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Researching rumen degradation behaviour of protein by FTIR spectroscopy

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ABSTRACT: The objectives of the present study were: (1) to detect protein secondary structures using the Fourier transform infrared spectroscopy (FTIR), and (2) to investigate the relationship between protein secondary structures and their rumen degradation kinetics in dairy cattle. The study was carried out using FTIR to estimate protein molecular structure of eight different types of samples, such as soybean meal, cottonseed meal, rapeseed meal, distilled dried grains with solubles, and corn. Nutritive values of these samples were determined using the Cornell Net Carbohydrate and Protein System. The results showed that: (1) different feed protein sources had different percentages and ratios of α-helixes and β-sheets in their protein secondary structures, (2) the higher protein α-helix to β-sheet ratio resulted in lower PB3 and PC, and higher PB1 and PB2 in the feeds, and (3) the α-helix to β-sheet ratio of the rumen degradation residues was changed along with the rumen degradation.

Keywords: cow; feed; protein secondary structure; rumen degradation characteristic

List of abbreviations: FTIR = Fourier transform infrared spectroscopy, CNCPS = Cornell Net Carbohydrate and Protein System, DM = dry matter, EE = ether extract, ASH = crude ash, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, CP = crude protein, NDICP = neutral detergent insoluble crude protein, ADICP = acid detergent insoluble crude protein, SCP = soluble crude protein, NPN = non-protein nitrogen, DDGS = distillers dried grains with solubles, PA, PB, PC = feed crude protein fractions (PA = NPN, PB = true protein, PC = unavailable protein), PB1, PB2, PB3 = PB subfractions

INTRODUCTION

Protein is known to be one of the most important nutrients. Traditionally, the conventional chemical analysis is used to determine the total chemical composition based on which nutritive and energy values are predicted. Traditional "wet" chemical analysis fails to reveal protein internal structure and fails to link protein molecular structural information to chemical and nutritional availability (Budevska 2004; Yu 2004). Feed protein quality, degradation characteristics, utilization, and availability are closely related not only to total protein chemical composition, but also to protein intrinsic structures such as protein secondary structures: α-helix, β-sheet, and their ratio (Yu et al. 2003b,c, 2004a,b). Protein secondary structures include mainly α-helix and β-sheet, and small amount of β-turn and random coil (Yu 2004). Studying the secondary structure of proteins leads to an understanding of the components that make up a whole protein (Dyson and Wright 1990).

Fourier transform infrared spectroscopy (FTIR) has been shown to be particularly sensitive to protein secondary structure based on the vibrational frequency of the Amide I (C = O) band, which is affected by different hydrogen-bonding environments for α-helix, β-sheet, β-turn, and random coil (Miller and Dumas 2010). These spectral features could be used to explain protein molecular structure makeup and to predict protein supply (Doiron et al. 2009; Yu et al. 2011). According to recent reports, the newly advanced synchrotron-based Fourier transform infrared microspectroscopy (S-FTIR) is a rapid, direct, non-invasive, and non-destructive bioanalytical technique (Wetzel et al.

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1998), and applicable in revealing protein structural features at a cellular level (Yu et al. 2004a,b) and protein molecular chemistry affected by heat processing within intact plant tissues (Yu 2007). In general, globar-sourced FTIR (G-FTIR) works with globar-source light, however, light for S-FTIR is provided from a synchrotron source. Because of the advantage of synchrotron light brightness (which is usually 100–1000 times brighter than a conventional globar source) and small effective source size, S-FTIR is capable of exploring the molecular chemistry within microstructures with a high signal to noise ratios at high spatial resolutions as fine as 3–10 μm (Yu et al. 2003b,c). Although S-FTIR has more advantages than G-FTIR, the later could be found in many laboratories. To date, S-FTIR and G-FTIR have not been widely used to study inherent feed structures in relation to nutrient utilization, digestive behaviour, and availability in animals (Yu 2004).

The primary objective of this study was: (1) to detect protein molecular spectral features of eight different types of feeds and their rumen degradation residues using a standard globar-sourced FTIR technique, (2) to reveal protein secondary structure in relation to degradation kinetics and effective degradability of the feeds in rumen, and (3) to determine nutritive values of the feeds using the Cornell Net Carbohydrate and Protein System (CNCPS).

