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Effect of Protein Secondary Structures in Mixed Feedstuff Detected by Fourier Transform Infrared Spectroscopy on Ruminal Protein Degradation Kinetics

Dasen Liu[1,2], Yanfang Li[1], Guangliang Zhang[1], Peng Zhang[2], Penghua Wu[1], Shai Wang[1], Xiuxiu Wang[1]*

[1]*College of Animal Science and Technology, Northeast Agricultural University, Harbin, P.R. China*

[2]*College of Science, Northeast Agricultural University, Harbin, P.R. China*

**Corresponding author: dasenliu@neau.edu.cn*

ABSTRACT

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The objective of this study was to investigate the relationship between the protein secondary structure and the protein rumen degradation kinetics and the protein fractions of mixed feedstuffs of soybean meal with distillers dried grains with solubles (DDGS) at five mixed ratios (DDGS to soybean meal: 100:0, 70:30, 50:50, 30:70, 0:100). The Fourier transform infrared (FTIR) as a novel and cheap approach was used to detect the protein secondary structure, and the *in situ* nylon bag method was used to measure degradation kinetics of protein. Protein fractions were classified based on the Cornell net carbohydrate protein system. The results showed that (1) with the increasing soybean meal rate, the ruminal degraded protein and fractions of PB1 and PB2 were changed, (2) a higher α-helix to β-sheet ratio could result in a higher ruminally degraded protein, lower PB3 and PC, and higher PB1 and PB2 fractions in the feedstuff. So, mixing processing changed the feedstuff protein molecular structure spectral feature, which could influence the rumen degradation kinetics and protein fractions. It was inferred that protein degradation rate in mixed feedstuff can be measured by FTIR.

Keywords*:* FTIR; secondary structure of protein; rumen degradation characteristic

Abbreviations: FTIR = Fourier transform infrared, CNCPS = Cornell net carbohydrate protein system, DDGS = distillers dried grains with solubles, 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 protein, ADICP = acid detergent insoluble protein, SCP = soluble crude protein, NPN = non-protein nitrogen, RD = ruminal degradation

There is an increasing need for protein structure evaluation and the studies on evaluating the protein nutritive values of feedstuff have become a global research focus. Protein is one of the most important nutrients in human and animal diet. Soybean meal and distillers dried grains with solubles (DDGS, co-product of fuel ethanol production) have high protein content and are mainly used as an important

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ingredient of diets for ruminants. Conventional "wet" analytical chemical method, in which protein content of a sample was calculated as N × 6.25 (N was determined by Kjeldahl method), was used for analyzing protein (Yu 2004); however, the chemical analysis destroyed the measured object and the information about the spatial origin and distribution of the component was lost (Budevska 2002). So, chemical analysis fails to reveal protein internal structure and link protein molecular structural information to chemical and nutritional availability (Budevska 2002; Yu 2004). Published results showed that plant protein nutritive quality and availability were closely related not only to total protein and ruminal degraded behaviours, but also to their inherent protein molecular structure (Yu et al. 2004; Yu 2007).

Fourier transform infrared (FTIR) spectrum plays a major role in detecting feedstuff protein molecular spectral features and is used to describe the feed nutritional value in terms of protein (Belanche et al. 2013), dry matter (DM), and neutral detergent fibre (NDF) (Belanche et al. 2014) in a wide range of feeds for ruminants. 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 (Haris and Severcan 1999). 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). Until the nineties, the quality of FTIR increased gradually to reach extremely good signal-to-noise ratios (Goormaghtigh et al. 2009). It was found that the newly advanced synchrotron technology (S-FTIR) as a rapid, direct, non-invasive, and non-destructive bio-analysis technique (Wetzel et al. 1998; Yu 2005a) could be used to reveal protein structures of feedstuff tissues affected by heat processing (Yu 2005a; Doiron et al. 2009) and investigate the relationship between protein molecular structures and protein degradation kinetics and nutritive value in the rumen (Yu 2005b; Yu and Nuez-Ortin 2010). S-FTIR has more advantages than globar-sourced FTIR. However, it has not been used widely because of shortage of available equipment for FTIR performance.

To date, some results on protein molecular structures in relation to nutritive value, digestive behaviours, and availability of protein in animals have been reported for single feedstuff (Yu 2004, 2007; Yu et al. 2004; Liu et al 2015). But fewer reports have been found for mixed feedstuff, using S-FTIR or globar-sourced FTIR. The primary objectives of this study were: (1) to reveal the changes in protein molecular structure in mixed feedstuff of DDGS with soybean meal; (2) to investigate the relationship between protein molecular structures and protein fractions and degradation kinetics in mixed feedstuff, using globar-sourced FTIR.

