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Crude protein fractionation, in situ ruminal degradability and FTIR protein molecular structures of different cultivars within barley, corn and sorghum cereal grains

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ABSTRACT

The objectives of current study was to determine protein characteristics, including crude protein fractionation according to the Cornell Net Carbohydrate and Protein System (CNCPS), in situ ruminal crude protein (CP) degradability and Fourier Transformed Infrared Spectroscopy (FTIR), among barley, corn, and sorghum cultivars. Cereal grains were collected from the central part of Iran and included 1) moderate, cold, and dry climate barley cultivars, 2) early and late maturity corn cultivars and 3) new and conventional sorghum cultivars. The main differences among cereal types were that corn and sorghum contained more starch and less fibre (NDF) than barley ($P < 0.05$), while soluble CP fractions (PA and PB1), slowly degradable true protein (PB3) and rate and extent of ruminal CP degradation (in situ) were greater ($P < 0.05$) for barley than corn and sorghum. The FTIR absorbance intensities for amide I, amide II, the ratio of amide I to amide II, α-helix and β-sheets were greater for barley compared with corn and sorghum grain ($P < 0.05$). Within barley, the moderate type cultivar contained less soluble CP and NPN and more ADICP than the cold and dry type cultivars ($P < 0.05$), while in situ CP degradation characteristics were similar among cultivars. Protein molecular structures in different cultivars of barley were similar. In corn grain, the early maturing cultivar contained more ash, EE, soluble CP and PB1 and less PB2, PB3 and NDICP than the late maturing cultivar ($P < 0.05$). Furthermore, FTIR protein molecular structures amide I and II had different between the two corn cultivars. In sorghum, the new cultivar contained less starch, more CP and PB3, and had slower degradation rate (kd) of CP than the old cultivar ($P < 0.05$). The FTIR protein molecular structures were similar between the sorghum cultivars according to univariate analysis, but cluster and principal component analysis largely separated the FTIR spectra of the two cultivars in the amide region. In conclusion, differences among three cereal grains and their cultivars were mainly observed for protein subfractionation, with minor effects on in situ ruminal protein degradability. Barley had different FTIR protein molecular structure compared with corn and sorghum.

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1. Introduction

Cereal grains have become an important component of the diet of ruminants in many countries because they have a high energy value which supports high milk production or rapid growth rates (Nocek, 1997). Corn (*Zea mays* L.), barley (*Hordeum Vulgare* L.) and sorghum (*Sorghum bicolor* L. Moench) are major cereal crops grown in terms of cultivated area. Barley and corn cereal are widely used in animal feeding in Iran as a primary source of dietary energy (Gholizadeh et al., 2014). However, more frequent and severer droughts have caused water scarcity for cereal production (Abbasi et al., 2008). Sorghum is more tolerant to heat and drought and maintains grain yield and therefore might be a suitable substitution for barley and corn in dry arid areas (Berenguer and Faci, 2001; Oliver et al., 2004; Gholizadeh et al., 2014). However, there is also variability in drought tolerance among cultivars within cereal type.

Different cereal grain types have a large variation in chemical composition and ruminally degradable starch and crude protein contents (Nocek and Tamminga, 1991; Nutrient Requirements of Dairy Cattle, 2001). The grain chemical structure (amylose vs. amylopectin content), crystal pattern, granule size and shape, as well as the presence of a protein matrix are the main factors responsible for the wide range in intrinsic degradation characteristics of the cereal grains (French, 1973). Protein molecular structures of feed cannot be determined using traditional wet chemical analysis because of destruction of the protein molecular structure during chemical processing (Peng et al., 2014; Tian et al., 2019), while fourier transformed infrared spectroscopy (FTIR) can be used to reveal primary protein molecular structures (e.g. amide I, amide II) and secondary protein structures (e.g. α-helix and β-sheet) (Walker et al., 2009; Liu and Yu, 2010a; Zhang and Yu, 2012; Peng et al., 2014; Tian et al., 2019). Differences in FTIR structures might influence degradation and availability of proteins to the microbial enzymes in the rumen, and also post-ruminally (Liu et al., 2012; Zhang and Yu, 2012; Peng et al., 2014; Ying et al., 2019; Tian et al., 2019).

The degree of protein binding on starch granula surface varies considerably among cereal grains. Interaction between protein and starch have been shown to be (at least in part) responsible for variation among cereal grains in the case of ruminal degradation. Crude protein sub-fractions and molecular structures of protein play important roles in its potential of gastrointestinal degradation rate (Ying et al., 2019) and might have relation with starch degradation potential in cereal grains (Yu et al., 2004). Previously, the FTIR structures of starch and its relation with in situ ruminal degradation among different types of cereals considered by Gholizadeh et al. (2014). The objectives were to determine the chemical composition, crude protein fractions, in situ crude protein degradability and FTIR protein olecular structures among cereal grain cultivars of barley, corn, and sorghum grown in central parts of Iran conditions.

2. Material and methods

2.1. Source of cereal grain cultivars

Cereal grains and cultivars within each type of grain used in the current study were:1) moderate, cold, and dry climate barley cultivars, 2) early and late maturity corn cultivars and 3) a new and a conventional sorghum cultivar. The new sorghum cultivar is bread for greater biomass yield under water deficient and salinity soil conditions. All cereal grain samples were obtained from the Plant Seed Improvement Institute (Karaj, Iran). All cultivars were grown under the same field conditions. One kilogram of clean subsample of each cultivar was collected after harvest.

Sub-samples were ground through a 2-mm screen (Laboratory Hammer Mill, Christry& Norris LTD, England) before in situ incubations, another sub-sample was ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments LTD, ON, Canada) before chemical analysis and a third sub-sample was ground to pass a 0.5 mm sieve (Retsch ZM200, Rose, Scientific Ltd., Canada) before FTIR spectroscopy analysis (Gholizadeh et al., 2014). Therefore, the results of the analysis of FTIR structures in the different grain types in the current study are directly relevant to livestock fed grain whole, ground or rolled. Processing of grain like pelleting, toasting and steam flaking will change the innate FTIR protein structures of each feed (Ying et al., 2019).