MATERIAL AND METHODS

The experiments were carried out at the Animal Nutrition Research Institute at Northeast Agricultural University, Harbin, P.R. China.

The following eight different types of samples were studied: soybean meal, cottonseed meal1, cottonseed meal2, rapeseed meal1, rapeseed meal2, DDGS1, DDGS2, and corn. Always two samples of each type were used in each experiment run.

Animals and diets. The *in situ* experiments were carried out at the Livestock Research Station, Northeast Agricultural University, P.R. China. Three Holstein cows (body weight (mean ± SD) = 500 ± 10 kg) with flexible rumen cannula were used in this experiment. The animals were housed in pens of approximately 1.5 × 3 m in the research barn. The cows were fed twice daily at 6:00 and 18:00 h with equal allotment of a diet, and had *ad libitum* access to water. The feed was formulated based on feeding standard of dairy cattle (Ministry of Agriculture of the P.R. China 2004), consisting of 42.72% *Leymus chinensis*, 15.82% corn silage, 13.22% corn, 7.44% corn gluten meal, 4.97% corn germ meal, 3.15% soybean meal, 5.37% distillers dried grains with solubles (DDGS), 3.76% wheat bran, 2.06% cottonseed meal, 0.99% beet molasses, and 0.50% Premix (contents per 1 kg: vitamin A 800 000 IU, vitamin D 700 000 IU, vitamin E 10 000 IU, Fe 1600 mg, Cu 1500 mg, Zn 10 000 mg, Mn 3500 mg, Se 80 mg, I 120 mg, Co 50 mg). The animal experiment was carried out according to the guidelines approved by the Ethical Committee of the Veterinary Faculty of the Northeast Agricultural University (P.R. China).

In situ rumen degradation kinetics. Rumen degradation kinetics was determined using the standard laboratory *in situ* method (McKinnon et al. 1995; Yu et al. 2003a). Each coded nylon bag (8 × 12 cm) with the pore size of 45 μm was filled with approximately 5 g samples. All nylon bags were placed in a polyester mesh bag (45 × 45 cm) with a 90 cm length of rope to be anchored to the cannula. In order to keep the samples in the liquid levels of the rumen, a plastic bottle (250 ml) filled with gravel was used. The sample bags were added to the polyester mesh bag according to the gradual addition-all out schedule and incubated for 48, 36, 24, 12, 8, 4, and 0 h. The numbers of bags for each treatment at each incubation time in each experiment run were 2. When the incubation was finished, the bags were removed from the rumen, including those samples for 0 h, rinsed under cold water to remove excessive ruminal contents, and washed six times in cool water without detergent. The washed bags were then dried in a forced air oven at 65°C for 48 h and the dried samples (degradation residue) were stored in a refrigerator at 4°C until chemical analysis.

Protein secondary structure determination. The FTIR spectrum of the feed samples and their residue samples were recorded on a Shimadzu FTIR 8400S spectrophotometer (Shimadzu, Kyoto, Japan). Original feed samples and their residue samples were ground (10 μm). All the samples were dried at 65°C and potassium bromide powder was dried at 120°C. Samples were prepared in potassium bromide disks (2 mg sample in 200 mg KBr). The scanning range was 400–4000 cm^{-1} with resolution of 4 cm^{-1}. IRSolution (Version 1.0.265.508, 2002) software (Shimadzu) was used to smooth the data, and perform a multipoint baseline correction. To determine the relative amounts

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of α-helixes and β-sheets in the protein secondary structures, two steps were applied. The first step was using Fourier self-deconvolution (FSD) with IRSolution software to obtain the FSD spectrum in the protein Amide I region in order to identify the protein Amide I component peak frequencies. The second step was using a multipeak fitting program with Gaussian and Lorentzian functions using Origin (Version 8.0, 2007) data analysis software to quantify the multicomponent peak areas in the protein Amide I band. The relative amounts of α-helixes and β-sheets based on the modelled peak areas were calculated according to the report generated by the software.

