# Relationship between Rumen Degradation Characteristics and Protein Molecular Structure of Corn Silage: Postprint

**Authors:** Wang Xiaofan, Zhang Xingyi, Hao Xiaoyan, Gao Hong, Ding Xue, Zhang Yonggen, Xin Hangshu

**Date:** 2017-10-23T00:00:00+00:00

## Abstract

This experiment aimed to investigate the correlation between rumen degradation characteristics and protein molecular structure of corn silage, and to establish fitting equations. The nylon bag method was used to determine the rumen degradation rates of dry matter, protein, and neutral detergent fiber in 11 corn silage varieties, and their degradation characteristics were calculated. Fourier transform infrared spectroscopy (FTIR) was employed to analyze the protein molecular structure (amide I band, amide II band, -helix, -sheet) of corn silage samples. The results showed that the peak height of amide II band was extremely significantly correlated with dry matter effective degradation rate (DMED) (r=-0.71, P<0.01), significantly correlated with degradation rate of the slowly degradable fraction of neutral detergent fiber (NDFc) (r=-0.52, P<0.05) and undegradable fraction (NDFu) (r=-0.46, P<0.05). The peak height ratio of amide I to II bands was significantly correlated with rapidly degradable fraction of dry matter (DMa) (r=0.57, P<0.05), slowly degradable fraction of dry matter (DMb) (r=-0.55, P<0.05), significantly correlated with undegradable fraction of protein (CPu) (r=-0.50, P<0.05), and tended to be correlated with protein effective degradation rate (CPED) (r=0.38, P<0.10). The peak height of -helix was extremely significantly correlated with undegradable fraction of dry matter (DMu) (r=0.59, P<0.01) and significantly correlated with rapidly degradable fraction of protein (CPa) (r=0.45, P<0.05). The peak height ratio of -helix to -sheet was extremely significantly correlated with degradation rate of the slowly degradable fraction of dry matter (DMc) (r=0.59, P<0.01), significantly correlated with degradation rate of the slowly degradable fraction of protein (CPc) (r=0.57, P<0.05), and significantly correlated with CPED (r=0.43, P<0.05). Protein molecular structure was not correlated with slowly degradable fraction of protein (CPb), rapidly degradable fraction of neutral detergent fiber (NDFa), or slowly degradable fraction of neutral detergent fiber

(NDFb) (P>0.10). Protein molecular structure of corn silage showed the best fit for DMED (R2=0.50) and CPED (R2=0.48). Preliminary results demonstrated that FTIR technology can be used to analyze the correlation between rumen degradation characteristics and protein molecular structure of corn silage, and to establish regression equations. The quantitative relationship between the two can be utilized for rapid, non-destructive analysis of the nutritional value of corn silage, thereby reducing the disadvantages of traditional chemical analysis such as being time-consuming, labor-intensive, and causing environmental pollution.

## Full Text

# Relationship between Rumen Degradation Characteristics of Corn Silage and Protein Molecular Structure

**WANG Xiaofan, ZHANG Xingyi, HAO Xiaoyan, GAO Hong, DING Xue, ZHANG Yonggen*, XIN Hangshu*** *(College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China)*

### Abstract

This study aimed to explore the correlation between rumen degradation characteristics of corn silage and its protein molecular structure, and to establish predictive equations. The nylon bag technique was employed to determine the rumen degradation rates of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) in 11 corn silage varieties, and their degradation characteristics were calculated. Fourier transform infrared spectroscopy (FTIR) was used to analyze the protein molecular structures (amide I band, amide II band, -helix, -sheet) of corn silage samples. Results showed that the peak height of amide II was very significantly correlated with the effective degradability of DM (DMED) ($r = -0.71$, $P < 0.01$), significantly correlated with the degradation rate of the slowly degraded fraction of NDF (NDFc) ($r = -0.52$, $P < 0.05$) and the undegradable fraction of NDF (NDFu) ($r = -0.46$, $P < 0.05$). The peak height ratio of amide I to amide II was significantly correlated with the rapidly degraded fraction of DM (DMa) ($r = 0.57$, $P < 0.05$) and the slowly degraded fraction of DM (DMb) ($r = -0.55$, $P < 0.05$), significantly correlated with the undegradable fraction of CP (CPu) ($r = -0.50$, $P < 0.05$), and tended to be correlated with the effective degradability of CP (CPED) ($r = 0.38$, $P < 0.10$). The peak height of -helix was very significantly correlated with the undegradable fraction of DM (DMu) ($r = 0.59$, $P < 0.01$) and significantly correlated with the rapidly degraded fraction of CP (CPa) ($r = 0.45$, $P < 0.05$). The peak height ratio of -helix to -sheet was very significantly correlated with the degradation rate of the slowly degraded fraction of DM (DMc) ($r = 0.59$, $P < 0.01$), significantly correlated with the degradation rate of the slowly degraded fraction of CP (CPc) ($r = 0.57$, $P < 0.05$), and significantly correlated with CPED ($r = 0.43$, $P < 0.05$). No correlation was found between protein molecular structures and the slowly degraded fraction of CP (CPb), the rapidly degraded fraction of

NDF (NDFa), or the slowly degraded fraction of NDF (NDFb) ($P > 0.10$).