2.2. Chemical analysis

Standard procedures described by the Association of Official Analytical Chemists (AOAC 1990) were used to determine dry matter (DM; AOAC 930.15), ash (AOAC 942.05), crude protein (CP; AOAC 984.13) and ether extract (EE; AOAC 954.02). Neutral detergent fiber assayed with heat stable alpha-amylase (aNDF) and acid detergent fiber (ADF) were determined with the ANKOM A200 Filter Bag technique (Ankom Technology, Fairport, NY, USA) according to Van Soest et al. (1991). Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were determined by Kjeldahl-N analysis of the aNDF and ADF bag residues, respectively, as described by Licitra et al. (1996). The reported ADF and aNDF were corrected for NDICP and ADICP, respectively, but not for ash. Starch was analysed using the Megazyme total starch assay kit (Megazyme International Ltd, Wicklow, Ireland; McCleary et al., 1997). Non-protein N (NPN), not precipitated by trchloroacetic acid, and sodium bicarbonate/phosphate buffer soluble crude protein (SCP) were determined according to Licitra et al. (1996).

2.3. Protein fractionation

The Cornell Net Carbohydrate and Protein system (CNCPS) was used to define five CP sub-fractions (Sniffen et al., 1992; Lanzas et al., 2007a, b). The CP was fractionated into a instantaneously soluble protein A (PA; i.e. NPN), a completely undegradable CP (PC; i.e. ADICP) and a potentially degradable true protein (PB; i.e. CP-NPN-ADICP) fraction. The PB fraction was further sub-divided into rapidly (PB1; i.e. SCP-NPN), intermediately (PB2; i.e. PB-PB1-PB3), and slowly (PB3; i.e. NDICP-ADICP) degradable true protein.

2.4. Rumen incubation procedure

Three rumen fistulated, non-pregnant, dry Holstein Frisian dairy cows were used for in situ filter bag incubations. The animal trial adhered to the guidelines for care and use of animals and was approved by the Animal Care Committee of the University of Saskatchewan (Animal use protocol # 19910012). Cows were individually housed in pens at the experimental farm of the University of Saskatchewan (Saskatoon, SK, Canada) and were cared for according to the Canadian Council on Animal Care guidelines (1993). The cows had free access to water and were fed 15 kg DM/day total mixed ration twice daily in equal portions at 08:00 AM and 04:00 PM. The total mixed ration consisted in g/kg DM of 550 g barley silage, 125 g alfalfa hay, 50 g dehydrated alfalfa and 275 g concentrates. Ruminal degradability of CP was determined at 0, 3, 6, 12, 36 and 72 h of incubation by the 'all out method' (Yu et al., 2004). Number of bags, amount of sample per bag surface area and washing procedure after withdrawal from rumen were described by Yu et al. (2004). Rumen incubation was carried out in two runs and incubation residues from the treatment bags were combined within time per run.

2.5. FTIR spectroscopy

The FTIR spectroscopy analysis was performed at Department of Animal and Poultry Science, University of Saskatchewan (Saskatoon, Canada). Spectral data from all samples were collected using a JASCO FTIR-ATR-4200 (JASCO Corporation, Tokyo, Japan). The IR spectrometer was equipped with a MIRacle ATR accessory module and a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI, USA). Spectra were generated from the mid-infrared range from 700 to 4000 cm^{-1} in transmission mode with 256 scans per spectrum at a spectral resolution of 4 cm^{-1} (Fig. 1). Each sample was scanned 5 times. Molecular spectral data interpretation and analyses were performed using OMNIC 7.3 software (Spectra Tech, Madison, WI, USA).

2.6. Proteins molecular structures

Molecular spectral features associated with protein functional groups were identified according to published reports (Wetzel et al., 1998; Marinkovic et al., 2002). The regions in relation to protein molecular structures were: amide I (peak area and height, region and baseline: 1,716 to 1,574 cm^{-1}), and amide II (peak area and height, region and baseline: 1,574 to 1,483 cm^{-1}). The relative contribution of α-helix and β-sheet protein secondary structure to the amide I absorption band was determined using the second derivative spectrum to find the α-helix (*ca.* 1,655 cm^{-1}) and β-sheet (1,630 cm^{-1}) component peaks (Jackson and Mantsch, 1995; Marinkovic et al., 2002; Tian et al., 2019). Different peak area and height intensity ratios were also calculated.

2.7. Statistical analysis

Univariate spectral data analysis was performed by MIXED procedure of SAS 9.2 (Statistical Analysis System, 2003). The model used for the analysis was as follows:

$$Y_{ij} = \mu + T_i \text{ or } V(T)j + e_{ij}w$$

here, $Y_{ij}$is the observation of the dependent variable (the protein molecular spectral peak bands), μ is the population mean for the variable; T_i is the effect of cereal type (barley, corn and sorghum) as a fixed effect; $V(T)_j$ cultivar nested within each type of cereal as a fixed effect, and e_{ij} is the random error associated with the observation *ij*. For all statistical analyses, significance was declared at $P \leq 0.05$ unless otherwise stated. Differences among the multiple-treatments means were evaluated using the Tukey-method.

Multivariate spectral data analysis was performed using cluster analysis and principal component analysis of the amide spectral region (1,720–1,480 cm^{-1}) by Statistica 8.0 software (Stat Soft, Inc., USA) according to the procedure described by.