Chemical analysis. Feed samples were ground to pass a 1 mm sieve for subsequent chemical analysis. All samples were analyzed according to AOAC, 1990 methods for the content of dry matter (DM) (method 930.15), ether extract (EE) (method 920.39), starch (method 966.11), and total crude ash (ASH) (method 942.05). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were analyzed by Ankom filter bag method (Ankom A200 Filter bag technique; Ankom Technology, Fairport, USA) according to the procedures of Van Soest et al. (1991). Crude protein (CP) was determined by the Kjeldahl method (AOAC method 984.13). Neutral detergent insoluble protein (NDICP), which is CP in neutral detergent fibre, was determined after NDF determination, and acid detergent insoluble protein (ADICP), which is CP in acid detergent fibre, was determined after ADF determination (Lacitra et al. 1996). Soluble crude protein (SCP) was determined using the method of Roe et al. (1990). Non-protein nitrogen (NPN) was analyzed based on precipitation of true protein in the filtrate with trichloroacetic acid (TCA, final concentration 10%), and NPN value was the difference between total N and the N content of the residue after filtration (Roe et al. 1990). All N analyses were conducted using the FOSS Kjeltec™ 2300 Analyzer (Foss Companies, Hillerød, Denmark).

Fractionation of protein fractions. Feed crude protein was partitioned into three fractions: PA, PB, and PC, according to the CNCPS (Sniffen et al. 1992; Chalupa and Sniffen 1994). The characterizations of the CP fractions used in this system are as follows: the PA fraction is NPN, the PB fraction is true protein, and the PC fraction represents unavailable protein. The PB fraction is further subdivided into PB1, PB2, and PB3. The PB1 fraction is soluble in buffer (Roe et al. 1990), precipitated by TCA (Van Soest et al. 1981; Krishnamoorthy et al. 1983), and is mostly available to ruminal microorganisms. It is calculated as buffer-soluble protein minus NPN (PB1 = SCP – NPN). he PB2 fraction has a slower degradation rate than PB1 fraction and some of it escapes to the lower gut, which is estimated by the difference between feed CP and buffer-insoluble protein minus neutral detergent insoluble protein (PB2 = (feed CP – SCP) – NDICP). The PB3 fraction is insoluble in neutral detergent but it is soluble in acid detergent and has an even slower ruminal degradation rate (Goering and Van Soest 1970; Krishnamoorthy et al. 1982). PB3 is calculated as NDICP – ADICP. The PC fraction is insoluble in acid detergent solution and is considered to be unavailable, which is represented by ADICP.

Rumen degradation characteristics. The disappearance of CP (%) was calculated as the difference between protein in the feed and in the residues after incubation in the rumen. Degradation data obtained were then fitted by the mathematical model of Orskov et al. (1980) using the PROC NLIN procedure of SAS (Statistical Analysis System, Version 9.1.3, 2006):

$$Y(t) = S + D \times e^{-K_d(t-t_1)};\ U = 100 - S - D$$

where:
$Y(t)$ = degraded feed at time t
S = soluble fraction (%)
D = insoluble (but potentially degradable) fraction (%)
U = undegradable fraction (%)
K_d = degradation rate
t = time (h)
t_1 = lag phase (h)

The effective degradability of CP was calculated using the parameters S, D, K_d, and rumen outflow rate of 0.08 per h:

$$RD = S + D\left(\frac{K_d}{K_d - K_p}\right)$$

where
RD = effective degradability of CP
K_p = estimated rumen outflow rate per h

Statistical analysis. All data were analyzed by one way ANOVA using the Mixed Procedure of SAS (Statistical Analysis System, Version 9.1.3., 2005) and differences among the means were compared by Duncan's Multiple Range Test. For all statistical analyses, significance was declared at $P < 0.05$.

