Heat-induced protein structure and subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle

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ABSTRACT

The objectives of this study were to reveal protein structures of feed tissues affected by heat processing at a cellular level, using the synchrotron-based Fourier transform infrared microspectroscopy as a novel approach, and quantify protein structure in relation to protein digestive kinetics and nutritive value in the rumen and intestine in dairy cattle. The parameters assessed included 1) protein structure α -helix to β -sheet ratio; 2) protein subfractions profiles; 3) protein degradation kinetics and effective degradability; 4) predicted nutrient supply using the intestinally absorbed protein supply (DVE)/degraded protein balance (OEB) system for dairy cattle. In this study, Vimy flaxseed protein was used as a model feed protein and was autoclave-heated at 120°C for 20, 40, and 60 min in treatments T1, T2, and T3, respectively. The results showed that using the synchrotron-based Fourier transform infrared microspectroscopy revealed and identified the heat-induced protein structure changes. Heating at 120°C for 40 and 60 min increased the protein structure α -helix to β -sheet ratio. There were linear effects of heating time on the ratio. The heating also changed chemical profiles, which showed soluble CP decreased upon heating with concomitant increases in nonprotein nitrogen, neutral, and acid detergent insoluble nitrogen. The protein subfractions with the greatest changes were PB1, which showed a dramatic reduction, and PB2, which showed a dramatic increase, demonstrating a decrease in overall protein degradability. In situ results showed a reduction in rumen-degradable protein and in rumen-degradable dry matter without differences between the treatments. Intestinal digestibility, determined using a 3-step in vitro procedure, showed no changes to rumen undegradable protein. Modeling results showed that heating increased total intestinally absorbable protein (feed DVE value) and decreased degraded protein bal-

ance (feed OEB value), but there were no differences between the treatments. There was a linear effect of heating time on the DVE and a cubic effect on the OEB value. Our results showed that heating changed chemical profiles, protein structure α -helix to β -sheet ratio, and protein subfractions; decreased rumen-degradable protein and rumen-degradable dry matter; and increased potential nutrient supply to dairy cattle. The protein structure α -helix to β -sheet ratio had a significant positive correlation with total intestinally absorbed protein supply and negative correlation with degraded protein balance.

Key words: synchrotron, heat-induced protein structure and fraction, nutrient availability, rumen and intestine

INTRODUCTION

Recent published reviews show that the effects of heat processing on protein nutritive value and performance in animals are equivocal (Yu et al., 2002, 2004). Part of reason is that heating conditions inside a feed may not be optimal (either heating duration is too long or temperature too high), resulting in the feed being either underheated or overheated. One novel approach proposed here to check effects of heat processing on protein value and nutrient availability was to look at the magnitude of changes to the intrinsic protein structures in terms of protein α -helix to β -sheet ratio within the intact tissue at a cellular level. The protein structure profiles partly affect access to gastrointestinal digestive enzymes, which affects protein availability. However, studies on protein secondary structures in relation to nutritive value and digestive behaviors of protein in animals are extremely rare.

Recently, flaxseed has garnered interest as a functional food source in humans. This is due in large part to the high content of α -linolenic acid in flaxseed, but it is also a good source of high quality protein and lignin. All of these have benefits to the health of the consumer (Oomah, 2001). Flaxseed is generating interest as a functional feed source for ruminant production systems as well. The interest in flaxseed for the produc-

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tion system is caused by the same chemical components (such as α -linolenic acid); however, the benefits are multifaceted. First, ruminants fed flaxseed show improvements in reproductive health indicators. Petit and Twagiramungu (2006) found a decrease in embryo mortality, and consequently improvement in the establishment of pregnancy when feeding flaxseed compared with other fat sources. Ambrose et al. (2006) found a similar decrease in pregnancy losses when feeding flaxseed compared with sunflower seed. Second, the fatty acid profile of both milk and meat products changes upon feeding flaxseed as demonstrated by Petit (2002) and Scollan et al. (2001).

Often, in highly productive animals, there is an improvement in indicators of productivity when a greater amount of the dietary amino acids can reach the small intestine for absorption. This is commonly achieved for oilseeds and oilseed meals by heating (McKinnon et al., 1995; Mustafa et al., 2003a). The effects that thermal processing will have on degradability depend on the method used to apply heat, the presence of moisture, temperature, and time. Petit et al. (2002) demonstrated that a moist heating environment was more efficient than dry heat. At 100°C, there was large difference in RUP between the heating methods, but at 120°C there was much less difference between the heating methods. This reveals the complexity of the heating effects on a feed. Overall seed structure was not changed during heating, yet somehow the presence of moisture is a critical factor at certain temperatures, perhaps acting as a catalyst for the reactions that cause changes in protein degradability. When extrusion is used as a method of heat application, the consequences to RUP can be the opposite; Mustafa et al. (2003b) have demonstrated this while extruding at a temperature of 155°C and with a 43-s residence time. The conclusion from that study was that, under the conditions of extrusion used in the study, it was ineffective at increasing the post-ruminal supply of dietary amino acids. The abrogation of the oil/protein matrix could explain this result. To date, little information is available about flaxseed and few studies examine how variations in the same heating process would influence protein degradability. This study was part of a large flaxseed research program and focused on protein molecular structure and nutritive value in dairy cattle.

The objectives of this study were to reveal molecular chemistry of protein structures of feed tissues affected by heat processing at a cellular level, using the advanced synchrotron-based Fourier transform infrared microspectroscopy technology as a novel approach, and to quantify protein secondary structures in relation to protein digestive behaviors and nutritive value in the rumen and intestine in dairy cattle. The parameters

assessed included: 1) molecular structural differences in protein α -helix to β -sheet ratio; 2) protein subfraction profiles; 3) protein degradation kinetics and effective degradability; 4) predicted nutrient supply using the DVE/OEB system (Tamminga et al., 1994, 2007) in terms of the intestinally absorbed protein supply (DVE) and degraded protein balance (OEB).

MATERIALS AND METHODS

The animals used in this study were cared for according to the guidelines put forward by the Canadian Council on Animal Care (1993).