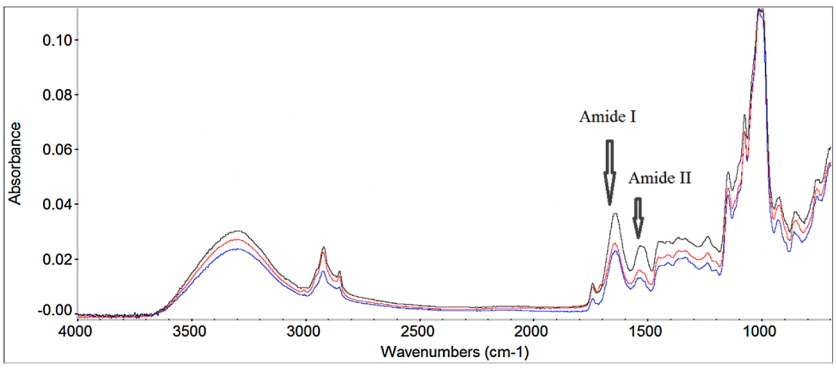

Fig. 1. Typical Fourier transform infrared spectroscopy (FTIR-ATR) of full molecular spectrum in barley (black), corn (red) and sorghum (blue) grain in the region ca. 4000 to 700 cm^{-1}. The regions in relation to protein molecular structures were: amide I (1716 to 1574 cm^{-1}), and amide II (1574 to 1483 cm^{-1}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Chemical composition and protein fractions in barley, corn and sorghum and cultivars within a type of grain.

	Grain Type			SEM[2]	Barley: Cultivar effect			SEM[2]	Corn : Maturity effect		SEM[2]	Sorghum: cultivar effect		SEM
Item[1]	Barley	Corn	Sorghum		Moderate	Cold	Dry		Early	Late		New	Old	
Basic chemical profile (g/kg DM)														
Ash	24.2[a]	13.3[c]	18.7[b]	0.90	22.5	25.0	25.0	0.51	15.5[a]	11.1[b]	0.90	19.3	18.1	0.30
EE	18.0[c]	42.3[a]	30.0[b]	2.21	17.9	17.4	18.7	1.87	52.0[a]	32.5[b]	4.10	28.4	31.7	1.50
aNDF	177.6[a]	111.1[b]	121.6[b]	3.42	173.5	185.2	173.9	5.51	111.9	110.2	2.14	128.7	114.5	4.30
ADF	66.2[a]	30.5[b]	63.4[a]	6.41	68.6	66.7	63.5	2.14	30.9	30.1	0.62	62.4	64.4	4.91
ADL	10.3	5.9	9.41	0.81	11.1	11.2	8.7	0.82	6.0	5.8	0.42	5.6	13.2	2.42
Starch	516.2[b]	670.5[a]	665.6[a]	15.7	520.5	515.8	512.2	7.50	672.8	668.7	7.11	654.3[b]	680.7[a]	6.61
CP	111.8[b]	97.8[b]	128.4[a]	3.43	112.9	118.6	103.9	4.52	101.5	94.1	2.21	142.4[a]	114.4[b]	6.42
Protein sub-fractions (g / kg CP)														
SCP	268.6[a]	214.6[b]	142.3[c]	10.71	242.8[b]	282.7[a]	280.2[a]	6.42	235.5[a]	193.6[b]	8.41	140.2	144.3	4.42
NPN	76.6[a]	50.7[ab]	36.3[b]	5.31	54.7[b]	104.1[a]	69.4[ab]	9.01	60.6	40.7	6.02	30.5	40.20	4.31
PB1	191.9[a]	163.8[b]	105.9[c]	7.92	188.1	178.6	214.6	7.31	174.8[a]	152.8[b]	5.51	109.6	102.2	5.51
NPN (g / kg SCP)	281.9	232.7	255.6	15.91	225.5	366.0	245.0	28.31	257.7	207.8	23.72	218.9	292.4	28.82
PB2	580.1[b]	651.5[a]	614.5[b]	7.21	592.6	569.8	577.8	5.92	642.0[b]	660.9[a]	4.01	594.5	634.4	13.21
NDICP	151.2[b]	133.8[b]	243.1[a]	10.01	164.4	147.4	141.8	6.72	122.3[b]	145.3[a]	5.73	265.1	221.2	14.21
PB3	116.7[a]	93.9[b]	44.5[c]	6.81	125.71	112.7	111.6	6.30	81.6[b]	106.1[a]	5.81	56.5[a]	28.4[b]	7.21
ADICP	34.5[b]	39.9[b]	203.6[a]	14.72	38.7[a]	34.6[ab]	30.1[b]	1.21	40.6	39.2	0.81	208.5	198.8	9.21

CP=Crude protein, EE=Ether extract, aNDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, NDICP=Neutral detergent insoluble crude protein, ADICP=Acid detergent insoluble crude protein, SCP=Soluble crude protein, NPN= non-protein nitrogen; PA = NPN; PB1 = rapidly degradable protein fraction; PB2 = intermediately degradable protein fraction; PB3 = slowly degradable protein fraction; PC = fraction of undegradable protein,calculated as ADICP.
[2]SEM, standard error of means; Means with adifferent letter in the same row differ ($P < 0.05$).

Table 2
In situ rumen degradation characteristics of CP in barley, corn and sorghum cereal grains and cultivars within a type of grain.

	Grain type				Barley; cultivar effect				Corn: maturity effect			Sorghum; cultivar effect		
Item[1]	Barley	Corn	Sorghum	SEM[2]	Moderate	Cold	Dry	SEM[2]	Early	Late	SEM[2]	New	Old	SEM[2]
Kd (%h^{-1})	11.77[a]	3.44[b]	1.89[b]	1.52	14.13	11.92	9.26	2.07	2.95	3.94	0.59	1.22[b]	2.57[a]	0.40
T0 (%h^{-1})	1.94	5.83	4.30	0.99	0	2.70	3.13	0.95	2.55	9.12	2.82	3.52	5.09	1.23
Soluble fraction (g / kg CP)	271.2	302.3	193.8	28.71	246.3	192.0	375.2	63.9	286.4	318.2	10.21	198.41	189.2	9.61
Potentially degradable fraction (g / kg CP)	664.4	697.6	723.3	28.11	663.51	742.1	587.8	45.42	713.5	681.7	10.22	635.9	810.7	77.40
Un-degradable fraction (g / kg CP)	64.3	0.1	82.7	24.61	90.1	65.8	36.91	20.81	0.0	0.0	0.0	165.5	0.0	82.7

[1] Kd = degradation rate, T0= lag time.
[2] SEM, standard error of means; Means with a different letter in the same row differ ($P < 0.05$).

