52	14.49	2.084	0.214	0.669	0.643
S (% of starch)	29.2	36.6	14.9	29.0	21.8	8.99	0.408	0.832	0.766
D (% of starch)	70.8	63.4	85.1	71.0	78.2	8.99	0.408	0.832	0.766
%BST	21.3	19.3	24.1	22.7	26.1	2.89	0.214	0.694	0.832
BST (g/kg of DM)	130 ^a	99 ^a	85 ^{ab}	49 ^{bc}	15 ^c	8.1	<0.001	0.406	0.572
%EDST	78.7	80.7	75.9	77.3	73.9	2.89	0.214	0.694	0.832
EDST (g/kg of DM)	482 ^a	415 ^b	268 ^c	167 ^d	43 ^e	8.1	<0.001	0.075	0.082
FOM (g/kg of DM)	632 ^a	600 ^{ab}	561 ^{bc}	545 ^c	518 ^c	9.6	<0.001	0.401	0.921

^{a-e}Means with superscript letters in the same row are significantly different ($P < 0.05$).

¹B100 = 100% barley; B75BD25 = 75% barley + 25% distillers dried grains with solubles (DDGS) blend; B50BD50 = 50% barley + 50% DDGS blend; B25BD75 = 25% barley + 75% DDGS blend; BD100 = 100% DDGS blend.

²K_d = the rate of degradation of the d fraction; S = soluble fraction in the in situ incubation; D = insoluble but potentially degradable fraction in the in situ incubation; %BST = starch fraction of the bypass rumen; BST = starch of the bypass rumen; %EDST = effectively degradable fraction of starch in the rumen; EDST = effectively degradable starch in the rumen; FOM = fermentable OM (FOM data were reported in Zhang and Yu, 2012).

which could improve the feed quality of both the co-product blend and the hull-less barley in dairy cattle.

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Table 4. Correlation between carbohydrate (CHO) molecular structural spectral profiles vs. CHO nutrient profiles of the mixtures of co-product blend with barley

Item ¹	Lignin area		Structural CHO area		Cellulose area		CHO area	
	R	<i>P</i> -value	R	<i>P</i> -value	R	<i>P</i> -value	R	<i>P</i> -value
Basic chemical profile (g/kg of DM)								
Total CHO	-0.988	0.002	0.787	0.114	-0.905	0.035	0.945	0.015
Starch	-0.996	0.000	0.738	0.155	-0.922	0.026	0.918	0.028
NFC	-0.992	0.000	0.773	0.125	-0.920	0.027	0.936	0.019
NDF	0.994	0.001	-0.764	0.133	0.929	0.022	-0.929	0.022
ADF	0.992	0.001	-0.771	0.127	0.928	0.023	-0.932	0.021
ADL	0.992	0.001	-0.765	0.132	0.922	0.026	-0.931	0.022
Hemicellulose	0.994	0.001	-0.761	0.135	0.929	0.022	-0.928	0.023
Cellulose	0.991	0.001	-0.773	0.125	0.928	0.023	-0.932	0.021
CHO fractions partitioned by CNCPS								
CA (g/kg of CHO)	0.820	0.089	-0.679	0.207	0.606	0.278	-0.844	0.072
CB ₁ (g/kg of CHO)	-0.996	0.000	0.738	0.155	-0.922	0.026	0.918	0.028
CB ₂ (g/kg of CHO)	0.991	0.001	-0.709	0.180	0.970	0.006	-0.884	0.047
CC (g/kg of CHO)	0.992	0.001	-0.720	0.170	0.959	0.010	-0.894	0.041
Truly digestible nutrients								
tdNFC (g/kg of DM)	-0.992	0.001	0.774	0.125	-0.920	0.027	0.936	0.019
tdNDF (g/kg of DM)	0.996	0.000	-0.749	0.145	0.943	0.016	-0.918	0.028
TDN _{1x} (g/kg of DM)	-0.992	0.001	0.753	0.142	-0.937	0.019	0.920	0.027
Energy value								
NE _{L3x} (Mcal/kg of DM)	0.609	0.276	-0.915	0.029	0.409	0.494	-0.869	0.056
NE _M (Mcal/kg of DM)	0.173	0.781	-0.496	0.395	-0.092	0.883	-0.442	0.457
NE _G (Mcal/kg of DM)	0.173	0.781	-0.496	0.395	-0.092	0.883	-0.442	0.457
FOM supply								
FOM (g/kg of DM)	-0.976	0.005	0.774	0.125	-0.866	0.058	0.940	0.018

¹CNCPS = Cornell Net Carbohydrate and Protein System; CA = fraction composed of sugars and organic acids, which had a rapid degradation rate of 300%/h (calculated as CA = NFC - CB₁); CB₁ = fraction including starch and pectin, which had an intermediate degradation rate of 20 to 50%/h (CB₁ = starch + pectin); CB₂ = digestible cell wall fraction, which had a slow degradation rate of 2 to 10%/h and equaled NDF_n minus CC [CB₂ = (NDF - NDICP) - CC], where NDICP is neutral detergent insoluble CP; CC = unavailable cell wall fraction, which were unavailable CHO in the rumen and were calculated by lignin [CC = (lignin × 2.4)/100]; tdNFC = total truly digestible NFC; tdNDF = total truly digestible NDF; TDN_{1x} = total digestible nutrients at a maintenance level; NE_{L3x} = NE_L at 3 times maintenance intake; FOM = fermentable OM.

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