Flaxseed Heating and Processing

Vimy flaxseed protein was used as a model feed protein and was heated (autoclaved) at the same temperature but for different durations. Flaxseed (*Linum usitatissimum* cv. Vimy) grown near Moose Jaw, Saskatchewan, Canada, during the 2005 growing year was provided by Shamrock Seeds Ltd. (Saskatoon, Saskatchewan, Canada). Samples of flaxseed (3 kg) were heated by autoclave (Amsco Eagle SG-3031, Steris Corp., Mentor, OH) at 120°C in gravity mode for 20, 40, or 60 min in an autoclave tray, as treatments T1, T2, and T3, respectively. The temperature and duration was chosen based on the preliminary study of Mustafa et al. (2003a). Treatments were then repeated on a separate set of 3-kg samples, providing 2 series, A and B, of treatments. Control treatments were kept raw. Samples were subsequently cooled to room temperature and then placed in the refrigerator to minimize sticking and clumping while grinding. The samples were ground using a Retsch SM 2000 (Retsch Inc., Newtown, PA) fitted with a 2-mm screen. Samples were fed slowly into the grinder to further prevent sticking and clumping during the grinding process and to ensure the flaxseed was cracked open and not "extruded" through the screen.

Animals and Diets

Two nonlactating Holstein dairy cows fitted with flexible rumen cannula (10 cm internal diameter (Bar Diamond Inc., Parma, ID) were used to determine the in situ degradation kinetics of flaxseed. The cows were housed in the research barn at the University of Saskatchewan during the period of study. Pens were approximately 6 × 9 m. The cows were fed a 50:50 barley silage (26.8% DM) to concentrate diet according to NRC maintenance requirement (NRC, 2001). The cows were fed half of the ration twice daily at 0800 and 1600 h. Water was available ad libitum.

Rumen Incubation

Rumen degradation kinetics were determined using the standard department in situ method (McKinnon et al., 1995; Yu et al., 2003a). Samples (7 g) were placed into numbered bags measuring 10 × 20 cm made of Nitetex 03-41/31 monofilament open mesh fabric (Screentec Corp., Mississauga, ON, Canada) with a pore size of 41 µm. The seams of the bags were sewn shut and sealed with glue to prevent samples from escaping during incubation. The sample bags were placed in a polyester mesh bag (45 × 45 cm with a 90-cm length of rope to anchor it to the cannula) weighed down with a plastic bottle (250 mL) filled with gravel to keep the samples in the liquid strata of the rumen. Bags were added according to the “gradual addition/all out” schedule and were incubated for 72, 48, 24, 12, 8, 4, 2, and 0 h at 2100 (d 1), 2100 (d 2), 2100 (d 3), 0900 (d 4), 1300 (d 4), 1700 (d 4), 1900 h (d 4), respectively. The number of bags incubated from each sample was increased based on incubation time to ensure an adequate amount of residue would remain for analysis. Estimates for the amount of sample required for incubation were obtained using data from Mustafa et al. (2003a). All treatments (from A and B series) for every incubation period were carried out in 2 runs in 2 nonlactating cows. For each cow at each run, there were 2, 2, 2, 2, 2, 3, 5, and 6 bags for 0, 2, 4, 8, 12, 24, 48, and 72 h incubations, respectively. After incubation, bags were rinsed with cold water, and then washed in groups of 10 with 2 L of water 5 times so the water from the last rinse cycle would run clear. Washed bags were then dried at 55°C in a forced air oven for 48 h. Dry samples were stored in a refrigerated room (4°C) until chemical analysis was performed.

Chemical Analysis

Dried samples were ground through a 1-mm screen using a Retsch ZM 100 (Retsch Inc.). Samples were then analyzed for DM (AOAC, 1990; method 930.15), ash (AOAC, 1990; method 942.05), ether extract (EE; AOAC, 1990; method 920.39), CP (AOAC, 1990; method 984.13; Kjeltex 2400 analyzer unit, Foss Tecator, Hillerød, Denmark), ADIN (Licitra et al. 1996), neutral detergent insoluble nitrogen (NDIN; Licitra et al. 1996), NPN (Roe et al., 1990), and starch (AOAC, 1990; method 996.11, using the Total Starch assay kit, Megazyme, Bray, Ireland); acid detergent insoluble protein (ADIP) and neutral detergent insoluble protein (NDIP) were calculated as ADIP = 6.25 × ADIN and NDIP = 6.25 × NDIN, respectively. Soluble CP (SCP) was determined by incubating the sample with bicarbonate-phosphate buffer and filtering through

Whatman #54 filter paper (Roe et al., 1990). Acid detergent fiber, NDF, and acid detergent lignin were analyzed (Van Soest et al., 1991) by Ankom filter bag method (Ankom A200 Filter Bag technique, Ankom Technology, Fairport, NY) with a modified fat extraction procedure, which included an initial 2-h ether extraction before the standard Ankom acetone fat extraction protocol, to prevent the high fat content of the flaxseed samples from giving erroneously high values for the fiber measurements. Neutral detergent fiber was analyzed without the addition of sodium sulfite and with the inclusion of heat stable α-amylase as described previously (Van Soest et al. 1991). Total carbohydrate (CHO) was calculated as: CHO = 100 – EE – CP – ash (NRC, 2001). Nonfiber carbohydrate was calculated as: NFC = 100 – (NDF – NDIP) – EE – CP – ash (NRC, 2001).

Fractionation of Protein Fractions According to CNCPS

Crude protein fractions were partitioned according to the Cornell Net Carbohydrate Protein System (CNCPS; Sniffen et al., 1992). The characterizations of the CP fractions used in this system are as follows: fraction **PA** is NPN, fraction **PB** is true protein, and fraction **PC** is unavailable protein. Fraction **PB** is further divided into 3 fractions (**PB1**, **PB2**, and **PB3**) that are believed to have different rates of degradation in the rumen. Fraction **PB1** was calculated as PB – PB2 – PB3. Buffer-insoluble protein minus fraction **PB3** is used to estimate fraction **PB2**. Fraction **PB2** is insoluble in buffer but soluble in neutral detergent, whereas fraction **PB3** is insoluble in both buffer and neutral detergent, but is soluble in acid detergent. Fraction **PB2** is fermented in the rumen at a lower rate than buffer-soluble fractions, and some of the **PB2** fraction escapes to the lower gut. Fraction **PB3** is believed to be more slowly degraded in the rumen than fractions **PB1** and **PB2** because of its association with the plant cell wall; a large proportion of **PB3** is thus believed to escape the rumen. Fraction **PC** is the ADIN, which is highly resistant to breakdown by microbial and mammalian enzymes, and it is assumed unavailable for the animal (Sniffen et al., 1992). The relative rumen degradation rates of the 5 protein fractions have been described by Sniffen et al. (1992) as follows: fraction **PA** is assumed to be infinity, fraction **PB1** is 120 to 400 %/h, fraction **PB2** is 3 to 16%/h, fraction **PB3** is 0.06 to 0.55%/h. Fraction **PC** is considered to be undegradable.