Protein molecular structures provided the best fit for DMED ($R^2 = 0.50$) and CPED ($R^2 = 0.48$). These findings preliminarily demonstrate that FTIR technology can be used to analyze the relationship between rumen degradation characteristics and protein molecular structures in corn silage, and to establish regression equations. Using this quantitative relationship, the nutritional value of corn silage can be rapidly and non-destructively analyzed, thereby overcoming the drawbacks of traditional chemical analysis methods, which are time-consuming, labor-intensive, and environmentally polluting.

**Keywords:** rumen degradation characteristics; Fourier transform infrared spectroscopy; protein molecular structure; correlation; multiple regression

## Introduction

Optimizing nutrient utilization in ruminant feeding requires a simple, rapid, and accurate method for estimating the nutritional value of dietary ingredients [**?**, **?**]. Current approaches primarily involve determining the chemical composition, rumen degradation characteristics, and digestibility of feeds. Rumen degradation characteristics not only affect feed nutritional value but also serve as a basis for diet formulation to meet energy and nitrogen balance requirements, thereby synchronizing rumen nutrition, enhancing microbial protein synthesis, and improving feed utilization efficiency [**?**].

Yu et al. [**?**] compared two types of barley with similar chemical composition (malting barley and feed barley) and found they exhibited different rumen degradation extents, with malting barley showing stronger degradation than feed barley. Liu et al. [**?**] reported that different barley varieties with similar chemical composition showed significant differences in degradation characteristics. These findings indicate that traditional chemical analysis methods destroy the intrinsic molecular structure and physicochemical properties during processing or measurement, providing only information on chemical components but failing to reveal the relationship between protein intrinsic molecular structure and nutritional value or utilization efficiency [**?**, **?**]. Lu et al. [**?**] noted that FTIR technology is a powerful tool for quantitatively calculating protein secondary structures. FTIR analysis is a rapid, direct, and minimally destructive technique that can reveal the molecular structural characteristics of biological feed components [**?**, **?**]. Since each biological component has specific molecular structural information, it possesses unique spectral signatures. For example, the protein molecular spectral region is divided into amide I band (primarily 80% C=O and 20% C–N stretching vibrations) and amide II band (primarily 60% N–H bending vibrations and 40% C–N stretching vibrations) [**?**]. The secondary structures in the amide I region mainly include -helix, -sheet, and small amounts of -turn and random coil [**?**]. Each functional group is closely related to the nutritional components and biological characteristics of the sample.

Therefore, this study aimed to use FTIR technology to scan corn silage samples, obtain spectral characteristics in the protein region, and conduct correlation and regression analyses with rumen degradation characteristics to explore whether protein molecular structural features of corn silage are related to its rumen degradation characteristics.

## Materials and Methods

### 1.1 Experimental Materials

The corn silage samples (n = 11) used in this study were collected from May to September 2014 from dairy farms located in Harbin, Qiqihar, and Daqing cities in Heilongjiang Province. The corn varieties are listed in Table 1 .

Collected samples were dried at 65 °C for 48 h, ground through a 100-mesh sieve for spectral analysis, through a 1-mm sieve for chemical analysis, and through a 2-mm sieve for rumen degradation experiments.

**Table 1** Basic information of experimental samples

| Corn silage No. | Producing area | Variety |
|---|---|---|
| Harbin (Wuchang) | Xianyu 335, Yangguang No. 1, Jinling 17 | |
| Qiqihar (Fularji) | Jinling 17, Dajingjiu 23 | |
| Jiamusi (Fujin) | Yangguang No. 1 | |
| Daqing (Lindian) | Ping' an 14 | |
| Daqing (Dorbod) | Ping' an 14 | |
| Jixi (Mishan) | Zhongyuandan 32 | |
| Suihua (Zhaodong) | Suiyu 7, Kendan 10, Yuqing 23 | |

### 1.2 Experimental Animals and Diet

Three healthy Holstein dairy cows fitted with permanent rumen fistulas were used for rumen degradation experiments at the Xiangfang Experimental Base of Northeast Agricultural University. The diet was formulated according to Chinese dairy feeding standards, with composition and nutrient levels shown in Table 2 . Cows were fed at 08:00 and 16:00 daily, had free access to water, and were housed individually.