MATERIAL AND METHODS

Mixed feedstuff of soybean meal and corn DDGS. In this study, DDGS (D; by-product of corn ethanol production; obtained from Hua Run Bioethanol Ltd., Harbin, China) and soybean meal (S; by-product of soybean oil production; obtained from Jiu San Oil Company, Harbin, China) were used for studying the effect of protein secondary structures on ruminal protein degradation kinetics. The two by-products were mixed at five ratios (D : S), being 100 : 0, 70 : 30, 50 : 50, 30 : 70, and 0 : 100, respectively, and the mixed feedstuffs were named as D100, D70S30, D50S50, D30S70, and S100.

Protein secondary structure determination. Detecting was performed with a FTIR spectrophotometer 8400S (Shimadzu, Japan), with scans of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The experimental methods were conducted according to Xie and Liu (2002). The percentage of each secondary protein structure was calculated based on the subpeak areas of the amide I in this manuscript. A small amount of each feed sample was mixed with potassium bromide (KBr), which worked as diluents and did not absorb mid-infrared, and was compressed into tablet (2 mg sample in 200 mg KBr). IR Solution software (Shimadzu) was used to smooth the data, and perform a multipoint baseline correction. The baseline was corrected by automatic baseline correction function based on IR Solution software. Then Origin 8.0 software (Origin Lab, USA) was used to fit curve and calculate the area of peaks, and contents of protein secondary structure of α-helix, β-sheet, β-turn, and random coil were obtained based on the area.

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Protein infrared spectra have two primary features, amide I and amide II. Amide I absorbs at ca. 1650 cm^{-1} (base region from ca. 1600–1700 cm^{-1}), and amide II absorbs at ca. 1550 cm^{-1} (base region from ca. 1480–1560 cm^{-1}) (Marinkovic et al. 2002). Amide I can be used to analyze the secondary structure of protein. For α-helix, β-sheet, β-turn, and random coil, the protein amide I is typically in the range of ca. 1648–1660 cm^{-1}, ca. 1625–1640 cm^{-1}, ca. 1660~1700 cm^{-1}, and ca. 1640~1650 cm^{-1}, respectively (Xie and Liu 2002).

Animal and diet. Three non-lactating Holstein cows weighing approximately 500 ± 10 kg with flexible rumen cannula were used in this study to determine the *in situ* degradation kinetics of the mixed feedstuff. All cows had free access to water and were housed in pens of approximately 1.5 × 3 m in the research barn at the livestock research station, Northeast Agricultural University, China. Twice daily (at 6:00 h and 18:00 h) the cows were offered with equal allotment of a diet consisting of 42.72% *Leymus chinensis*, 13.22% corn, 7.44% corn glut meal, 4.97% corn germ meal, 15.82% corn silage, 3.15% soybean meal, 5.37% DDGS, 3.76% wheat bran, 2.06% cottonseed meal, 0.99% molasses beet, and 0.50% premix (1 kg of premix contains the following: 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) according to the dairy nutrient requirements by feeding standard of dairy cattle(NRC 2001). Animal care and handling used in this study was in accordance with the guidelines approved by the Ethical Committee of the Veterinary Faculty, Northeast Agricultural University, China.

In situ rumen degradation kinetics. Rumen degradation parameters were determined using the *in situ* method (McKinnon et al. 1995; Yu et al. 2003). All nylon bags (8 × 12 cm; 45 μm of pore size) were filled with approximately 5 g feedstuff sample and were closed using rubber bands. A polyester mesh bag was used to hold the bags in the rumen, which was 45 × 45 cm with a 90 cm length of rope to be anchored to the cannula. Based on the incubation time period the sample bags with different degradation time were in scheduled intervals put into the rumen, and then all removed at the same time. Two bags were used for each treatment. Treatments were randomly assigned to the three cows in two *in situ* experimental runs. After removal from the incubation, the bags, including the samples for 0 h, were washed 6 times with tap water without detergent. Washed bags were dried at 65°C in a forced air oven for 48 h, and then were stored in a refrigerator (4°C) for analysis of crude protein (CP) content.