Rumen Degradation Characteristics

Rumen degradation kinetics of the raw (control) and autoclaved flaxseed were determined for DM and CP

degradation. Degradation kinetics were determined using a first-order rumen degradation equation modified to include lag time outlined by Dhanoa (1988):

$$Y(t) = S + D \times (1 - \exp^{-K_d(t-T_0)}),$$

where $Y(t)$ represents the feed disappearance (%) remaining at time t ; D represents the degradable part of the insoluble fraction (%); S represents the soluble fraction (%); U represents the undegradable fraction (%), calculated as $100 - S - D$; K_d represents the degradation rate (%/h); and T_0 represents the lag time for feed to begin degrading. The model was fitted to the data using PROC NLIN (nonlinear) of SAS (version 9.1.3; SAS Institute Inc., Cary, NC) via the Gauss-Newton method, which is an iterative nonlinear regression protocol.

The ruminally degraded (RD) fractions for DM and CP were also considered and calculated according to formulas outlined in NRC (2001):

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

where K_p represents the rate of passage and is assumed to be 6%/h (Tamminga et al., 1994).

Intestinal Protein Digestion Determination

Intestinal digestibility of rumen undegraded feed protein was determined according to the protocol for ruminants outlined in Calsamiglia and Stern (1995). Briefly, nylon bags containing feed samples were incubated in the rumen for 12 h. Residue containing ca. 15 mg of N after ruminal exposure was incubated for 1 h in 10 mL of a 0.1 N HCl solution containing 1 g of pepsin/L. After incubation, pH was neutralized with 0.5 mL of 1 N NaOH and 13.5 mL of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin were added to the solution and incubated at 38°C. After a 24-h incubation, 3 mL of a 100% (wt/vol) TCA solution was added to precipitate undigested proteins (Calsamiglia and Stern, 1995). The pellets were analyzed for N.

Nutrient Supply with the DVE/OEB System

The DVE/OEB system is outlined by Tamminga et al. (1994) and revised in 2007 (Tamminga et al., 2007). It is often used to evaluate feed protein value (Yu et al., 2000). It constitutes a 2 part system in which each feed has a DVE and OEB value. The DVE value comprises digestible feed protein, microbial protein, and an endogenous protein loss correction. The OEB value,

or degraded protein balance, reflects the difference between potential microbial protein synthesis based on degraded feed protein and the potential microbial protein synthesis based on the energy available for microbial fermentation in the rumen.

The DVE value was calculated as

$$DVE = AMCP + ARUP - ENDP,$$

where **AMCP** is absorbable fraction of microbial crude protein (**MCP**), **ARUP** is the absorbable fraction of ruminally undegraded feed protein, and **ENDP** is a correction factor for endogenous protein lost during the digestion process.

The degradable protein balance or OEB value of a feed is the difference between the potential MCP synthesis based on RDP (**MCP_{RDP}**, in the DVE/OEB system) and the potential MCP synthesis based on energy extracted from anaerobic fermentation (**MCP_{FOM}**). Therefore,

$$OEB = MCP_{RDP} - MCP_{FOM},$$

where **MCP_{RDP}** was calculated as $MCP_{RDP} = CP \times [1 - (1.11 \times RUP(\%CP)/100)]$ and **MCP_{FOM}** was calculated as $MCP_{FOM} = FOM \times 0.15$. The factor 1.11 in the formula was taken from the French PDI system (Vérité and Geay, 1987) and Dutch DVE/OEB system, the regression coefficient of in vivo on in situ degradation data (Tamminga et al., 1994, 2007). The factor 0.15 means that for each kilogram of rumen fermented OM (**FOM**), 150 g of microbial protein CP is assumed to be synthesized (Tamminga et al., 1994, 2007).

Synchrotron Light Source and Midinfrared Microspectroscopy

Synchrotron-based Fourier transform infrared microspectroscopy was performed on beamline U2B at the National Synchrotron Light Source located in Brookhaven National Laboratory (US Department of Energy, Upton, NY). Collimated light from the synchrotron was directed to a Fourier transform infrared spectrometer (Nicolet Magna 860, Thermo-Nicolet, Madison, WI) with a KBr beam splitter and a nitrogen cooled mercury cadmium telluride detector through a port on the spectrometer. The spectrometer was interfaced with a Nic Plan IR microscope using a single image plane mask set to give an aperture of $10 \mu\text{m} \times 10 \mu\text{m}$. Spectra were generated in transmission mode with the mid-IR (4,000 to 800 cm^{-1}) portion of the electromagnetic spectrum by 128 co-added scans with a spectral resolution of 4 cm^{-1} . Spectral analysis (protein secondary structures) was done with Omnic 6.0

(Thermo-Nicolet) software. Spectra were generated from randomly selected regions of the endosperm of the flaxseed sections. The detailed methodology has been reported in Yu (2004).

Statistical Analysis

Chemical Profile and Protein Subfraction Studies. Statistical analyses were performed using the PROC MIXED of SAS. The model used for the analysis was: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij ; μ was the population mean for the variable; T_i was the effect of the heating treatments, as a fixed effect; and e_{ij} was the random error associated with the observation ij .

Synchrotron-Based Protein Secondary Structure Study. Statistical analyses were performed using the PROC MIXED of SAS using a completed nested design. The model used for the analysis was: $Y_{ij} = \mu + T_i + S(T)_j + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij ; μ was the population mean for the variable; T_i was the effect of the heating treatments, as a fixed effect; $S(T)_j$ is the seeds nested within treatments, as a random effect; and e_{ij} was the random error associated with the observation ij . The detailed methodology was reported in Yu (2004).