**Table 2** Composition and nutrient levels of the experimental diet (air-dry basis), %

| Items | Content |
|---|---|
| **Ingredients** | |
| Corn | |
| Wheat bran | |
| Molasses | |

| Items | Content |
|---|---|
| Soybean meal | |
| Dried distillers grain | |
| Cottonseed meal | |
| Corn fiber meal | |
| Corn germ meal | |
| Premix[1] | |
| Chinese wild rye | |
| Corn silage | |
| **Total** | |
| **Nutrient levels[2]** | |
| NEL/(MJ/kg) | |
| CP | |
| NDF | |
| ADF | |
| Ca | |

[1] Each kilogram of premix contained: VA 800,000 IU, VD 700,000 IU, VE 10,000 IU, Fe 1,600 mg, Cu 1,500 mg, Zn 10,000 mg, Mn 3,500 mg, Se 80 mg, I 120 mg, Co 50 mg.
[2] NEL was a calculated value [**?**], while other nutrient levels were measured values.

### 1.3.1 Routine Nutrient Determination

Dry matter (DM) and crude protein (CP) were determined according to AOAC methods [**?**], and neutral detergent fiber (NDF) was determined according to Van Soest et al. [**?**].

### 1.3.2 Rumen Nylon Bag Technique

Following the method of Peng et al. [**?**], approximately 7 g of ground sample was placed in nylon bags (10 cm × 20 cm, 50  m pore size) of known weight, tied with nylon string 2 cm from the bag opening, resulting in a sample-to-surface-area ratio of approximately 19 mg/cm². Each sample was prepared in triplicate. Following the principle of "sequential insertion and simultaneous removal," bags were randomly placed in a 45 cm × 45 cm rumen mesh bag secured to the rumen fistula with a 90 cm rope before feeding, and incubated for 72, 48, 36, 24, 16, 12, 8, 4, and 0 h. No more than 28 bags were placed in the rumen per cow per time point. After removal (including 0 h), bags were rinsed under tap water until the water ran clear, then dried at 65 °C for 48 h to constant weight. The total weight of residue and nylon bag was recorded, and the residue was ground through a 1-mm sieve and stored in sealed bags for subsequent analysis.

### 1.3.3 Mid-Infrared Spectroscopy Detection

A BRUKER ALPHA mid-infrared spectrometer (Bruker Optics, Germany) was used. Following the method of Jiao et al. [**?**], under infrared lamp illumination and dry conditions, with potassium bromide (spectroscopic grade) as background, 2 mg of sample was thoroughly ground and mixed with 200 mg KBr in an agate mortar, pressed into pellets, and analyzed. Spectral acquisition parameters followed Kim et al. [**?**]: scanning range 700–4,000 cm $^{1}$ (Figure 1 [Figure 1: see original paper]), 128 scans, resolution 4 cm $^{1}$, with each sample loaded five times (Figure 2 [Figure 2: see original paper]) and repeated twice.

**Figure 1** The region of amide I and amide II of FTIR protein spectroscopy in 11 kinds of corn silages

**Figure 2** The region of amide I and amide II of FTIR protein spectroscopy in 11 kinds of corn silages

### 1.4.1 Calculation of Degradation Characteristics Parameters

The rumen disappearance rate of a component (%) was calculated as:
100 × (mass of component –mass of component in residue) / mass of component.

Rumen degradation kinetics were calculated using the exponential model of Ørskov et al. [**?**]:
Y = a + b(1 -e )
where Y is the rumen disappearance rate (%) after incubation time t, a is the rapidly degraded fraction (%), b is the slowly degraded fraction (%), c is the degradation rate of the slowly degraded fraction (%/h), and t is rumen incubation time (h).

The undegradable fraction was calculated as: u = 1 -(a + b), where u is the undegradable fraction (%).

Effective degradability was calculated as: ED = a + [(b × c)/(c + k)], where k is the rumen outflow rate (0.031/h) [**?**] and ED is effective degradability (%).

### 1.4.2 Mid-Infrared Spectral Analysis

Spectral processing software Ominic 8.2 (Spectra Tech, Madison, WI, USA) was used to analyze the protein region amide band segment (1,720–1,480 cm $^{1}$), with baseline correction applied to both amide I and amide II bands. The amide I region was subjected to second-derivative treatment, and the resulting spectrum was smoothed to identify corresponding protein secondary structure positions in the original spectrum [**?**]: -helix appears at 1,658–1,648 cm $^{1}$, and -sheet appears at 1,640–1,620 cm $^{1}$. This study analyzed peak height, peak area, peak height ratio, and peak area ratio of amide I and amide II bands, as well as peak height and peak height ratio of protein secondary structures -helix and -sheet, to explore potential relationships between corn silage protein molecular structures and rumen degradation characteristics of DM, CP, and NDF.