3. Results

3.1. Chemical composition

Among cereal types, barley had the greatest ash and NDF content and lowest EE content, corn had the greatest EE content and lowest ash content and sorghum had the greatest CP content (Table 1). Corn and sorghum had a greater starch content compared with barley. Basic chemical composition was similar among barley cultivars, while early maturing corn contained greater ash and EE than late maturing corn, and the new sorghum cultivar had less starch and greater CP than the old sorghum cultivar.

3.2. Crude protein sub-fractions

As a proportion of CP, NPN (i.e. PA), SCP, PB1 and PB3 were greatest in barley; NDICP and ADICP (i.e. PC) were greatest and PB1 and PB3 were least in sorghum; and PB2 was greatest in corn (Table 1). Among barley cultivars, moderate cultivar contained least PA and SCP and greatest ADICP, while cold cultivar had the greatest NPN, and the dry cultivar the least ADICP. Among corn cultivars, early cultivar had greater SCP and PB1 and less PB2, PB3 and NDICP compared with late cultivar. The new sorghum cultivar had a greater PB3 fraction compared with old cultivar with other CP fractions being similar between the two cultivars.

3.3. In situ ruminal CP degradation ruminal CP degradation

Barley grain had greater kd and efective degradable CP fraction compared with other two grain types, while barley and corn had smaller RUP content comppared with sorghum (Table 2). The new sorghum cultivar had a slower Kd fraction compared to the old cultivar. Other in situ parameters were similar between cultivars within barley, corn and sorghum.

3.4. Univariate analysis FTIR protein molecular structures in cereal types and cultivars within cereal type

Univariate analysis revealed that amide I, amide II, α-helix, β-sheets and the ratios of amide I to amide II were greater for barley cereal than for corn and sorghum cereals (Table 3).

Among barley cultivars, height ratio of amide I to amide II was greater in moderate climate type cultivar compared with cold and dry type cultivars (Table 4). Between corn grain cultivars, peak height of amide I and the peak area of amide II were greater for the early than for the late maturing cultivar. The α-helix and β-sheets structures were similar among cultivars in all three cereal types.

3.5. Multivariate molecular structures analysis

Protein molecular structures among cereal types, in the region of 1720 to 1480 /cm, were largely separated by cluster and principle component analysis (Fig. 2Wand 3W). The FTIR spectra in amide region was not fully separated by cluster and principal component analysis among cultivars within each type of grain (Fig. 2X to 2Z, and 3 X to Z).

4. Discussion

4.1. Effect of grain type

The main differences among cereal types in the current study were that corn and sorghum contained more starch and less fibre (NDF) than barley, while soluble CP fractions (PA and PB1), slowly degradable true protein (PB3) and rate and extent of ruminal CP degradation were greater for barley than corn and sorghum. The rate and extent of starch digestion in the rumen differs among the cereal grain cultivars (McAllister et al., 2006). Corn and sorghum grain were previously found to contain dense protein matrices within

Table 3
Fourier transform infrared spectroscopy (FTIR-ATR) protein molecular structural makeup characteristics in barley, corn and sorghum cereal grains.

	Grain Type			
Item	Barley	Corn	Sorghum	SEM[1]
Amide I peak area	2.42[a]	1.42[b]	1.38[b]	0.069
Amide I peak height	0.04[a]	0.02[b]	0.02[b]	0.001
Amide II peak area	0.48[a]	0.37[b]	0.37[b]	0.013
Amide II peak height	0.01[a]	0.007[b]	0.007[b]	0.001
Amide I + amide II peak area	2.90[a]	1.78[b]	1.75[b]	0.080
Alpha helix peak height	0.04[a]	0.02[b]	0.02[b]	0.001
Beta sheet peak height	0.03[a]	0.02[b]	0.02[b]	0.001
Area ratio Amide I : amide II	5.08[a]	3.83[b]	3.71[b]	0.097
Height ratio Amide I : amide II	3.67[a]	3.1[b]	3.03[b]	0.056
Height ratio alpha helix: beta sheet	1.12	1.14	1.13	0.006

[1] SEM, standard error of means; Means with a different letter in the same row differ ($P < 0.05$).

Table 4
Fourier transform infrared spectroscopy (FTIR-ATR) protein molecular structural makeup characteristics in different cultivars within a type of grain (barley, corn and sorghum).

	Barley cultivars[2]				Corn cultivars[3]			Sorghum cultivars[4]		
Item	Moderate	Cold	Dry	SEM[1]	Early	Late	SEM[1]	New	Old	SEM[1]
Amide I peak area	2.791	2.523	2.210	0.079	1.577	1.288	0.079	1.369	1.483	0.063
Amide I peak height	0.034	0.038	0.033	0.001	0.026[a]	0.021[b]	0.001	0.021	0.024	0.001
Amide II peak area	0.435	0.506	0.460	0.019	0.396[a]	0.304[b]	0.022	0.369	0.401	0.017
Amide II peak height	0.008	0.010	0.009	0.001	0.008	0.007	0.001	0.007	0.008	0.001
Amide I + amide II peak area	2.714	3.029	2.670	0.095	1.953	1.592	0.094	1.738	1.884	0.079
Alpha helix peak height	0.033	0.037	0.033	0.001	0.025	0.021	0.001	0.021	0.023	0.001
Beta sheet peak height	0.030	0.034	0.029	0.001	0.022	0.018	0.001	0.018	0.020	0.001
Area ratio amide I : amide II	5.266	5.114	4.808	0.112	3.907	3.761	0.096	3.708	3.727	0.085
Height ratio amide I : amide II	3.944[a]	3.594[b]	3.427[b]	0.088	3.112	3.032	0.053	2.915	3.066	0.048
Height ratio alpha helix :beta sheet	1.119	1.113	1.139	0.012	1.139	1.152	0.006	1.128	1.127	0.009

[1]SEM, standard error of means; Means with a different letter in the same row differ ($P < 0.05$).