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Table 1. Nutrition components of eight investigated feeds

	Soybean meal	Cottonseed meal1	Cottonseed meal2	Rapeseed meal1	Rapeseed meal2	DDGS1	DDGS2	Corn
DM (%)	90.6 ± 0.12	89.5 ± 0.08	88.9 ± 0.01	90.3 ± 0.04	91.0 ± 0.02	88.3 ± 0.02	87.0 ± 0.02	86.3 ± 0.01
CP (% of DM)	45.1 ± 0.10	46.9 ± 0.08	42.3 ± 0.14	39.9 ± 0.06	41.9 ± 0.33	29.8 ± 0.08	31.3 ± 0.32	8.46 ± 0.03
EE (% of DM)	0.65 ± 0.12	2.74 ± 0.11	2.36 ± 0.02	4.65 ± 0.56	5.02 ± 0.58	4.65 ± 0.29	4.13 ± 0.35	3.36 ± 0.08
ASH (% of DM)	6.36 ± 0.22	6.54 ± 0.43	6.22 ± 0.07	7.13 ± 0.16	7.68 ± 0.15	4.65 ± 0.11	4.33 ± 0.13	1.23 ± 0.03
NPN (% of SCP)	24.5 ± 2.74	24.6 ± 0.38	22.2 ± 1.12	51.4 ± 1.35	48.6 ± 2.68	51.4 ± 0.93	59.4 ± 1.97	76.2 ± 1.50
SCP (% of CP)	18.3 ± 1.07	26.9 ± 2.49	30.1 ± 1.32	10.6 ± 0.82	16.3 ± 0.89	17.6 ± 0.84	12.9 ± 0.26	21.3 ± 0.74
ADICP (% of CP)	2.78 ± 0.31	2.31 ± 0.27	4.25 ± 0.29	6.77 ± 0.60	3.9 ± 0.28	19.8 ± 0.25	23.6 ± 2.29	6.24 ± 0.61
NDICP (% of CP)	6.63 ± 0.63	8.28 ± 0.33	9.82 ± 0.64	19.5 ± 0.21	18.3 ± 0.86	43.5 ± 2.26	45.3 ± 1.41	14.5 ± 1.28
ADF (% of DM)	7.96 ± 0.73	15.9 ± 0.83	19.4 ± 0.21	19.0 ± 1.30	16.0 ± 0.54	19.0 ± 1.06	23.1 ± 0.15	3.95 ± 0.33
NDF (% of DM)	14.2 ± 0.90	31.91 ± 1.77	33.7 ± 0.16	27.8 ± 0.40	21.8 ± 0.58	42.8 ± 0.59	49.5 ± 1.17	12.7 ± 0.86
Starch (% of DM)	8.89 ± 0.95	5.88 ± 0.45	4.42 ± 0.34	4.23 ± 0.43	4.59 ± 0.61	9.05 ± 1.50	7.80 ± 1.06	46.8 ± 2.22
ADL (% of DM)	1.04 ± 0.06	9.34 ± 0.97	8.09 ± 0.53	1.15 ± 0.29	1.03 ± 0.23	1.69 ± 0.27	2.81 ± 0.41	0.88 ± 0.15

DM = dry matter, CP = crude protein, EE = ether extract, ASH = crude ash, NPN = non-protein nitrogen, SCP = soluble crude protein, ADICP = acid detergent insoluble crude protein, NDICP = neutral detergent insoluble crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre, ADL = acid detergent lignin, DDGS = distillers dried grains with solubles

RESULTS

Table 1 presents chemical analysis data on composition of each feed. DM and CP of the eight feeds ranked as: cottonseed meal1 > soybean meal > cottonseed meal2 > rapeseed meal2 > rapeseed meal1 > DDGS1 > DDGS2 > corn. Cottonseed meal1, cottonseed meal2, and soybean meal were lower in NPN and SCP than the others.

Table 2 shows the relative proportions and ratios of α-helix and β-sheet in protein internal structures of feed. Comparing the feed protein, soybean meal contained a higher ($P < 0.05$) proportion of α-helix than the other feeds; however, cottonseed meal1 contained a lower ($P < 0.05$) proportion of β-sheet than the others.

Results for the CP subfractions using CNCPS are presented in Table 3. The PA content of the corn and the PB1 content of the cottonseed meal2 were significantly higher ($P < 0.05$) than the others. The PC content of the DDGS2 was the highest in all the feeds.