Rumen Degradation and Intestinal Digestibility Studies. Statistical analyses were performed using the PROC MIXED of SAS. The model used for the analysis was: $Y_{ijk} = \mu + T_i + S_k + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ijk ; μ was the population mean for the variable; T_i was the effect of the heating treatments, as a fixed effect, S_k was the run effect, as a random effect, and e_{ijk} was the random error associated with the observation ijk .

Correlation Analysis. Relationship between the changes in protein structure α -helix to β -sheet ratios and changes in chemical composition, CNCPS protein fractions, in situ degradation kinetics and predicted nutrient supply to dairy cattle in the raw (control) and autoclave-treated Vimy flaxseed were analyzed using the PROC CORR of SAS using a nonparametric correlation method (Spearman) because some of nutrient data are not normal distributed. The normality tests were performed using PROC UNIVARIATE of SAS.

For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Differences among the treatments were evaluated using a multiple comparison test following the Tukey-Kramer method. Orthogonal polynomial contrasts were also used to examine the linear, quadratic and cubic effects of heating time (0, 20, 40, and 60 min). The linear, quadratic, and cubic contrast coefficients were obtained from Steel and Torrie (1980).

RESULTS AND DISCUSSION

Detection of Changes in Chemical Profile

Dry matter and EE both increased ($P < 0.05$) upon autoclave heating, whereas ash and OM remained unchanged (Table 1). Results for ash content were similar to those published by Gonthier et al. (2004). There was no difference ($P > 0.05$) between the treatments for both DM and EE. Values for EE of the raw Vimy flaxseed samples were very similar to those reported by Oomah and Kenaschuk (1995). The slight increase in the EE fraction is interesting to note and has been observed in ground sunflower seeds treated similarly by Mustafa et al. (2003a) but also in roasted whole soybean and cottonseed by Mohamed et al. (1988). This would appear to be an artifact as heating somehow increases the amount of fat that is soluble in ether. This effect is used advantageously in the oil extraction industry and it is held that the heating results in the rupture of the oil cells and permits the coalescing of larger droplets of oil (Booth, 2004). Under autoclave or roasting conditions, with no physical disruption directly from heating, there is a physicochemical reaction taking place, which affects the physical properties of the components making up the oil bodies, resulting in the change in solubility. The disruption of oil bodies through the heating process is likely to occur in 1 of 2 ways: a) through a physical change such as denaturation, which would remove the effect of steric hindrance exposing the inside of the oil bodies to one another allowing them to coalesce, or b) through changes to the charged surfaces of the protein responsible for the structure of the oil protein matrix (Huang, 1992).

Total CHO compositions of the raw and treated flaxseed show a decrease ($P < 0.01$) upon autoclaving from 30.4 to 28.1 (%DM; Table 1). This difference arises from the calculation of total CHO through the difference method where ash (%DM), EE (%DM), and CP (%DM) are subtracted from 100. Because of the change in the EE fraction, less CHO was found in the treatments. Neutral detergent fiber and ADF both decreased ($P < 0.05$) relative to the control, although there was no difference ($P > 0.05$) between the treatments themselves. These results for ADF and NDF are paralleled in a study by Mustafa et al. (2003a) in which both NDF and ADF decreased initially upon autoclave heating; however, with longer heating times, the decrease became less prominent. The results from this study showed a similar inflection in NDF and ADF values with increasing heating time; however, the values were not different from one another ($P > 0.05$) between the autoclaved treatments. The NDF and ADF values from this study were similar to those reported for raw

Table 1. Chemical profiles: comparison of raw (control) versus autoclave-treated Vimy flaxseed

Item	Treatment ¹					Contrast, <i>P</i> -value			
	Control	T1	T2	T3	SEM	Control vs. treatments	Linear	Quadratic	Cubic
Basic chemical profile									
DM, %	90.7 ^b	92.5 ^a	92.8 ^a	92.8 ^a	0.07	<0.001	<0.001	<0.001	0.016
Ether extract, % of DM	42.1 ^b	44.1 ^a	44.5 ^a	44.2 ^a	0.34	0.005	0.011	0.025	0.611
Ash, % of DM	3.5	3.6	3.6	3.5	0.01	0.351	0.650	0.093	0.549
Carbohydrate/fiber profile, % of DM									
Total carbohydrate ²	30.4 ^a	28.7 ^{ab}	28.1 ^b	28.5 ^{ab}	0.35	0.007	0.014	0.040	0.808
NDF	17.2 ^a	14.0 ^b	13.9 ^b	14.5 ^b	0.31	0.002	0.006	0.006	0.156
ADF	10.2 ^a	7.7 ^b	7.8 ^b	7.9 ^b	0.31	0.001	0.003	0.005	0.034
Acid detergent lignin	2.2	2.4	2.7	2.8	0.14	0.073	0.027	0.822	0.720
NFC ³	17.0	18.8	18.6	19.7	0.69	0.069	0.067	0.661	0.363
Starch	0.3	0.3	0.3	0.3	0.23	0.477	0.372	0.354	0.092
Protein profile, % of DM									
Total CP	23.9	23.7	23.8	23.8	0.13	0.296	0.704	0.316	0.438
Soluble CP	12.4 ^a	8.5 ^b	5.4 ^c	4.5 ^c	0.32	<0.001	<0.001	0.009	0.386
NPN	1.4 ^b	1.3 ^b	1.5 ^{ab}	2.0 ^a	0.10	0.240	0.011	0.027	0.675
NDIP (= 6.25 × NDIN ⁴)	3.8 ^b	4.1 ^b	4.4 ^b	5.7 ^a	0.13	0.004	<0.001	0.015	0.089
ADIP ⁵ (= 6.25 × ADIN)	0.5 ^b	0.6 ^{ab}	0.7 ^{ab}	0.8 ^a	0.04	0.018	0.009	0.821	0.919

^{a-c}Means within a row with different superscripts differ ($P < 0.05$). Mean separation was done by using the Tukey-Kramer method.

¹T1 = autoclaved at 120°C for 20 min; T2 = autoclaved at 120°C for 40 min; T3 = autoclaved at 120°C for 60 min.

²Total carbohydrate was calculated as: carbohydrate = 100 – ether extract (EE) – CP – ash (NRC, 2001).

³NFC = 100 – [NDF – neutral detergent insoluble protein (NDIP)] – EE – CP – ash (NRC, 2001).

⁴NDIN = neutral detergent insoluble nitrogen.