### 1.4.3 Statistical Analysis

Data were initially processed using Excel 2010. The PROC MIXED procedure in SAS 9.2 software was used to analyze rumen degradation parameters (rapidly degraded fraction, slowly degraded fraction, degradation rate of slowly degraded fraction, undegradable fraction, effective degradability) and protein molecular spectral data (peak area, peak height, peak area ratio, and peak height ratio of amide I and amide II bands; peak height and peak height ratio of secondary structures -helix and -sheet). The PROC CORR procedure was used to analyze Pearson correlations between rumen degradation characteristics and protein spectral data ($P < 0.01$ = very significant correlation; $P < 0.05$ = significant correlation; $P < 0.10$ = tendency to correlate; P 0.10 = no correlation). The PROC REG procedure was used for multiple regression analysis ($R^2 < 0.2$ = weak fit; $R^2 < 0.4$ = moderate fit; $R^2 < 0.6$ = moderately strong fit; $R^2 > 0.6$ = strong fit).

## Results

### Rumen Degradation and Protein Molecular Structure Parameters

The rumen degradation parameters of the 11 corn silages are summarized in Table 3. The rapidly degraded fraction of DM (DMa) ranged from 14.86% to 32.80%, the slowly degraded fraction (DMb) ranged from 43.58% to 67.36%, the degradation rate of the slowly degraded fraction (DMc) ranged from 1.46%/h to 4.93%/h, the undegradable fraction (DMu) ranged from 11.75% to 31.16%, and DMED ranged from 40.99% to 54.58%. For CP, CPa ranged from 35.80% to 61.02%, CPb ranged from 12.24% to 46.09%, CPc ranged from 0.85%/h to 7.07%/h, CPu ranged from 10.41% to 44.66%, and CPED ranged from 45.85% to 69.35%. For NDF, NDFa ranged from 1.03% to 22.70%, NDFb ranged from 46.34% to 88.05%, NDFc ranged from 1.23%/h to 4.12%/h, NDFu ranged from 8.81% to 35.39%, and NDFED ranged from 32.98% to 47.59%. The substantial variation in rumen degradation parameters among corn silages from different regions provided robust data support for subsequent correlation and regression analyses.

**Table 3** Summary of rumen degradation characteristics data of corn silages

| Items | Mean | Range | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| **DM** | | | | | |
| a (%) | | | | | |
| b (%) | | | | | |
| c (%/h) | | | | | |
| u (%) | | | | | |
| ED (%) | | | | | |
| **CP** | | | | | |
| a (%) | | | | | |

| Items | Mean | Range | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| b (%) | | | | | |
| c (%/h) | | | | | |
| u (%) | | | | | |
| ED (%) | | | | | |
| **NDF** | | | | | |
| a (%) | | | | | |
| b (%) | | | | | |
| c (%/h) | | | | | |
| u (%) | | | | | |
| ED (%) | | | | | |

*a, rapidly degraded fraction; b, slowly degraded fraction; c, degradation rate of b; u, undegradable fraction; ED, effective degradability. The same as below.*

The protein molecular structural information of the 11 corn silages is presented in Table 4. Amide I peak area (A_ ) ranged from 4.66 to 11.68, amide II peak area (A_ ) ranged from 0.39 to 1.00, amide I peak height (H_ ) ranged from 0.06 to 0.14, amide II peak height (H_ ) ranged from 0.02 to 0.05, -helix peak height ( ) ranged from 0.05 to 0.10, -sheet peak height ( ) ranged from 0.06 to 0.14, amide I to II peak area ratio (A_ _ ) ranged from 5.53 to 22.02, amide I to II peak height ratio (H_ _ ) ranged from 1.72 to 4.34, and -helix to -sheet peak height ratio ( _ ) ranged from 0.58 to 1.00. Substantial variation was observed in protein molecular structure parameters among the 11 corn silages from different regions.

**Table 4** Summary of protein structural characteristics data of corn silages

| Items | Mean | Range | Minimum | Maximum |
|---|---|---|---|---|
| A_ | | | | |
| A_ | | | | |
| A_ _ | | | | |
| H_ | | | | |
| H_ | | | | |
| H_ _ | | | | |
| _ | | | | |

*A_ , peak area of amide I; A_ , peak area of amide II; A_ _ , ratio of amide I peak area to amide II; H_ , peak height of amide I; H_ , peak height of amide II; H_ _ , ratio of amide I peak height to amide II; , peak height of -helix; , peak height of -sheet; _ , ratio of -helix peak height to -sheet. The same as below.*

**Correlations between Rumen Degradation Characteristics and Protein Molecular Structures**

**2.2.1 Dry Matter Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table 5 , DMa was significantly negatively correlated with A_ (r = -0.47, P = 0.03) and significantly positively correlated with A_ _ (r = 0.45, P = 0.03) and H_ _ (r = 0.57, P = 0.01). DMb tended to be negatively correlated with A_ _ (r = -0.37, P = 0.09) and was significantly negatively correlated with H_ _ (r = -0.55, P = 0.01). DMc tended to be negatively correlated with H_ (r = -0.37, P = 0.09) and positively correlated with (r = 0.37, P = 0.09), and was very significantly positively correlated with _ (r = 0.59, P = 0.004). DMu was very significantly positively correlated with (r = 0.59, P = 0.004) and tended to be positively correlated with (r = 0.43, P = 0.05). DMED was very significantly negatively correlated with A_ and H_ (r = -0.61, P = 0.003; r = -0.71, P = 0.0002), significantly positively correlated with A_ _ (r = 0.44, P = 0.04), and very significantly positively correlated with H_ _ (r = 0.61, P = 0.003).