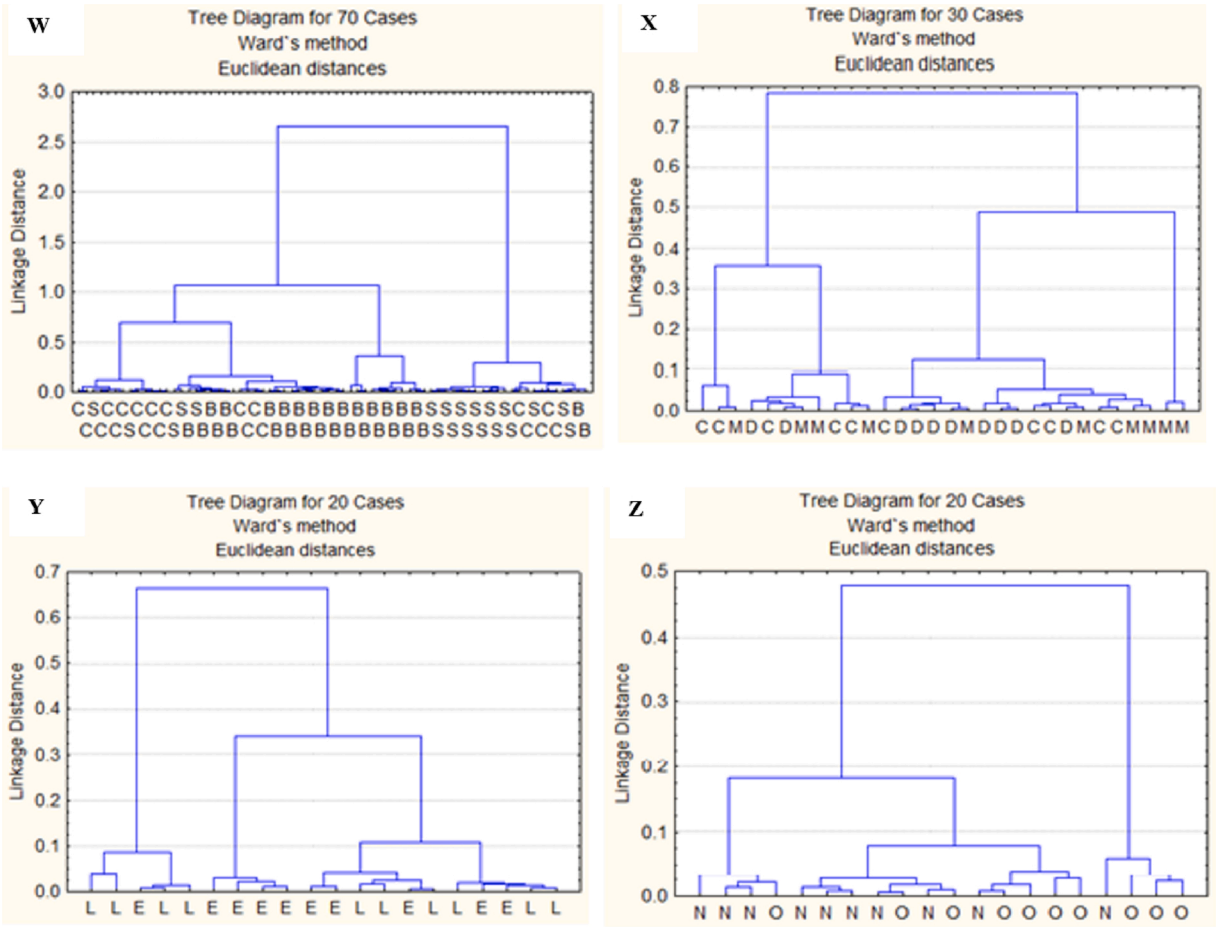

Fig. 2. Agglomerative hierarchical cluster analysis was performed on the whole spectra of amide region (1480 to 1720 cm^{-1}) for (W), barley (code B) vs. corn (code C) vs. sorghum (code S); (X), barley cultivars moderate (code M) vs. cold (code C) vs. dry (code D); (Y), corn cultivars early maturity (code E) vs.late maturity (code L), and (Z), sorghum cultivars new (code N) vs. conventional (code O).

the vitreous endosperm that surround starch granules, which is extremely resistant to invasion by ruminal microbes for digestion and limit the access of amylolytic microbes to starch granules. On the other hand, protein matrices of barley were more diffusible and did not prevent the access of rumen microbes to granules (McAllister et al., 2006). These might explain the general finding that barley starches have rapid rates and large extents of degradation in the rumen compared with sorghum and corn starch (Nutrient Requirements of Dairy Cattle, 2001; Benninghoff et al., 2015).

Those differences in protein matrices among cereal types might also affect their FTIR protein molecular structures. Spectral bands