Rumen degradation characteristics of CP were calculated using the NLIN procedure of the SAS software (Statistical Analysis System, Version 9.1.3, 2005). The first-order kinetics equation for CP is as follows (Orskov et al. 1980):

$$Y(t) = S + D \times \exp^{(-K_d \times (t-t_1))}$$

where:

Y(t) = residue of the incubated material after t h of rumen incubation (%)

S, D = undegradable and potentially degradable fractions, respectively (%)

t_1 = lag time (h)

K_d = degradation rate (%/h)

Ruminal degradation (RD) for CP was calculated according to the formula (NRC 2001):

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

where:

K_p = rate of passage (assumed to be 0.08%/h; Tamminga et al. 1994)

Chemical analysis. Mixed feedstuff samples (D100, D70S30, D50S50, D30BD70, and S100) and rumen incubation residues of them for chemical analysis were ground through a 1 mm screen. Dry matter (DM), ether extract (EE), starch, total crude ash (ASH), and crude protein (CP) were measured as described by the Association of Official Analytical Chemists (AOAC 1990). The 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, USA) according to the procedures of Van Soest et al. (1991). The neutral detergent insoluble protein (NDICP) and acid detergent insoluble protein (ADICP) were determined by Kjeldahl-N analysis of the NDF and ADF filter residue, respectively (Lacitra et al. 1996). Total soluble crude protein (SCP) analysis followed Roe et al. (1990). The non-protein nitrogen (NPN) was measured using the tungstic acid method (Lacitra et al. 1996). All N analyses were conducted using the FOSS Kjeltec™ 2300 Analyzer (Foss Company, Denmark).

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Protein fractions partitioning. By Cornell net carbohydrate protein system (CNCPS), CP fractions of mixed feedstuff samples are partitioned (Sniffen et al. 1992; Chalupa and Sniffen 1994), and the fractions of CP are named fraction A (PA), fraction B (PB), and fraction C (PC). The PA fraction, representing non-protein N, is determined as the proportion of CP that is not precipitated by trichloroacetic acid and is soluble in borate-phosphate buffer (Lacitra et al. 1996). PB is true protein, which can be further subdivided into PB1, PB2, and PB3. The PB1 fraction is soluble in buffer (Roe et al. 1990), precipitated by trichloroacetic acid (Van Soest et al. 1981; Krishnamoorthy et al. 1983) and is mostly available to ruminal microorganisms. Fraction PB2, a little of which can escape to the lower gut, is intermediately degradable true protein and estimated as the difference between CP and the sum of PB1 + PB3 + PC. The PB3 fraction is insoluble in neutral detergent but soluble in acid detergent and has an even slower ruminal degradation rate (Goering and Van Soest 1970; Krishnamoorthy et al. 1982). The PC fraction is measured as acid detergent insoluble protein (ADICP) and assumed to be unavailable to ruminant.

Statistical analysis. All the data were analyzed by one way ANOVA using the MIXED procedure of the SAS software (Statistical Analysis System, Version 9.1.3, 2005).

Rumen degradation analyses. For studies on the effect of mixed feedstuffs of DDGS with soybean meal on *in situ* degradation kinetics, statistical analyses were performed using the following model:

$$Y_{ij} = \mu + P_i + R_j + e_{ij}$$

where:

Y_{ij} = observation of the dependent variable *ij*

μ = population mean for the variable

P_i = effect of combinations, as a fixed effect

R_j = *in situ* experimental run, as a random effect

e_{ij} = random error associated with the observed *ij* variable

Protein molecular structure infrared data analysis. The model used for chemical and spectral studies was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where:

T_i = effect of a feedstuff (fixed effect)

Orthogonal polynomial contrasts were used to examine the linear, quadratic, and cubic effects of the DDGS to soybean meal ratio. Differences between the means were compared by the Tukey's Multiple Range Test. For all statistical analyses, significance was declared at $P < 0.05$.

RESULTS

In situ ruminal digestion kinetics and ruminally degraded characters of the mixed feedstuffs are shown in Table 1. For the characteristics of *in situ* rumen CP degradation, there were significant differences in K_d, S, D, U, and RD between D100 (DDGS) and S100 (soybean meal). D100 had significantly higher U ($P < 0.05$), but S100 had higher K_d and RD ($P < 0.05$). The RD significantly increased ($P < 0.05$) from 23.09% (D100) to 54.19% (S100) with increasing inclusion ratio of soybean meal to DDGS. Values of D, K_d, and U differed between the five feedstuff samples ($P < 0.05$); however, S in S100 was higher than in others ($P < 0.05$) with no differences between the other four treatments.