**Table 5** Correlation between rumen degradation characteristics of dry matter and protein structural characteristics of corn silages

| Items | A_ | A_ | A_ _ | H_ | H_ | H_ _ | _ |
|---|---|---|---|---|---|---|---|
| **DM** | | | | | | | |
| a | | | | | | | |
| b | | | | | | | |
| c (%/h) | | | | | | | |
| u | | | | | | | |
| ED | | | | | | | |

*r, correlation coefficient; P, P-value. The same as below.*

**2.2.2 Crude Protein Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table 6 , CPa was significantly positively correlated with (r = 0.45, P = 0.04) and tended to be positively correlated with H_ _ and _ (r = 0.37, P = 0.09; r = 0.39, P = 0.07). CPc tended to be negatively correlated with A_ and (r = -0.38, P = 0.08; r = -0.38, P = 0.08), was significantly negatively correlated with A_ _ (r = -0.46, P = 0.03), and significantly positively correlated with _ (r = 0.57, P = 0.01). CPu was significantly positively correlated with A_ (r = 0.50, P = 0.02), significantly negatively correlated with A_ _ (r = -0.48, P = 0.03), and significantly positively correlated with H_ _ (r = 0.50, P = 0.02). CPED tended to be negatively correlated with A_ (r = -0.40, P = 0.06), positively correlated with and H_ _ (r = 0.38, P = 0.09; r = 0.38, P = 0.08), and significantly positively correlated with _ (r = 0.43, P = 0.04).

**Table 6** Correlation between rumen degradation characteristics of crude protein and protein structural characteristics of corn silages

| Items | A_ | A_ | A_ _ | H_ | H_ | H_ _ | _ |
|---|---|---|---|---|---|---|---|
| **CP** | | | | | | | |
| a | | | | | | | |
| b | | | | | | | |
| c (%/h) | | | | | | | |
| u | | | | | | | |
| ED | | | | | | | |

**2.2.3 Neutral Detergent Fiber Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table 7 , NDFa showed no correlation with protein molecular structures (P > 0.10). NDFb tended to be positively correlated with H_ (r = 0.39, P = 0.08). NDFc was significantly negatively correlated with H_ (r = -0.52, P = 0.01) and tended to be positively correlated with _ (r = 0.39, P = 0.08). NDFu was significantly negatively correlated with H_ (r = -0.46, P = 0.03). NDFED tended to be positively correlated with _ (r = 0.42, P = 0.05).

**Table 7** Correlation between rumen degradation characteristics of neutral detergent fiber and protein structural characteristics of corn silages

| Items | A_ | A_ | A_ _ | H_ | H_ | H_ _ | _ |
|---|---|---|---|---|---|---|---|
| **NDF** | | | | | | | |
| a | | | | | | | |
| b | | | | | | | |
| c (%/h) | | | | | | | |
| u | | | | | | | |
| ED | | | | | | | |

**Regression Analysis between Rumen Degradation Characteristics and Protein Molecular Structures**

**2.3.1 Regression Analysis for Dry Matter Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table 8 , H_ _ was the best independent variable for fitting regression equations of DMa and DMb, with determination coefficients ($R^2$) of 0.32 and 0.30, respectively, indicating moderate fit. was the best independent variable for fitting DMu ($R^2$ = 0.34, moderate fit). _ was the best independent variable for fitting DMc ($R^2$ = 0.35, moderate fit). H_ showed good fit with DMED ($R^2$ = 0.50).

**2.3.2 Regression Analysis for Crude Protein Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table

8, could fit CPa ($R^2$ = 0.20). No fitting equation was obtained for CPb with protein molecular structures. _ moderately fitted CPc ($R^2$ = 0.33). H_ _ was the best independent variable for fitting both CPu and CPED, with $R^2$ values of 0.25 and 0.48, respectively, showing the best fit for CPED.

**2.3.3 Regression Analysis for Neutral Detergent Fiber Rumen Degradation Characteristics and Protein Molecular Structures** As shown in Table 8, protein molecular structures showed weak correlations with NDFa and NDFb, and no fitting equations were obtained. H_ could fit both NDFc and NDFu, with $R^2$ values of 0.27 and 0.21, respectively. _ could fit NDFED, but the fit was weak ($R^2$ = 0.18).