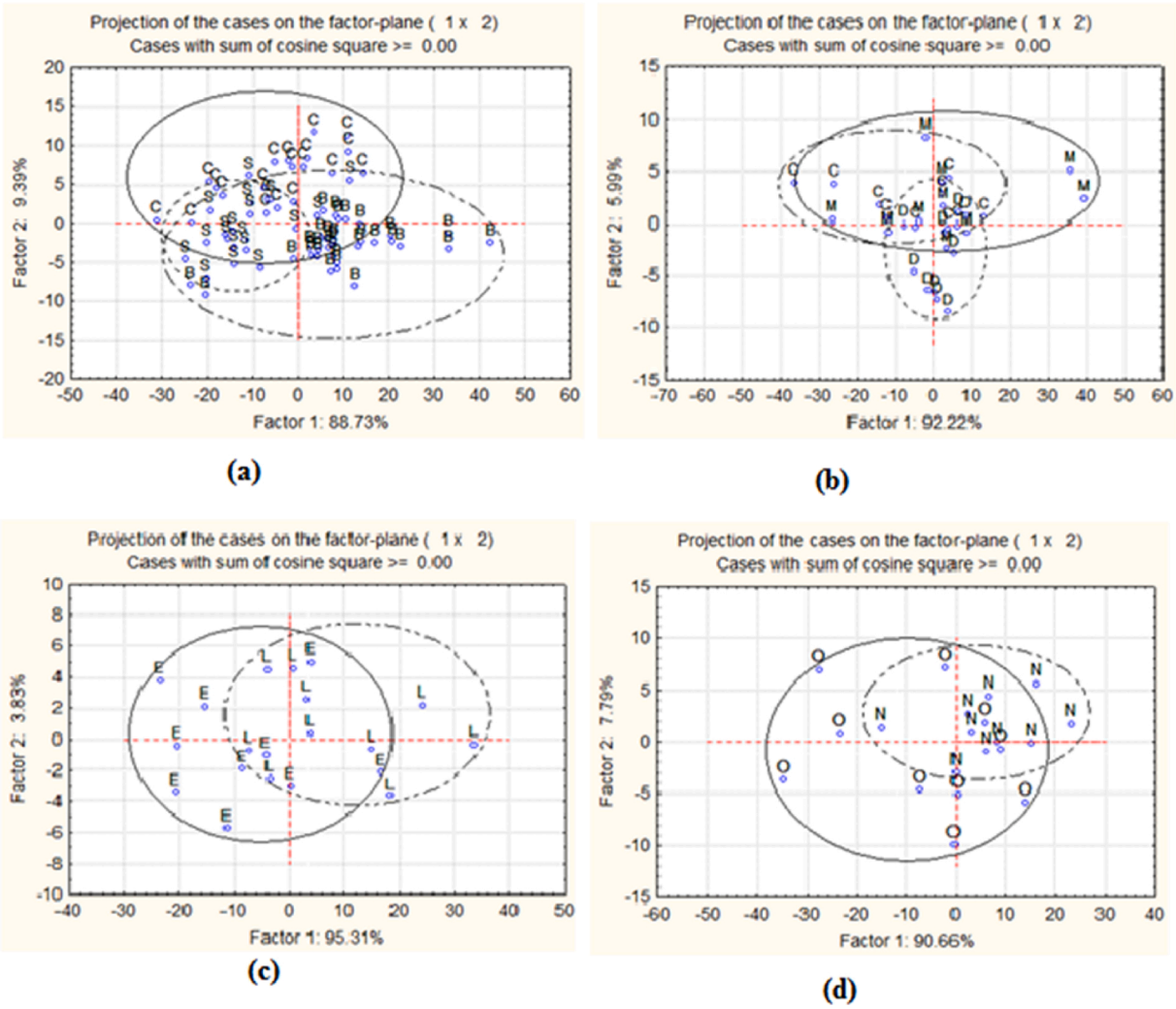

Fig. 3. Principle component analysis was performed on the whole spectra of amide region (1480 to 1720 cm^{-1}) for (a), barley (code B) vs. corn (code C) vs. sorghum (code S); (b), barley cultivars moderate (code M) vs. cold (code C) vs. dry (code D); (c), corn cultivars early maturity (code E) vs. late maturity (code L), and (d), sorghum cultivars new (code N) vs. conventional (code O).

mainly related to protein molecular structures were presented in the region of ca. 1716 to 1483 cm^{-1} in the current study, which was similar to wavenumbers reported by Liu et al. (2012) and Peng et al. (2014). Barley had the greatest absorbance peak in amide I height and area, amide II height and area, amide (I + II) area as well as α- helix and β-sheet heights compared with corn and sorghum. Corn was also previously found to have lower spectral intensity related to protein molecular structures compared with barley grain (Abeysekara et al., 2011; Peng et al., 2014), and also compared with wheat grain (Yu and Nuez-Ortín, 2010).

The amide I and II region are related to most prominent vibration bands of protein backbone (Krimm and Bandekar, 1986; Susi and Byler, 1986). The protein primary structural bands, including peak height and peak area of amide I and amide II, indicate quantitative differences in protein functional groups, whereas their ratios indicate variation in protein molecular structures (Damiran and Yu, 2011). The frequency of the bands related to protein amide are regarded to be sensitive and can be used to predict protein nutritive value of feed samples (Diron et al., 2009; Yu et al., 2004) and rumen degradation residues (Xin and Yu, 2013; Peng et al., 2014). The area ratio of amide I:amdie II was previously found to have a strong correlation with the content of rumen degradable crude protein (Yu and Nuez-Ortín, 2010; Peng et al., 2014). In the current study the area and height ratio of amide I:II were higher for barley than those of both corn and sorghum. The differences in FTIR protein molecular structures may be one of the possible reasons for the greater ruminal degradability of crude protein in barley compared with corn and sorghum. However, in this study correlation between in situ ruminal degradability kinetics and CNCPS protein fractions with FTIR molecular structures were not statistically significant (data not shown), even do there were differences in protein sub-fractions and in situ degradation kinetics between cereal grain types.

The FTIR protein structures were similar between sorghum and corn (only small numerical differences) while they different from barley. The PCA and cluster analysis did not fully distinguished samples from each cereal grain type in the protein area. For sorghum and corn the FTIR results and in situ ruminal degradation characteristics are in line with each other, while different from CNCPS

fractionation and chemical composition analysis. Previously Gholizadeh et al. (2014) reported that the FTIR carbohydrates (starch) structural features were similar between corn and sorghum, while different from the barley.

4.2. Effect of cultivar

Besides the general differences among cereal grain types, one has also to consider variations between different cultivars within cereal type (Ramos et al., 2009). In the current study, some parameters different among cultivars within cereal type.

The moderate type barley cultivar contained less SCP and NPN and more ADICP than the cold and dry type cultivars, while in situ CP degradation characteristics were similar among cultivars. Ghorbani and Hadj-Hussaini (2002) also reported little variation in in situ ruminal dry matter degradability characteristics among Iranian barley grain cultivars ($n = 10$), indicating minor effects of genetic selection on degradation parameters. The chemical composition of the barley cultivars in the current study had a similar composition to those reported by Ghezeljeh et al. (2011) and Ghorbani and Hadj-Hussaini (2002) for barley cultivares from different parts of Iran (n = 16 and 10), respectively.

Protein molecular structures in different cultivars within barley cultivar were similar, indicating that cereal breeding program did not change protein molecular structural make-up greatly. The FTIR results for barley cultivars in the current study were similar to findings by Damiran and Yu (2010), but different from previous findings among barley cultivars from western Canada (Yu et al., 2010, Walker et al., 2009; Liu and Yu, 2010a, 2010b). Furthermore, Gholizadeh et al. (2014) found some differences in FTIR carbohydrate molecular structures among barley cultivars (same samples as in current study).