⁵ADIP = acid detergent insoluble protein.

flaxseed by Gonthier et al. (2004). Acid detergent lignin showed a trend for a small increase ($P = 0.073$), from 2.2 (%DM), upon autoclaving to 2.8 (%DM). There were quadratic ($P < 0.01$), cubic ($P < 0.05$), and linear ($P < 0.05$) effects of heating time on NDF, ADF, and acid detergent lignin, respectively. Starch composition of the flaxseed samples was minimal in all cases. No differences were detected ($P > 0.05$) for nonfiber carbohydrates between the raw and heated treatments, although there was a numerical increase in the values in the heated treatments. This value is determined by the difference method and contains the artifact associated with the EE value, which would skew the results downward about 2% on a DM basis for the treatments compared with the raw.

No changes ($P > 0.05$) were observed in the CP measurements upon autoclaving (Table 1). Crude protein values are in accordance with those reported in Gonthier et al. (2004). Soluble CP showed a marked decrease ($P < 0.05$) upon autoclaving but the change appeared to plateau for the treatments (at 120°C for 40 and 60 min), which were not different ($P > 0.05$) from one another. The decrease in SCP is mirrored by the results for micronized flaxseed reported by Gonthier et al. (2004), as well as for autoclaved sunflower in Mustafa et al. (2003a). It is of interest to note that the amount of SCP in flaxseed increases upon extrusion (Gonthier et al., 2004; Mustafa et al., 2003b), which is the oppo-

site effect of autoclaving. The difference is likely caused by the physical aspect of extrusion, which would lend itself to abrogating the oil/protein matrix more readily, thus possibly leading to an increase in both EE and SCP. Because the oil/protein matrix is physically different from a starch/protein matrix, as part of the oil/protein matrix is in a liquid form, it may also account for the differences observed when extruding oilseed versus cereals. There was a quadratic ($P < 0.01$) effect of autoclave time on SCP with lower values in the treatments. Nonprotein nitrogen did not show any increase with the exception of the treatment at 120°C for 60 min, for which NPN changed ($P < 0.05$) from 1.4 to 2.0 (%DM). These results differ from those of Mustafa et al. (2003a), in which they report a decrease in NPN as treatment time increases using autoclaved sunflower seed; yet upon extrusion, NPN in flaxseed has been demonstrated to increase (Mustafa et al., 2003b). Neutral detergent insoluble protein shows a similar pattern to NPN in that only treatment T3 was different ($P < 0.05$) from the raw samples and it increased from 3.8 to 5.7 (%DM). This is also in accordance with NDIP results for micronized flaxseed reported in Gonthier et al. (2004), as well as for autoclaved sunflower seed Mustafa et al. (2003a). Acid detergent insoluble protein results showed a small incremental increase ($P < 0.05$) increasing from 0.5 (%DM) for raw Vimy flaxseed to 0.8 (%DM) for treatment T3. From these results for the

Table 2. Protein subfraction profiles using the Cornell Net Carbohydrate and Protein System (CNCPS) and protein secondary structure α -helix to β -sheet ratios in the endosperm of flaxseed using synchrotron-based Fourier transform infrared microspectroscopy: comparison of raw (control) versus autoclave-treated Vimy flaxseed

Item	Treatment ¹					Contrast, <i>P</i> -value			
	Control	T1	T2	T3	SEM	Control vs. treatments	Linear	Quadratic	Cubic
Protein secondary structure									
Protein α -helix to β -sheet ratios	0.86 ^b	0.88 ^b	1.03 ^a	1.03 ^a	0.033	0.002	<0.001	0.798	0.068
Protein subfractions, ² % of CP									
PA	6.0 ^b	5.3 ^b	6.4 ^{ab}	8.5 ^a	0.41	0.199	0.009	0.024	0.697
PB1	45.9 ^a	30.7 ^b	16.5 ^c	10.5 ^d	0.99	<0.001	<0.001	0.009	0.168
PB2	43.0 ^c	58.3 ^b	71.0 ^a	73.3 ^a	1.09	<0.001	<0.001	0.004	0.183
PB3	3.3 ^b	3.5 ^b	3.7 ^b	4.9 ^a	0.15	0.014	0.002	0.027	0.138
PC	1.9 ^b	2.2 ^{ab}	2.5 ^{ab}	2.8 ^a	0.11	0.017	0.009	0.850	0.858
True protein, ³ % of true protein	92.2 ^a	92.6 ^a	91.1 ^{ab}	88.7 ^b	0.46	0.065	0.005	0.037	0.697
PB1	49.8 ^a	33.2 ^b	18.1 ^c	11.8 ^c	1.21	<0.001	<0.001	0.013	0.241
PB2	46.6 ^c	63.0 ^b	77.9 ^a	82.6 ^a	1.10	<0.001	<0.001	0.006	0.147
PB3	3.6 ^c	3.8 ^b	4.0 ^b	5.6 ^a	0.16	0.008	0.001	0.014	0.115

^{a-d}Means within a row with different superscripts differ ($P < 0.05$). Mean separation was done by using the Tukey-Kramer method.

¹T1 = autoclaved at 120°C for 20 min; T2 = autoclaved at 120°C for 40 min; T3 = autoclaved at 120°C for 60 min.

²Protein subfractions using 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 the sum of fractions PA, PB1, PB3 and PC; PB3 = calculated as the difference between the portions of total CP recovered with NDF and ADF; PC = fraction of CP recovered with ADF and is considered to be undegradable. It contained proteins associated with lignin and tannins and heat-damaged proteins such as the Maillard reaction products.

³True protein = PB1 (% of CP) + PB2 (% of CP) + PB3 (% of CP).

nitrogen fractions of flaxseed, it is clear that autoclaving as a form of heat treatment is not akin to extrusion, likely because of the physical changes that extrusion imparts to the feed sample.