**Table 8** Regression relationships between protein structural characteristics and rumen degradation characteristics of corn silages

| Predicted variables (Y) | Variable selection (x) | Prediction equations | $R^2$ | P-value |
|---|---|---|---|---|
| **DM** | | | | |
| a | H_ _ | Y = 8.982 + 4.343 × x | 0.32 | |
| b | H_ _ | Y = 71.949 - 6.095 × x | 0.30 | |
| c (%/h) | _ | Y = -2.019 + 5.683 × x | 0.35 | |
| u | | Y = 3.531 + 269.132 × x | 0.34 | |
| ED | H_ | Y = 58.106 - 353.667 × x | 0.50 | |
| **CP** | | | | |
| a | | Y = 27.200 + 229.081 × x | 0.20 | |
| c (%/h) | _ | Y = 4.352 + 5.510 × x1 + 39.033 × x2 | 0.33 | |
| u | H_ _ | Y = -5.575 + 10.646 × x | 0.25 | |
| ED | H_ _ | Y = 54.331 - 6.826 × x | 0.48 | |
| **NDF** | | | | |
| c (%/h) | H_ | Y = 4.424 - 57.506 × x | 0.27 | |
| u | H_ | Y = 40.446 - 500.980 × x | 0.21 | |
| ED | _ | Y = 23.206 + 18.736 × x | 0.18 | |

## Discussion

### 3.1 Relationship between Dry Matter Rumen Degradation Characteristics and Protein Molecular Structure

Protein molecular structures showed better fit for DMb than DMa, possibly because DMa is more closely associated with protein molecular structures than DMb. The correlation between DMc and protein molecular structures partially disagreed with Zhang et al. [**?**], who used air as background for direct sample detection—a principle consistent with this study. The discrepancy may be attributed to different research objects, as their study investigated the relationship between five different ratios of corn distillers dried grains with solubles (DDGS) and hulless barley mixtures and rumen degradation characteristics. Different feed types have different nutritional values, which may lead to variations in the relationship between protein molecular structures and rumen degradation characteristics.

The ability to establish relationships between DM degradation characteristics and protein molecular structures, with some degradation features showing good fit, may be due to associations with aromatic compound vibrations in fiber, which concentrate in the 1,498–1,587 cm $^{1}$ spectral range [**?**] within the protein structural spectral region, thus creating certain connections.

### 3.2 Relationship between Crude Protein Rumen Degradation Characteristics and Protein Molecular Structure

Protein molecular spectral characteristics (spectral intensities of amide I and II bands, secondary structure intensities, and their ratios) affect CP quality and utilization efficiency [**?**, **?**], primarily because protein structure influences microbial and gastrointestinal digestive enzyme access to CP. Therefore, studying protein molecular structure is crucial for understanding CP composition, quality, and rumen degradation characteristics. Feed CP rumen degradation characteristics are closely linked to molecular structure [**?**].

Liu et al. [**?**] used diffuse reflectance infrared spectroscopy to study degradation differences between hull and seed of six barley varieties, finding that CPa was significantly positively correlated with the -helix to -sheet peak height ratio—a conclusion consistent with this study. However, they did not find the significant positive correlation between CPa and -helix peak height observed in our study, possibly due to differences in protein sources and processing methods. Zhang et al. [**?**] found no relationship between CPc and -helix peak height, consistent with our results, but our finding that CPc was negatively correlated with -sheet peak height and significantly positively correlated with -helix to -sheet peak height ratio disagreed with their report of no correlation. Our conclusion that the -helix to -sheet peak height ratio showed no correlation with CPu but significant positive correlation with CPED, with good fit, aligns with reports by Jiao et al. [**?**], Wu et al. [**?**], Liu et al. [**?**], and Yari et al. [**?**], indicating that higher -helix to -sheet peak height ratios in feed protein correspond to

higher effective degradability. Zhang et al. [?] noted that the -helix to -sheet peak height ratio is closely related to protein nutritional value and utilization efficiency, though the amide I to II peak area ratio is not completely correlated. Our study found no correlation between CPED and amide I to II peak area ratio, consistent with Liu et al. [?].

Compared to barley, oats, and wheat, feathers contain up to 84% -sheet but have the lowest protein utilization efficiency, closely related to structural features including -sheet content, keratin content, and disulfide bonds [?]. This demonstrates that the nutritional value of plant feed proteins is not only related to protein type and total protein and amino acid content but also to sensitivity to hydrolytic enzymes in the rumen and, more importantly, to protein molecular structure. The proportion of -sheet is typically associated with accessibility to gastrointestinal digestive enzymes. Yu [?] found in studies on different processing methods for golden flaxseed that heating increased protein-lignin linkages and Maillard reaction products, while increased -sheet proportion in protein secondary structure affected protein absorption and utilization. Therefore, similar protein contents with different proportions of -helix and -sheet secondary structures can lead to different degradation characteristics [?], with high -sheet proportions generally resulting in low nutritional value [?, ?, ?].