In corn grain, the early maturing cultivar contained more Ash, EE, SCP and PB1 and less PB2, PB3 and NDICP than the late maturing cultivar. Furthermore, FTIR protein molecular structures Amide I and II were different between the two corn cultivars in the current study, which was similar to previous findings among corn silages from different cultivars (Abeysekara et al., 2013). These differences between the corn cultivars did, however, not result in different in situ CP degradation characteristics.

In sorghum, the new cultivar contained less starch, more CP and PB3, and had slower degradation rate (kd) of CP than the old cultivar. The FTIR protein molecular structures were similar between the sorghum cultivars according to univariate analysis, but cluster and principal component analysis largely separated the FTIR spectra of the two cultivars in the amide region. A similar trend was observed for the FTIR spectra of the same samples in the carbohydrate regions (Gholizadeh et al., 2014).

5. Conclusions

Barley had different FTIR protein molecular structures of amide I, amide II, amide I to amide II ratio, α-helix, and β-sheet compared with corn and sorghum, which had similar FTIR protein molecular structures. The molecular structures were different between corn cultivars while these structures were mostly similar for cultivars within barley and sorghum. The higher FTIR protein amide I:amide II ratio in barley may explain its greater in situ crude protein degradability compared to corn and sorghum.

CRediT authorship contribution statement

Hojjat Gholizadeh: Conceptualization, Data curation, Formal analysis, Investigation. **Abbas A. Naserian:** Supervision, Conceptualization, Project administration. **Mojtaba Yari:** Writing - original draft, Writing - review & editing. **Arjan Jonker:** Writing - review & editing. **Peiqiang Yu:** Methodology, Software, Validation, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Abbasi, F., Heydari, N., Sohrab, F., 2008. Water use efficiency in Islamic Republic of Iran: status, challenges and opportunities. In: Proceeding of AARINENA Water Use Efficiency Network. Aleppo, Syria, pp. 58–64.

Abeysekara, S., Damiran, D., Yu, P., 2011. Spectroscopic impact on protein and carbohydrate inherent molecular structures of barley, oat and corn combined with wheat DDGS. Spectroscopy 26, 255–277. https://doi.org/10.3233/SPE-2011-0546.

Abeysekara, S., Christensen, D.A., Niu, Z., Theodoridou, K., Yu, P., 2013. 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. J. Dairy Sci. 96, 6631–6643. https://doi.org/10.3168/jds.2013-6841.

Benninghoff, J., Paschke-Beese, M., Südekum, K.-H., 2015. In situ and in vitro ruminal degradation of maize grain and untreated or xylose-treated wheat, barley and rye grains. Anim. Feed Sci. Technol. 210, 86–93. https://doi.org/10.1016/j.anifeedsci.2015.10.002.

Berenguer, M.J., Faci, J.M., 2001. Sorghum (Sorghum Bicolor L. Moench) yield compensation processes under different plant densities and variable water supply. Eur. J. Agron. 15, 43–55. https://doi.org/10.1016/S1161-0301(01)00095-8.