Detection of Changes in Protein Subfractions

Results for the CNCPS CP subfractions are presented in Table 2. Fraction PA was only higher ($P < 0.05$) for the 1-h autoclave treatment. Fraction PB1 was the only fraction to decrease ($P < 0.05$) in concentration upon autoclave treatment, with each treatment showing a significant difference from the next. Compared with the raw, treatments dramatically reduced PB1 4.4 times. The PB1 fraction degrades in the rumen, so it would follow that the autoclave treatments are decreasing the overall degradation rate of protein in the rumen, given that changes to PA are not large enough to offset the changes in degradability caused by the decrease in PB1. Results for the PB1 fraction changes are similar to those for autoclaved sunflower seed (Mustafa et al., 2003a). The PB2 fraction was increased ($P < 0.05$) by autoclave treatment, reaching a plateau of 73 (%CP) for the 1-h treatment from a basis of 43 (%CP) for the raw flaxseed. The shift observed in this fraction upon autoclaving accounts for the majority of the loss in the PB1 fraction, hence we expect that the overall degradation rate will decrease upon autoclaving as a result of this shift from the ruminally degraded PB1 fraction to the partially ruminally degraded PB2 fraction. The PB3

fraction was shown to increase ($P < 0.05$) after heating, but only the flaxseed exposed to the 1-h treatment was demonstrably different. The increase in the PB3 fraction from 3.3 (%CP) for the raw flaxseed to 4.9 (%CP) for the 1-h treatment, although statistically significant, is not likely to be a major factor in the reduction of ruminal degradability that would be observed upon autoclaving, simply as a result of the relatively small shift that is taking place. This is despite the fact that PB3 is more slowly degraded in the rumen than fraction PB2. Fraction PC is not considered degradable in the rumen and shows a gradual progression upward with increasing autoclave treatment time, increasing ($P < 0.05$) from 1.9 (%CP) for the raw to 2.8 (%CP) for the 1-h autoclave treatment. The protein in this fraction is found bound to other feed constituents such as lignin and tannins, and as such it is not available to the animal (Sniffen et al., 1992). The minuscule shift observed in this fraction suggests that the autoclave treatments used in this experiment would not be detrimental to the protein quality of flaxseed in terms of its use as a feed ingredient. There were quadratic ($P < 0.05$) effects of heating time on all protein fractions except the PC fraction, which showed a linear response to autoclave time.

Changes in Protein Structure α -Helix to β -Sheet Ratio

Results from the protein structure analysis are presented in Table 2. The results showed that the α -helix

Table 3. In situ degradation kinetics profile of DM and CP and intestinal digestibility of RUP: comparison of raw (control) versus autoclave-treated Vimy flaxseed

Item	Treatment ¹					Contrast, <i>P</i> -value			
	Control	T1	T2	T3	SEM	Control vs. treatments	Linear	Quadratic	Cubic
In situ rumen degradation kinetics of DM									
Degradation rate (K_d), %/h	6.8	7.5	7.8	6.0	0.98	0.716	0.523	0.099	0.551
Lag time (T_0), h	0.5	0.5	0.0	0.0	0.25	0.257	0.108	0.942	0.444
Soluble fraction in situ (S), % of DM	22.8 ^{ab}	15.2 ^b	18.6 ^{ab}	25.6 ^a	2.66	0.240	0.237	0.006	0.448
Undegradable fraction (U), % of DM	12.7 ^b	19.1 ^a	20.6 ^a	19.0 ^a	0.90	<0.001	<0.001	<0.001	0.583
Degradable fraction (D), % of DM	64.5 ^a	65.8 ^a	60.9 ^{ab}	55.4 ^b	2.12	0.134	0.005	0.127	0.556
Rumen-degradable DM, % of DM	57.1 ^a	51.4 ^b	52.7 ^b	53.2 ^b	0.73	<0.001	0.008	0.001	0.038
In situ rumen degradation kinetics of CP									
Degradation rate (K_d), %/h	7.2 ^a	6.1 ^{ab}	6.1 ^{ab}	5.5 ^b	0.48	0.017	0.016	0.552	0.319
Lag time (T_0), h	0.9	0.6	0.0	0.7	0.37	0.269	0.475	0.177	0.370
Soluble fraction in situ (S), % of CP	24.3 ^a	12.6 ^b	14.2 ^b	18.4 ^{ab}	1.72	<0.001	0.047	<0.001	0.153
Undegradable fraction (U), % of CP	6.5 ^b	13.3 ^a	17.0 ^a	16.6 ^a	1.07	<0.001	<0.001	0.002	0.799
Degradable fraction (D), % of CP	69.2 ^{ab}	74.2 ^a	68.8 ^{ab}	64.5 ^b	2.10	0.976	0.076	0.059	0.229
RDP, % of CP	62.0 ^a	49.7 ^b	48.9 ^b	49.2 ^b	1.09	<0.001	<0.001	<0.001	0.055
RDP, % of DM	14.4 ^a	11.6 ^b	11.4 ^b	11.4 ^b	0.27	<0.001	<0.001	<0.001	0.088
Intestinal digestibility of RUP, %	61.7	58.6	58.4	60.3	1.94	0.107	0.463	0.075	0.899

^{a,b}Means within a row with different superscripts differ ($P < 0.05$). Mean separation was done by using the Tukey-Kramer method.

¹T1 = autoclaved at 120°C for 20 min; T2 = autoclaved at 120°C for 40 min; T3 = autoclaved at 120°C for 60 min.

to β -sheet ratio in the endosperm area increased ($P < 0.05$) upon heating. These changes in the protein structure ratio are likely caused by α -helices or β -sheets being denatured or destroyed during the autoclaving process. There was a linear response ($P < 0.05$) of the protein α -helix to β -sheet ratio to the autoclave time.

Changes in Protein Degradation Kinetics

As shown in Table 3, autoclave heating had no effect ($P > 0.05$) on the DM degradation rate of flaxseed, nor was any effect on the DM lag time found by this study. The soluble fraction of DM demonstrated some interesting results with the 20-min autoclave treatment, causing the largest reduction in DM solubility, whereas the 1-h autoclave treatment caused an increase in the soluble fraction of DM. Mustafa et al. (2003a) also demonstrated a decrease in soluble fraction upon autoclaving sunflower seed; however, despite temperatures being similar, the longest treatment in that study was only 30 min, which may not have been long enough to demonstrate the pattern seen in the present study. The decrease in soluble DM was also accompanied by a decrease in soluble CP, which was responsible for a portion of the change. In a separate study by Mustafa et al. (2003b) using extruded flaxseed, an increase in soluble DM was reported when compared with a raw sample; this increase was also accompanied by an increase in soluble CP. This study shows a similar pattern in soluble CP and DM, suggesting that the majority of the change in the S fraction of DM between the treatments is largely the result of changes to the S fraction of CP. The undegradable fraction of DM increased (P

< 0.05) upon autoclaving from 12.7 to 20.6 (%DM) but there were no significant differences between any of the treatments. The potentially degradable fraction of DM decreased ($P < 0.05$) from 64.5 to 55.4 (%DM). The only treatment significantly different from the raw sample was treatment T3. These results are the opposite effects of those reported by Mustafa et al. (2003a) for autoclaved sunflower seed. The ruminally effectively degradability of DM also decreased ($P < 0.05$) upon autoclave treatment; however, there were no differences ($P > 0.05$) between any of the treatments. Once again, this trend is mirrored by the Mustafa et al. (2003a) study, showing a decrease in the effectively degradable DM of sunflower seed, but the opposite effect was observed upon flaxseed extrusion in the Mustafa et al. (2003b) study.