### 3.3 Relationship between Neutral Detergent Fiber Rumen Degradation Characteristics and Protein Molecular Structure

The rumen degradation characteristics of NDF are more closely related to the carbohydrate spectral region [?, ?], with limited research on its relationship with protein molecular structures. Our results also indicated weak associations between protein molecular structures and NDF rumen degradation characteristics, with no fitting equations obtained for NDFa and NDFb, and low fit for NDFED. This weak relationship may similarly be due to the presence of aromatic compounds in lignin that are not utilized by microorganisms, with some aromatic compound vibrations interfering with protein amide bands [?].

## Conclusions

1. FTIR technology can obtain spectral information on corn silage protein molecular structures and establish regression equations based on correlations with rumen degradation characteristics. The peak height of amide II band was very significantly correlated with DMED and showed the best fit. The peak height ratio of amide I to II and the peak height ratio of protein secondary structures ( -helix and -sheet) showed the best fit for CPED. Protein secondary structures showed weak fit for NDFED.

2. FTIR technology can simultaneously analyze multiple rumen degradation characteristics with rapid analysis speed, offering advantages over conventional methods. Evaluating some rumen degradation characteristics using

corn silage protein molecular structures is feasible, with the best evaluation results for DM and CP effective degradability.

## References

[1] ALDERMAN G, COTTRILL B R. Energy and protein requirements of ruminants: an advisory manual prepared by the AFRC technical committee on responses to nutrients[M]. Wallingford, UK: CAB International, 1993.
[2] NRC. Nutrient requirements of dairy cattle[M]. 7th ed. Washington, D.C.: National Academy of Sciences, 2001.
[3] CASPER D P, MAIGA H A, BROUK M J, et al. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows[J]. Journal of Dairy Science, 1999, 82(8): 1779–1790.
[4] YU P, CHRISTENSEN D A, CHRISTENSEN C R, et al. Use of synchrotron FTIR microspectroscopy to identify chemical differences in barley endosperm tissue in relation to rumen degradation characteristics[J]. Canadian Journal of Animal Science, 2004, 84(3): 523–527.
[5] LIU N, YU P Q. Molecular clustering, interrelationships and carbohydrate conformation in hull and seeds among barley cultivars[J]. Journal of Cereal Science, 2011, 53(3): 379–383.
[6] YU P Q, JONKER A, GRUBER M. Molecular basis of protein structure in proanthocyanidin and anthocyanin-enhanced Lc-transgenic alfalfa in relation to nutritive value using synchrotron-radiation FTIR microspectroscopy: a novel approach[J]. Spectrochimica Acta Part A, 2009, 73(5): 846–853.
[7] YU P. Application of advanced synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and science: a novel approach[J]. British Journal of Nutrition, 2004, 92(6): 869–885.
[8] 卢雁, 张玮玮, 王公轲. FTIR 用于变性蛋白质二级结构的研究进展 [J]. 光谱学与光谱分析, 2008, 28(1): 88–93.
[9] YU P Q, MCKINNON J J, CHRISTENSEN C R, et al. Using synchrotron-based FTIR microspectroscopy to reveal chemical features of feather protein secondary structure: comparison with other feed protein sources[J]. Journal of Agricultural and Food Chemistry, 2004, 52(24): 7353–7361.
[10] YU P, MCKINNON J J, SOITA H W, et al. Use of synchrotron-based FTIR microspectroscopy to determine protein secondary structures of raw and heat-treated brown and golden flaxseeds: a novel approach[J]. Canadian Journal of Animal Science, 2005, 85(4): 437–448.
[11] DOIRON K, YU P, MCKINNON J J, et al. Heat-induced protein structure and subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle[J]. Journal of Dairy Science, 2009, 92(7): 3319–3330.
[12] SAMADI, YU P. Dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans[J]. Journal of Dairy Science, 2011, 94(12): 6092–6102.
[13] YU P Q. Protein molecular structures, protein subfractions, and protein availability affected by processing: a review[J]. American Journal of Biochemistry and Biotechnology, 2007, 3(2): 66–86.