For the CNCPS protein fraction correlation, the results showed that α-helix to β-sheet ratio of the protein structure had no correlation with the protein PA fraction ($P = 0.6562$, $R = –0.1883$), but had a very significantly negative correlation with the protein PB3 fraction ($P < 0.0001$, $R = –0.9456$) and the protein PC fraction ($P = 0.0157$, $R = –0.8098$); however, a positive correlation with the proteins PB1 ($P = 0.0561$, $R = 0.6947$) and PB2 ($P = 0.0372$, $R = 0.7365$) was observed (Table 4).

The CP degradation kinetics is shown in Table 5, and the correlation analysis between protein secondary structure (α-helix/β-sheet) and degradation rates are presented in Table 6. The α-helix to β-sheet ratio had a significantly positive correlation with the degradable part of the insoluble fraction

Table 2. Percentage of α-helixes, β-sheets, β-turn, and random coil in the protein secondary structures of eight feeds

Contents (%)	Soybean meal	Cottonseed meal1	Cottonseed meal2	Rapeseed meal1	Rapeseed meal2	DDGS1	DDGS2	Corn
α-Helix	30.5 ± 2.58	23.8 ± 2.60	24.6 ± 1.69	21.1 ± 2.23	22.6 ± 0.82	18.8 ± 0.73	18.7 ± 0.56	26.2 ± 1.55
β-Sheet	34.9 ± 3.80	28.4 ± 1.73	31.6 ± 2.01	33.4 ± 1.45	34.7 ± 1.1	33.3 ± 3.48	34.3 ± 1.26	35.9 ± 4.11
β-Turn	25.1 ± 1.12	35.8 ± 2.74	28.0 ± 2.54	28.0 ± 2.84	27.0 ± 2.67	26.6 ± 4.18	26.4 ± 1.95	31.4 ± 3.43
Random coil	9.46 ± 1.74	11.7 ± 1.10	15.8 ± 1.85	17.1 ± 1.03	15.7 ± 2.06	21.4 ± 1.09	20.3 ± 0.85	6.52 ± 2.36

DDGS = distillers dried grains with solubles

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Table 3. Crude protein fractions of feeds using the CNCPS

Protein subfractions[1]	Soybean meal	Cottonseed meal1	Cottonseed meal2	Rapeseed meal1	Rapeseed meal2	DDGS1	DDGS2	Corn
PA	4.48 ± 0.22	6.62 ± 0.21	6.69 ± 0.38	5.42 ± 0.36	7.89 ± 0.40	9.02 ± 0.12	7.66 ± 0.38	16.2 ± 0.10
PB1	13.8 ± 0.76	20.3 ± 1.14	23.4 ± 0.79	5.13 ± 0.40	8.36 ± 0.06	8.53 ± 0.44	5.24 ± 0.32	5.06 ± 0.44
PB2	75.2 ± 1.35	64.9 ± 1.66	60.2 ± 1.63	69.9 ± 0.34	65.4 ± 0.80	38.8 ± 1.17	41.9 ± 3.24	64.3 ± 0.71
PB3	3.85 ± 0.37	5.97 ± 0.41	5.57 ± 0.60	12.7 ± 0.22	14.4 ± 0.19	23.7 ± 0.64	21.7 ± 0.62	8.26 ± 0.18
PC	2.78 ± 0.31	2.31 ± 0.27	4.25 ± 0.29	6.77 ± 0.60	3.9 ± 0.28	19.8 ± 0.25	23.6 ± 2.29	6.24 ± 0.61

[1]protein subfractions (% of CP) using the CNCPS: PA = fraction of CP that is instantaneously solubilized at time zero, PB1 = fraction of CP that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid, PB2 = calculated as total CP minus sum of fractions PA, PB1, PB3, and PC, PB3 = calculated as the difference between the portions of total CP covered with NDF and ADF, PC = fraction of CP recovered with ADF and is considered to be undegradable, it contained protein associated with lignin and tannins and heat-damaged protein such as the Mailard reaction products
CNCPS = Cornell Net Carbohydrate and Protein System, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, DDGS = distillers dried grains with solubles

(P = 0.0092, R=0.8394) and the ruminally degraded part (P < 0.0001, R = 0.9478), but a significantly negative correlation with the undegradable fraction (P = 0.0027, R = −0.9025) was observed.