The degradation rate of CP upon autoclaving flaxseed decreased ($P < 0.05$) from 7.2 to 5.5%/h as shown in Table 3. The only treatment significantly different from the raw samples in this respect was the 1-h treatment. Decreases in CP degradation rates have also been reported for extruded flaxseed (Mustafa et al., 2003b), autoclaved sunflower seed (Mustafa et al., 2003a), and roasted sunflower seeds (Sarrazin et al., 2003) so in this respect the K_d of CP from full fat oilseeds appears to respond consistently to heating despite the method of heating used. No differences ($P > 0.05$) were observed for the lag time of CP in the present study. The soluble fraction of CP shows a parabolic type of response in which the shortest treatment exhibited the largest decrease ($P < 0.06$), from 24.3 to 12.6 (%CP), and the longer the autoclave treatment, the less it decreased the soluble fraction. Considering other full-

Table 4. Predicted protein supply to the small intestine using the DVE/OEB system: comparison of raw (control) versus autoclave-treated Vimy flaxseed

Item, g/kg of DM	Treatment ¹					Contrast, <i>P</i> -value			
	Control	T1	T2	T3	SEM	Control vs. treatments	Linear	Quadratic	Cubic
Fermentable OM	236.1 ^a	198.3 ^c	196.7 ^c	214.0 ^b	3.37	<0.001	<0.001	<0.001	0.266
Microbial CP	35.4 ^a	29.7 ^c	29.5 ^c	32.1 ^b	0.51	<0.001	<0.001	<0.001	0.267
Absorbable microbial CP	22.6 ^a	19.0 ^c	18.8 ^c	20.5 ^b	0.32	<0.001	<0.001	<0.001	0.267
Endogenous CP losses	17.0 ^a	16.2 ^b	15.7 ^c	14.7 ^d	0.002	<0.001	<0.001	<0.001	<0.001
Ruminally undegraded CP	103.9 ^b	134.7 ^a	138.7 ^a	139.2 ^a	2.15	<0.001	<0.001	<0.001	0.029
Absorbable ruminally undegraded CP	64.0 ^b	79.1 ^a	81.1 ^a	83.9 ^a	2.97	<0.001	<0.001	0.023	0.202
Total intestinally absorbed protein supply (DVE)	69.6 ^c	81.9 ^b	84.2 ^{ab}	89.7 ^a	2.88	<0.001	<0.001	0.136	0.185
Degraded protein balance (OEB)	100.0 ^a	72.1 ^b	69.6 ^b	66.8 ^b	2.16	<0.001	<0.001	<0.001	0.021

^{a-d}Means within a row with different superscripts differ ($P < 0.05$). Mean separation was done by using the Tukey-Kramer method.

¹T1 = autoclaved at 120°C for 20 min; T2 = autoclaved at 120°C for 40 min; T3 = autoclaved at 120°C for 60 min.

fat oilseeds, upon extrusion flaxseed shows an increase in the soluble fraction of CP (Mustafa et al., 2003b) but a decrease is observed for roasted and autoclaved sunflower seed (Mustafa et al., 2003a; Sarrazin et al., 2003). Here, physical changes imparted to the oilseed as a result of processing may be responsible for the difference in response. The undegradable CP fraction in this study increased ($P < 0.05$) with increasing treatment times. With the raw flaxseed having an undegradable CP fraction consisting of 6.5 (%CP), the first 20-min treatment doubles this fraction to 13.3 (%CP) and the 40-min treatment almost triples this fraction to a value of 17.0 (%CP) where it appears to reach a plateau. The degradable fraction shows an interesting response in which the 20-min autoclave treatment increases it from 69.2 to 74.2 (%CP) and the 40-min and 60-min treatments were 68.8 and 64.5 (%CP), respectively. However, it should be noted that the raw samples were not significantly different from any of the treatments and only treatments T1 and T3 were significantly different from one another. Presumably, there is a point at which no change to the degradable fraction as determined by this study would be observed under these conditions. Finally, the effective degradability of CP decreased ($P < 0.05$) from 62.0 to approximately 49.2 (%CP), with none of the treatments having been found to be significantly different from one another. The same response to effective degradability of CP was found for roasted full fat sunflower seed (Sarrazin et al., 2003) and autoclaved sunflower seed (Mustafa et al., 2003a), whereas once again extruded flaxseed exhibited the opposite response.

Changes in Protein Intestine Digestibility

Results of the intestinal digestibility estimation are presented in Table 3. No difference ($P > 0.05$) was detected between the raw and any of the autoclave treat-

ments. It is entirely possible that the temperature of the treatments or the time for the treatments was not sufficient to damage the flaxseed protein enough for there to be any detectable differences via this method. In the study outlining the protocol (Calsamiglia and Stern, 1995), soybean meal was used and a decrease in digestibility was only observed after heating for 2.5 h at a temperature of 165°C, this is clearly a much more extreme heat treatment than the one used in the present study. What these results suggest is that the digestibility of autoclaved flaxseed protein is not adversely affected when heated within the scope of the treatments used in this trial, so we can increase RUP without any accompanying decrease in digestibility in the small intestine as determined by this method.