[14] YU P Q. Protein secondary structures ( -Helix and -Sheet) at a cellular level and protein fractions in relation to rumen degradation behaviors of protein: a novel approach[J]. British Journal of Nutrition, 2005, 94(4): 655-665.
[15] DYSON H J, WRIGHT P E. Peptide conformation and protein folding[J]. Current Opinion in Structural Biology, 1993, 3(1): 60-65.
[16] KEMP W. Organic spectroscopy[M]. Edinburgh: W H Freeman & Co, 1991.
[17] JACKSON M, MANTSCH H H. The use and misuse of FTIR spectroscopy in the determination of protein structure[J]. Critical Reviews in Biochemistry and Molecular Biology, 1995, 30(2): 95-120.
[18] JACKSON M, MANTSCH H. Biomedical infrared spectroscopy[M]//MANTSCH H H, CHAPMAN D. Infrared Spectroscopy of Biomolecules. New York: Wiley-Liss Inc., 1996.
[19] (美) 国家科学研究委员会. 奶牛营养需要 [M]. 孟庆翔, 译. 北京: 中国农业大学出版社, 2002.
[20] AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists[M]. 7th ed. [S.l.]: Association of Official Analytical Chemists, 1950.
[21] VAN SOEST P J, ROBERTSON B R, LEWIS B A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition[J]. Journal of Dairy Science, 1991, 74(10): 3583-3597.
[22] PENG Q H, KHAN N A, WANG Z S, et al. Moist and dry heating-induced changes in protein molecular structure, protein subfractions, and nutrient profiles in camelina seeds[J]. Journal of Dairy Science, 2013, 97(1): 446-457.
[23] 焦培鑫, 刘大森, 郑帅, 等. 饲料原料蛋白质二级结构对奶牛瘤胃降解特性的影响 [J]. 饲料工业, 2012, 33(13): 48-51.
[24] KIM S, KIM H, REUHS H, et al. Differentiation of outer membrane proteins from Salmonella enterica serotypes using Fourier transform infrared spectroscopy and chemometrics[J]. Letters in Applied Microbiology, 2006, 42(3): 229-234.
[25] ØRSKOV E R, MCDONALD I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage[J]. Journal of Agricultural Science, 1979, 92(2): 499-503.
[26] 莫放, 冯仰廉. 常用饲料蛋白质在瘤胃的降解率 [J]. 中国畜牧杂志, 1995(3): 23-26.
[27] ZHANG X W, YU P Q. Using ATR-FT/IR molecular spectroscopy to detect effects of blend DDGS inclusion level on the molecular structure spectral and metabolic characteristics of the proteins in hulless barley[J]. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2012, 95: 53-63.
[28] BELANCHE A, WEISBJERG M R, ALLISON G G, et al. Measurement of rumen dry matter and neutral detergent fiber degradability of feeds by Fourier-transform infrared spectroscopy[J]. Journal of Dairy Science, 2014, 97(4): 2361-2375.
[29] WETZEL D L, SRIVARIN P, FINNEY J R. Revealing protein infrared spectral detail in a heterogeneous matrix dominated by starch[J]. Vibrational Spectroscopy, 2003, 31(1): 109-114.

[30] SEGUCHI M, TAKEMOTO M, MIZUTANI U, et al. Effects of secondary structures of heated egg white protein on the binding between prime starch and tailings fractions in fresh wheat flour[J]. Cereal Chemistry, 2004, 81(5): 633-636.
[31] YU P. Molecular chemistry imaging to reveal structural features of various plant feed tissues[J]. Journal of Structural Biology, 2005, 150(1): 81-89.
[32] 吴鹏华, 刘大森, 仝泳, 等. 干酒糟及其可溶物和豆粕混合饲料蛋白质二级结构与瘤胃降解特性的关系 [J]. 动物营养学报, 2013, 25(11): 2763-2769.
[33] YARI M, VALIZADEH R, NASERIAN A A, et al. Protein molecular structures in alfalfa hay cut at three stages of maturity and in the afternoon and morning and relationship with nutrient availability in ruminants[J]. Journal of the Science of Food and Agriculture, 2013, 93(12): 3072-3080.
[34] ZHANG X, YU P. Molecular basis of protein structure in combined feeds (hulless barley with bioethanol coproduct of wheat dried distillers grains with solubles) in relation to protein rumen degradation kinetics and intestinal availability in dairy cattle[J]. Journal of Dairy Science, 2012, 95(6): 3363-3379.
[35] WARD W H, LUNDGREN H P. The formation, composition, and properties of keratins[J]. Advances in Protein Chemistry, 1954, 9: 243-297.
[36] LAMMERS K, ARBUCKLE-KEIL G, DIGHTON J. FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning[J]. Soil Biology and Biochemistry, 2009, 41(2): 340-347.
[37] STEWART D. Fourier transform infrared microspectroscopy of plant tissues[J]. Applied Spectroscopy, 1996, 50(3): 357-365.
[38] HIMMELSBACH D S, KHALILI S, AKIN D E. FT-IR microspectroscopic imaging of flax (Linum usitatissimum L.) stems[J]. Cellular and Molecular Biology, 1998, 44(1): 99-108.

---

*Responsible editor: TIAN Yanming*

*Note: Figure translations are in progress. See original paper for figures.*