Damiran, D., Yu, P., 2010. Structural makeup, biopolymer conformation, and biodegradation characteristics of a newly developed super genotype of oats (CDC SO-I versus conventional varieties): a novel approach. J. Agric. Food Chem. 58, 2377–2387. https://doi.org/10.1021/jf903514t.
Damiran, D., Yu, P., 2011. Molecular basis of structural make-up of hulless barley in relation to rumen degradation kinetics and intestinal availability in dairy cattle: a novel approach. J. Dairy Sci. 94, 5151–5159. https://doi.org/10.3168/jds.2011-4564.
French, D., 1973. Chemical and physical properties of starch. J. Anim. Sci. 37, 1048–1061. https://doi.org/10.2527/jas1973.3741048x.
Ghezeljeh, A.E., Mesgaran, D.M., Moghaddam, N.H., Vakili, A., 2011. Bulk density, chemical composition and in vitro gas production parameters of Iranian barley grain cultivars grown at different selected climates. Afr. J. Agric. Res. 6 (5), 1226–1232. https://doi.org/10.5897/AJAR10.877.
Gholizadeh, H., Naserian, A.A., Xin, H., Valizadeh, R., Tahmasbi, A.M., Yu, P., 2014. Detecting carbohydrate molecular structural makeup indifferent types of cereal grains and different cultivars within each type of grain grown in semi-arid area using FTIR spectroscopy with uni- and multi-variate molecular spectral analyses. Anim. Feed Sci. Techno. 194, 136–144.
Ghorbani, G., Hadj-Hussaini, A., 2002. In situ degradability of Iranian barley grain cultivars. Small Rumin. Res. 44, 207–212. https://doi.org/10.1016/S0921-4488(02)00082-2.
Jackson, M., Mantsch, H.H., 1995. The use and misuse of FTIR spectroscopy in the determination of protein structure. Crit. Rev. Biochem. Mol. Biol. 30, 95–120. https://doi.org/10.3109/10409239509085140.
Krimm, S., Bandekar, J., 1986. Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. Adv. Protein Chem. 38, 181–364. https://doi.org/10.1016/S0065-3233(08)60528-8.
Lanzas, C., Tedeschi, L.O., Seo, S., Fox, D.G., 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J. Dairy Sci. 90 (50), 7–521. https://doi.org/10.3168/jds.S0022-0302(07)72653-X.
Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3.
Liu, N., Yu, P., 2010a. Characterization of the microchemical structure of seed endosperm within a cellular dimension among six barley varieties with distinct degradation kinetics, using ultraspatially resolved synchrotron-based infrared microspectroscopy. J. Agric. Food Chem. 58, 7801–7810. https://doi.org/10.1021/jf101233n.
Liu, N., Yu, P., 2010b. Using DRIFT molecular spectroscopy with uni- and multivariate spectral techniques to detect protein molecular structure differences among different genotypes of barley. J. Agric. Food Chem. 58, 6264–6269. https://doi.org/10.1021/jf1003545.
Liu, B., Thacker, P., McKinnon, J., Yu, P., 2012. In-depth study of the protein molecular structures of different types of dried distillers grains with solubles and their relationship to digestive characteristics. J. Sci. Food Agric. 93, 1438–1448. https://doi.org/10.1002/jsfa.5912.
Marinkovic, N.S., Huang, R., Bromberg, P., Sullivan, M., Toomey, J., Miller, L.M., Sperber, E., Moshe, S., Jones, K.W., Chouparova, E., Lappi, S., Franzen, S., Chance, M.R., 2002. Center for Synchrotron Biosciences' U2B beamline: an international resource for biological infrared spectroscopy. J. Synchrotron Radiat. 9, 189–197. https://doi.org/10.1107/s0909049502008543.
McAllister, T.A., Gibb, D.J., Beauchemin, K.A., Wang, Y., 2006. Starch type, structure and ruminal digestion. In: Cattle Grain Processing Symposium. Oklahoma State University, pp. 30–41.
McCleary, B.V., Gibson, C.C., Mugford, C.C., 1997. Measurements of total starch in cereal products by amyloglucosidase alpha-amylase method, collaborative study. AOAC Int. 80, 571–579.
Nocek, J.E., 1997. Bovine acidosis implications on laminitis. J. Dairy Sci. 80, 1005–1028. https://doi.org/10.3168/jds.S0022-0302(97)76026-0.
Nocek, J.E., Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74, 3598–3629. https://doi.org/10.3168/jds.S0022-0302(91)78552-4.
Nutrient Requirements of Dairy Cattle, 7th revised ed., 2001. National Academic Science, Washington, DC, USA.
Oliver, A.L., Grant, R.J., Pedersen, J.F., O'Rear, J., 2004. Comparison of brown midrib-6 and -18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows. J. Dairy Sci. 87, 637–644. https://doi.org/10.3168/jds.S0022-0302(04)73206-3.
Peng, Q., Khan, N.A., Wang, Z., Yu, P., 2014. Relationship of feeds protein structural make-up in common Prairie feeds with protein solubility, in situ ruminal degradation and intestinal digestibility. Anim. Feed Sci. Technol. 194, 58–70. https://doi.org/10.1016/j.anifeedsci.2014.05.004.
Ramos, B.M.O., Champion, M., Poncet, C., Mizubuti, I.Y., Nozière, P., 2009. Effects of vitreousness and particle size of maize grain on ruminal and intestinal in sacco degradation of dry matter, starch and nitrogen. Anim. Feed Sci. Technol. 148, 253–266. https://doi.org/10.1016/j.anifeedsci.2008.04.005.
Sniffen, C.J., O'Connor, J.D., VanSoest, P.J., Fox, D.G., Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim.Sci. 70, 3562–3577. https://doi.org/10.2527/1992.70113562x.
Statistical Analysis System, 2003. User Guide: Statistics, Version 9.2. SAS Institute, Inc., Cary, NC, USA.
Susi, H., Byler, D.M., 1986. Resolution-enhanced fourier transform infrared spectroscopy of enzymes. Methods Mol. Biol. 130, 290–311. https://doi.org/10.1016/0076-6879(86)30015-6.
Tian, Y., Zhang, X., Huang, R., Yu, P., 2019. Protein molecular structure, degradation and availability of canola, rapeseed and soybean meals in dairy cattle diets. Asian-Austr. J. Anim. Sci. 32, 1381–1388. https://doi.org/10.5713/ajas.18.0829.
Walker, A.M., Yu, P., Christensen, C.R., Christensen, D.A., McKinnon, J.J., 2009. Fourier transform infrared microspectroscopic analysis of the effects of cereal type and variety within a type of grain on structural make-up in relation to rumen degradation kinetics. J. Agric. Food Chem. 57, 6871–6878. https://doi.org/10.1021/jf901461u.
Wetzel, D.L., Eilert, A.J., Pietrzak, L.N., Miller, S.S., Sweat, J.A., 1998. Ultraspatially-resolved synchrotron infrared microspectroscopy of plant tissue in situ. Cell. Mol. Biol. 44, 145–168.
Xin, H., Yu, P., 2013. Detect changes in protein structure of carinata meal during rumen fermentation in relation to basic chemical profile and comparison with canola meal using ATR-FTIR molecular spectroscopy with chemometrics. Spectrochim Acta Part A: Mol. Biomol. Spectrosc. 112, 318–325. https://doi.org/10.1016/j.saa.2013.04.073.
Ying, Y., Zhang, H., Yu, P., 2019. Implications of recent research on microstructure modifications, through heat-related processing and trait alteration to bio-functions, molecular thermal stability and mobility, metabolic characteristics and nutrition in cool-climate cereal grains and other types of seeds with advanced molecular techniques. Crit. Rev. Food Sci. Nutr. 59, 2214–2224. https://doi.org/10.1080/10408398.2018.1442314.
Yu, P., Nuez-Ortín, W.G., 2010. Relationship of protein molecular structure to metabolisable proteins in different types of dried distillers grains with solubles: a novel approach. Br. J. Nutr. 104, 1429–1437. https://doi.org/10.1017/S0007114510002539.
Yu, P., McKinnon, J.J., Christensen, C.R., Christensen, D.A., 2004. Imaging molecular chemistry of Pioneer corn. J. Agric. Food Chem. 52, 7345–7352. https://doi.org/10.1021/jf049291b.
Yu, P., Damiran, D., Liu, D., 2010. Synchrotron infrared spectroscopy with multivariate spectral analyses potentially facilitates the classification of inherent structures of feed type of sorghum. Proceeding of AIP 1214, 60–62. https://doi.org/10.1063/1.3326350.
Zhang, X., Yu, P., 2012. Relationship of carbohydrate molecular spectroscopic features in combined feeds to carbohydrate utilization and availability in ruminants. Spectrochimica Acta Part A. 92, 225–233. https://doi.org/10.1016/j.saa.2012.01.070.