Table 2 shows the CP fractionations using the CNCPS. The results of CP partition using CNCPS model showed that fraction PA of S100 (2.89%) was lower than that of D100 (8.77%), D70S30 (9.61%), D50S50 (9.24%), and D30S70 (7.52%) ($P < 0.05$). In this study, the fractions PB1 (3.24%) and PB2 (47.51%) in D100 were significantly ($P < 0.05$) lower than those of PB1 (15.20%) and PB2 (72.14%) in S100 ($P < 0.05$). The highest PB3 fraction (22.06%) and PC fraction (18.42%) were in DDGS (D100), but soybean meal (S100) had the lowest PB3 fraction (6.10%) and PC fraction (3.66%). With increase of soybean meal in mixtures, PB1 fraction and PB2 fraction increased while PB3 fraction and PC fraction decreased.

The results of CP structure spectral intensities revealed using FTIR in the mixed feedstuffs are presented in Table 3. The α-helix of the mixed feedstuffs increased from 15.73% (D100) to 35.64% (S100). However, there was no significant difference in the β-sheet between D100 and D70S30 and, in addition, there was no significant difference between D30S70 and S100 for β-sheet. With increasing rate of soybean meal to DDGS, the ratio of α-helix to β-sheet changed dramatically, increasing from 0.74 (D100) to 1.15 (S100). However, there was no significant difference between D70S30 (0.80) and D50S50 (0.82).

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Table 1. *In situ* rumen degradation kinetics of different feedstuffs (data are means + standard error)

Items	D100	D70S30	D50S50	D30S70	S100	SEM	*P*-value	*P*-value		
								linear	quadratic	quartic
S	9.87 ± 0.92^{b}	9.56 ± 0.01^{b}	9.67 ± 0.13^{b}	9.94 ± 0.04^{b}	10.41 ± 0.19^{a}	0.0669	< 0.0001	< 0.0001	< 0.0001	< 0.0001
D	67.94 ± 2.41^{e}	69.08 ± 0.81^{d}	73.15 ± 2.26^{c}	75.32 ± 0.45^{b}	87.63 ± 0.18^{a}	0.9400	< 0.0001	< 0.0001	< 0.0001	0.2325
U	17.05 ± 2.50^{a}	15.36 ± 0.82^{b}	14.89 ± 2.34^{c}	12.05 ± 0.49^{d}	9.7 ± 0.01^{e}	0.0671	< 0.0001	< 0.0001	< 0.0001	0.0008
K_d	1.27 ± 0.04^{e}	1.57 ± 0.02^{d}	2.19 ± 0.18^{c}	4.99 ± 0.01^{b}	6.23 ± 0.03^{a}	0.0423	< 0.0001	< 0.0001	< 0.0001	< 0.0001
RD	23.09 ± 0.21^{d}	31.44 ± 0.04^{c}	35.13 ± 0.35^{b}	46.48 ± 0.29^{a}	54.19 ± 0.21^{a}	0.1077	< 0.0001	< 0.0001	< 0.0001	0.0289

D100 = 100% of distillers dried grains with solubles (DDGS), S100 = 100% of soybean meal, S = soluble fraction (%), D = insoluble but potentially degradable fraction (%), U = undegradable fraction calculated as U = 100 – S – D (%), K_d = degradation rate (%/h), RD = effective degradability of CP (%), SEM = standard error of the means

[a–e]means with different letters within the same row differ ($P < 0.05$)

Table 2. Crude protein fractions of feedstuffs using the Cornell net carbohydrate protein system (CNCPS) (data are means + standard error)

Items	D100	D70S30	D50S50	D30S70	S100	SEM	*P*-value	*P*-value		
								linear	quadratic	cubic
PA	8.77 ± 0.17^{b}	9.61 ± 0.37^{a}	9.24 ± 0.08^{ab}	7.52 ± 0.05^{c}	2.89 ± 0.05^{d}	0.0767	< 0.0001	0.001	0.001	0.001
PB1	3.24 ± 0.03^{e}	5.06 ± 0.05^{d}	6.8 ± 0.22^{c}	9.56 ± 0.26^{b}	15.20 ± 0.36^{a}	0.0923	< 0.0001	0.001	0.001	0.001
PB2	47.51 ± 0.07^{e}	52.93 ± 0.61^{d}	58.41 ± 0.84^{c}	68.23 ± 0.09^{b}	72.14 ± 0.46^{a}	0.2084	< 0.0001	0.001	0.1191	0.001
PB3	22.06 ± 0.36^{a}	18.92 ± 0.09^{b}	14.14 ± 0.98^{c}	9.00 ± 0.01^{d}	6.10 ± 0.44^{e}	0.2076	< 0.0001	0.001	0.8920	0.001
PC	18.42 ± 0.24^{a}	13.49 ± 0.28^{b}	11.37 ± 0.45^{c}	5.69 ± 0.22^{d}	3.66 ± 0.29^{e}	0.1255	< 0.0001	0.0001	0.0008	0.0617