Characteristics of the protein secondary structure of the rumen degradation residue at each time are presented in Table 7. The results showed that the α-helix to β-sheet ratio in the soybean meal, DDGS1, and corn decreased from 0.88 to 0.43, 0.56 to 0.21, and 0.73 to 0.32 from 0 h to 36 h, respectively, and then increased at 48 h. However, the α-helix to β-sheet ratio in the cottonseed meal1, cottonseed meal2, and rapeseed meal1, respectively lowered down from 0.84 to 0.24, 0.78 to 0.27, and 0.63 to 0.24 from 0 h to 12 h. The α-helix to β-sheet ratio in the rapeseed meal2 and DDGS2, respectively decreased from 0.66 to 0.18 and 0.55 to 0.17 from 0 h to 8 h.

Table 4. Correlation between protein structures (α-helix to β-sheet ratio) and chemical profiles

Protein subfractions[1]	Protein molecular structure	
	R	*P*
PA	–0.188	0.656
PB1	0.695	0.056
PB2	0.737	0.037
PB3	–0.946	< 0.0001
PC	–0.81	0.016

[1]protein subfractions (% of CP) using the CNCPS: PA = fraction of CP that is instantaneously solubilized at time zero, PB1 = fraction of CP that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid, PB2 = calculated as total CP minus sum of fractions PA, PB1, PB3, and PC, PB3 = calculated as the difference between the portions of total CP covered with NDF and ADF, PC = fraction of CP recovered with ADF and is considered to be undegradable, it contained protein associated with lignin and tannins and heat-damaged protein such as the Mailard reaction products
CNCPS = Cornell Net Carbohydrate and Protein System, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre

DISCUSSION

The present study detected the feed protein molecular spectral features of eight different types of feeds and their rumen undegradation residues using FTIR technology to reveal the protein secondary structure in relation to degradation kinetics and effective degradability in the rumen. Protein has unique molecular structures; therefore it has its own infrared spectrum (Krimm and Bandekar 1986). The FTIR spectrum of protein has two prominent features, the Amide I (1600–1700 cm^{-1}) and Amide II (1500–1560 cm^{-1}) bands, which arise primarily from the C = O and C-N stretching vibrations of the peptide backbone, respectively. As a first approximation, the Amide I band can be curve-fitted to predict the secondary structure of a protein (Sarver and Krueger 1991). Peak ratio images (obtained by the area under one functional group band) were divided by the area under another functional group band at each pixel, representing biological component ratio intensity and distribution in the plant tissues. The combination of resolution-enhancement

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Table 5. *In situ* rumen degradation kinetics of different feeds

Items	Feeds							
	soybean meal	cottonseed meal1	cottonseed meal2	rapeseed meal1	rapeseed meal2	DDGS1	DDGS2	corn
S (%)	6.26	23.2	20.2	16.43	17.2	11.3	8.61	26.9
D (%)	93.5	61.9	65.4	60.8	56.4	23.2	22.5	42.4
U (%)	0.2	14.9	14.4	22.8	26.4	65.5	68.9	30.7
K_d (%/h)	0.06	0.04	0.03	0.03	0.04	0.04	0.05	0.01
RD (%)	48.0	44.3	39.6	32.7	34.4	19.6	18.3	31.7

S = soluble fraction, *D* = insoluble but potentially degradable fraction, *U* = undegradable fraction (calculated as 100 – *S* – *D*), K_d = degradation rate, *RD* = effective degradability of crude protein, DDGS = distillers dried grains with solubles

Table 6. Correlation between protein structures (α-helix to β-sheet ratio) and *in situ* rumen degradation kinetics of CP

Items	*R*	*P*
S (%)	0.256	0.542
D (%)	0.839	0.009
U (%)	–0.903	0.003
K_d (%/h)	0.09	0.835
RD (%)	0.948	< 0.0001

CP = crude protein, *S* = soluble fraction, *D* = insoluble but potentially degradable fraction, *U* = undegradable fraction (calculated as 100 – *S* – *D*), K_d = degradation rate, *RD* = effective degradability of CP

methods with curve-fitting procedures allowed to quantitatively assign different secondary structures each band according to the frequency of its maximum. The area of each band was then divided by the sum of all areas. This gave the relative ratio of each secondary structure. Chemical functional groups were identified according to published reports: for α-helix, the Amide I is typically in the range of 1650–1658 cm^{-1}, and for β-sheet, the peak can be found within the range of 1640–1610 cm^{-1} (Xie and Liu 2002).

In our study, comparing the eight feeds, the chemical profiles in the feeds were completely different (Table 1). The eight feeds differed in protein secondary structure conformation in terms of the ratio of protein α-helix and β-sheet, indicating the differences in protein molecular structural make-up and features (Table 2). The results indicated that the proportion of α-helix and β-sheet in protein structures could influence protein nutritive value and quality. A published study also showed that plant varieties had differences in protein structures (Wetzel et al. 2003).

To detect features and characteristics of rumen degradation of protein, the CNCPS system can be used to partition protein fractions (Sniffen et al. 1992). It is assumed that most of the soluble protein (PA and PB1) is completely and quickly degraded in the rumen, and that varying proportions of the insoluble fractions (PB2, PB3, and

Table 7. The α-helix to β-sheet ratio at different time points

Rumen degradation time (h)	Protein molecular structure (α-helix to β-sheet ratio) of rumen degradation residue							
	soybean meal	cottonseed meal1	cottonseed meal2	rapeseed meal1	rapeseed meal2	DDGS1	DDGS2	corn
0	0.88	0.84	0.78	0.63	0.66	0.56	0.55	0.73
2	0.65	0.57	0.47	0.41	0.46	0.42	0.35	0.76
4	0.59	0.36	0.33	0.31	0.31	0.43	0.39	0.38
8	0.54	0.52	0.46	0.48	0.18	0.44	0.17	0.38
12	0.47	0.24	0.27	0.24	0.28	0.29	0.37	0.50
24	0.63	0.46	0.43	0.42	0.50	0.33	0.50	0.65
36	0.43	0.54	0.62	0.74	0.44	0.21	0.42	0.32
48	0.86	0.63	0.64	0.68	0.57	0.37	0.63	0.79

DDGS = distillers dried grains with solubles

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PC) escape rumen depending on the interactive effects of digestion and passage (Sniffen et al. 1992). The results (Tables 3 and 4) indicated that a higher α-helix to β-sheet ratio might result in lower PB3 and PC, and higher PB1 and PB2 in the feeds. This result is opposite to the previously published studies (Yu and Nuez-Ortin 2010). A wider variety of feed samples in the present study might be a part of the reason.

For *in situ* parameter correlation, the results showed that a higher α-helix to β-sheet ratio may result in a lower insoluble fraction and undegradable fraction, and a higher ruminally degraded part. This result was different from previous findings (Yu and Nuez-Ortin 2010; Zhang and Yu 2012), the reason of which might be the difference in test equipments and spectrum data analysis. Yu used the advanced S-FTIR, which could explore a very small area (a few microns) and provide higher accuracy and precision. In our study, the globar source was used to reveal a larger area than the synchrotron source.

The present work plus published results demonstrate that the α-helix to β-sheet ratio of the rumen degradation residues firstly decreased at one time and increased later on, demonstrating that protein secondary structure changed along with the rumen degradation (Tables 6 and 7). That was not in accordance with the knowledge on more easily degrading of α-helix than of β-sheet. The increase of the α-helix to β-sheet ratio in samples may be a result of rumen microorganism contamination, which had higher α-helix proportion. A large-scale *in vivo* study would be needed to determine the influence of microbial protein synthesis on rumen degradation behaviour of protein.

CONCLUSION

In conclusion, the proportion of α-helix and β-sheet in protein structures may influence protein nutritive value and quality and different feed protein sources have different ratios of α-helixes and β-sheets in their protein secondary structures. The results indicate that a higher α-helix to β-sheet ratio may result in a lower PB3 and PC, and a higher PB1 and PB2 in feeds. The α-helix to β-sheet ratio of the rumen degradation residues changed along with the rumen degradation. FTIR has recently been developed as a rapid and convenient technique to research rumen degradation behaviour of protein.

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