D100 = 100% of distillers dried grains with solubles (DDGS), S100 = 100% of soybean meal, SEM = standard error of the means

Protein sub-fractions using CNCPS: PA = fraction of crude protein (CP) that is instantaneously solubilized at time zero, PB1 = CP fraction that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid, PB2 = CP fraction calculated as total CP – Σ (PA + PB1 + PB3 + PC) fractions, PB3 = CP fraction calculated as the difference between the portions of total CP covered with neutral detergent fibre and acid detergent fibre, PC = CP fraction recovered with acid detergent fibre, it is considered undegradable and contains protein associated with lignin and tannins and heat-damaged protein such as the Mailard reaction products

[a–e]means with different letters within the same row differ ($P < 0.05$)

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Table 3. Percentage of α-helixes, β-sheets, β-turn, and random coil in the protein secondary structures of five feedstuffs (data are means + standard error)

Items	D100	D70S30	D50S50	D30S70	S100	SEM	*P*-value
α-Helix (%)	15.73 ± 0.48^{d}	17.09 ± 0.35^{d}	20.04 ± 1.63^{c}	30.68 ± 0.26^{b}	35.64 ± 0.29^{a}	0.2492	< 0.0001
β-Sheet (%)	21.34 ± 0.15^{c}	21.29 ± 0.48^{c}	24.51 ± 2.20^{b}	28.50 ± 0.16^{a}	31.02 ± 0.45^{a}	0.4218	< 0.0001
β-Turn (%)	29.31 ± 0.60^{a}	26.99 ± 3.51^{ab}	23.14 ± 0.64^{b}	18.28 ± 0.42^{c}	27.09 ± 0.13^{ab}	0.6660	< 0.0001
Random coil (%)	31.98 ± 0.87^{a}	32.49 ± 1.95^{a}	29.71 ± 2.90^{a}	22.55 ± 0.32^{b}	5.01 ± 0.26^{c}	0.6620	< 0.0001
α-Helix/β-sheet ratio	0.74 ± 0.01^{d}	0.80 ± 0.00^{c}	0.82 ± 0.01^{c}	1.08 ± 0.00^{b}	1.15 ± 0.01^{a}	0.0089	< 0.0001

D100 = 100% of distillers dried grains with solubles (DDGS), S100 = 100% of soybean meal, SEM = standard error of the means
$^{a–d}$means with different letters within the same row differ ($P < 0.05$)

The correlation analysis of protein secondary structure (α-helix/β-sheet) and CP fractions are presented in Table 4. The results showed that there was negative significant correlation between the protein secondary structures and the fraction PA in the mixtures ($P < 0.0001$, $r = 0.0045$). The ratio of α-helix to β-sheet had significantly positive correlation with fraction PB1 ($P < 0.0001$, $r = 0.9000$) and fraction PB2 ($P < 0.0001$, $r = 0.643$), but significantly negative correlation with fraction PB3 ($P < 0.0001$, $r = -0.9214$) and fraction PC ($P < 0.0001$, $r = 0.9286$).

Table 4. Correlation between protein structures (α-helix/β-sheet ratio) and protein sub-fractions and degradation rate

Items	Correlation	
	r	*P*-value
Protein sub-fractions		
PA	–0.6893	0.0045
PB1	0.9000	< 0.0001
PB2	0.9643	< 0.0001
PB3	–0.9214	< 0.0001
PC	–0.9286	< 0.0001
Degradation rate		
S	–0.1679	0.5499
D	0.3893	0.1515
U	–0.1072	0.7036
K_d	0.8509	< 0.0001
RD	0.9464	< 0.0001

Protein sub-fractions using Cornell net carbohydrate protein system: PA = fraction of crude protein (CP) that is instantaneously solubilized at time zero, PB1 = CP fraction that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid, PB2 = CP fraction calculated as total CP – Σ (PA + PB1 + PB3 + PC) fractions, PB3 = CP fraction calculated as the difference between the portions of total CP covered with neutral detergent fibre and acid detergent fibre, PC = CP fraction recovered with acid detergent fibre, it is considered undegradable and contains protein associated with lignin and tannins and heat-damaged protein such as the Mailard reaction products
S = soluble fraction (%), D = insoluble but potentially degradable fraction (%), U = undegradable fraction, calculated as U = 100 – S – D (%), K_d = degradation rate (%/h), RD = effective degradability of CP (%)

The results showed that there were no correlations between the ratio of α-helix to β-sheet and S, D, and U, and strongly positive correlations with RD ($P < 0.0001$, $r = 0.9765$) and K_d ($P = 0.0031$, $r = 0.9811$) (Table 4).

DISCUSSION

Protein molecular spectral features of mixed feedstuff samples (D100, D70S30, D50S50, D30BD70, and S100) were detected using FTIR technology to reveal the relationship of protein secondary structure with degradation kinetics and effective degradability in the rumen and using CNCPS to detect CP fraction. Each biological component has a unique molecular structural feature, thus each has its own unique infrared spectrum (Yu 2007). Protein has a unique molecular structure, so it has its own infrared spectrum (Krimm and Bandekar 1986). The vibrational frequency of the amide I band is particularly sensitive to protein secondary structure and can be used to predict protein secondary structure (Wetzel et al. 2003). The parameters in terms of α-helix and β-sheet peak area and their ratios were determined for protein molecular structure. For the α-helix and β-sheet, the peak falls within the range of 1650 to 1658 cm^{-1} and 1640–1610 cm^{-1}, respectively (Xie and Liu 2002).

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In our study, the five mixtures differed in protein secondary structure conformation in terms of the ratio of protein α-helix to β-sheet, indicating the differences in protein molecular structural make-up and features and there was a significant difference in the ratio of α-helix to β-sheet between mixtures of different ratios (Table 3). The ratio of α-helix to β-sheet increased from 0.74 (D100) to 1.15 (S100). The results indicated that the components of samples not only made rumen degradation kinetics and CP fractionations different, but also produced different spectra.

In regard to CP fractionations, PA (namely NPN) is rapidly degradable with an assumed infinity degradation rate in the rumen. However, in our study, PA was not increased with the increase of soybean meal inclusion, the reason of which needs to be studied. Fraction PB1 is rapidly degradable true protein and can be degraded by digestible enzyme in the rumen. Degradation of PB2 fraction, which is partly degraded in the rumen, depended on digestion and passage rates. With the increasing inclusion of soybean meal in mixture, PB1 fraction and PB2 fraction increased. However, PB3 fraction and PC fraction decreased. PB3 is insoluble in neutral detergent but soluble in acid detergent and PC is acid detergent insoluble protein. This indicated that soybean meal had a higher level of ruminally degradable protein than DDGS.

For the *in situ* ruminal degradation kinetics and characteristic of the mixed feedstuffs, it was observed that the rate of ruminal degradation increased with the increase of soybean meal rate in mixture, and K_d, S, D, and U fractions were changed with increasing of soybean meal, which was similar to previous findings (Yu and Nuez-Ortin 2010; Zhang and Yu 2012). Their research showed that α-helix to β-sheet ratio had no correlation with rumen degradation kinetics and the protein structure α-helix to β-sheet ratio had a positive correlation with the protein PC fraction but it had no correlation with PB1, PB2, and PB3 fractions, the reason of which could be the different materials. In the present research, the materials were mixed feedstuffs of soybean meal with DDGS at differently mixed ratios, and soybean meal was of higher protein quality (high α-helix to β-sheet ratio) to ruminants compared with DDGS, so with the increase of soybean a higher α-helix to β-sheet ratio might result in a higher ruminal degradation rate and PB1 and PB2 fractions. However, Yu and Nuez-Ortin (2010) used different types of DDGS samples and similar protein quality of different types of DDGS samples (similar α-helix to β-sheet ratio) and no correlation with rumen degradation kinetics and PB1, PB2 fractions was found. This indicates that when detecting protein secondary structure by Fourier transform infrared spectroscopy, it is necessary to study, besides the protein secondary structures, also the effects of chemical structure of the other feedstuffs.

CONCLUSION

The changes of the protein molecular structures in the mixed feedstuff highly correlated with CP subfractions for *in situ* degradation kinetics. The results showed that a higher α-helix to β-sheet ratio may result in a higher ruminally degraded protein, lower PB3 and PC, and higher PB1 and PB2 fractions in the feedstuff. The results indicated the protein molecular structures could be used as a predictor for nutrient availability in mixed feedstuff.

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