Protein Supply to Dairy Cattle Using the DVE/OEB System

The results from estimation of nutrient supply to the small intestine using the DVE/OEB model (Tamminga et al., 1994) are presented in Table 4. The DVE/OEB system takes into consideration some factors not considered by the model in NRC (2001). The detailed comparison between the NRC and DVE/OEB system was reported in Yu et al. (2003b). Fermented OM results show decreases for autoclaved treatments, FOM consists of digestible OM corrected for the fat contained in the feed, which is not considered degradable in the rumen, as well as RUP, so in light of the EE fraction and RUP fraction increasing upon autoclaving, these results are to be expected. Fermented OM has been demonstrated to decrease for several different feedstuffs in (Yu et al., 2002) upon heating. In the DVE/OEB system, changes in FOM are responsible for the calculated changes in MCP and AMCP. Endogenous CP was reduced for flaxseed upon autoclaving from 145.7 to 112.7 g/kg of DM yet there was no significant dif-

Table 5. Correlation between protein structure α -helix to β -sheet ratios and chemical profiles, Cornell Net Carbohydrate and Protein System (CNCPS) protein fractions, in situ degradation kinetics, and nutrient availability in raw (control) and autoclave-treated Vimy flaxseed

Item	Correlation with protein α -helix to β -sheet ratios	
	Spearman's correlation coefficient, <i>r</i>	<i>P</i> -value
Chemical profiles, % of DM		
Ether extract	0.95	0.051
Total carbohydrate	-0.95	0.051
NDF	-0.63	0.368
ADF	-0.32	0.684
Acid detergent lignin	0.95	0.051
NFC	0.63	0.367
Total CP	-0.33	0.667
Soluble CP	-0.95	0.051
Neutral detergent insoluble protein (NDIP) ¹	0.95	0.051
Acid detergent insoluble protein (ADIP) ²	0.95	0.051
Protein fractions, ³ % of CP		
PA	0.74	0.261
PB1	-0.95	0.051
PB2	0.95	0.051
PB3	0.95	0.051
PC	0.95	0.051
In situ rumen degradation kinetics of DM		
Degradation rate (<i>K_d</i>), %/h	0.11	0.895
Soluble fraction in situ (S), % of DM	0.21	0.789
Undegradable fraction (U), % of DM	0.63	0.367
Degradable fraction (D), % of DM	-0.74	0.262
Rumen-degradable DM, % of DM	-0.32	0.684
In situ rumen degradation kinetics of CP		
Degradation rate (<i>K_d</i>), %/h	-0.83	0.167
Soluble fraction in situ (S), % of CP	-0.32	0.684
Undegradable fraction (U), % of CP	0.95	0.051
Degradable fraction (D), % of CP	-0.74	0.261
RDP, % of CP	-1.00	<0.001
Intestinal digestibility of RUP, %	-0.63	0.368
Modeling nutrient supply, g/kg of DM		
Absorbable microbial CP (AMCP)	-0.63	0.368
Endogenous CP losses (ENDP)	-0.95	0.051
Absorbable RUP (ARUP)	0.95	0.051
Total intestinally absorbed protein supply (DVE)	0.95	0.051
Degraded protein balance (OEB)	-0.95	0.051

¹NDIP = $6.25 \times$ neutral detergent insoluble nitrogen.²ADIP = $6.25 \times$ ADIN.³Protein subfractions 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 the sum of fractions PA, PB1, PB3, and PC; PB3 = calculated as the difference between the portions of total CP recovered with NDF and ADF; PC = fraction of CP recovered with ADF and is considered to be undegradable. It contained proteins associated with lignin and tannins and heat-damaged proteins such as the Maillard reaction products.

ference between the treatments. Ruminally undegraded feed protein, as in the NRC model, showed a significant increase upon autoclaving from 103.9 to 139.2 g/kg of DM with, again, no significant difference between autoclave treatments. Absorbable protein in the intestine (DVE value) was increased ($P < 0.05$) upon autoclaving flaxseed. In this case, the relative increase of RUP was greater than the decreases observed for AMCP, AECp, and ARUP; so the trend among the treatments was for this value to increase ($P < 0.05$) from 69.6 g/kg of DM

for raw flaxseed to 89.7 g/kg of DM for the flaxseed exposed to the autoclave for 1 h. The OEB values for all treatments and the raw flaxseed was determined to be positive without any significant difference between the treatments. The OEB value decreased ($P < 0.05$) upon autoclave treatment, suggesting that the potential loss of N from the rumen was mitigated when the flaxseed was autoclaved; however, according to the model, there would still be potentially significant N losses from the rumen. There was a linear effect of heating time on the

DVE ($P < 0.01$) and a cubic effect on the OEB value ($P < 0.05$).

Correlations Between Protein Structure α -Helix to β -Sheet Ratios with Nutrient Profiles

There were negative correlation ($P = 0.051$) between protein structure α -helix to β -sheet ratios and total CHO and SCP with $r = -0.95$, but positive correlation ($P = 0.051$) with ADIP and NDIP with $r = 0.95$. This result indicated that increasing protein α -helix to β -sheet ratios resulted in decreasing total CHO and SCP and increasing both ADIP and NDIP (Table 5). With CNCPS protein fractions correction, the results show that the protein structure α -helix to β -sheet ratios had no correlation with PA fraction ($P = 0.261$), but negative correlation ($P = 0.051$) with PB1 fraction and positive correlation ($P = 0.051$) with PB2, PB3, and PC fractions with $r = 0.95$. The results indicated that autoclaving reduced rapidly degradable protein fraction and increased slowly degradable and undegradable fractions. With in situ parameters correction, the results show that the protein α -helix to β -sheet ratios had no correlation with in situ DM degradation kinetics ($P > 0.10$) except lag time ($P = 0.057$), but positive correlation ($P = 0.051$) with the U fraction of CP, but strongly negative correlation ($P < 0.01$) with RDP and RUP. The result indicated that the protein α -helix to β -sheet ratios had a close relationship with CP degradation but not DM degradation. There was no correlation between the protein α -helix to β -sheet ratios and intestinal digestibility of RUP in vitro (Table 5). With predicted nutrient supply correction, the results show that the protein α -helix to β -sheet ratios had positive correlation with ARUP and total intestinally absorbed protein supply (the feed DVE value) and negative correlation with endogenous CP losses and degraded protein balance. It needs to be mentioned that only 4 heating treatments were evaluated in this study. To make a conclusion, large feed protein samples with a wide range of chemical compositions need to be evaluated.

CONCLUSIONS

Using the synchrotron-based analytical technique, the heat-induced protein structure changes were revealed and identified. Heating significantly increased the protein secondary structure α -helix to β -sheet ratio. The heating significantly changed chemical profiles. The protein subfractions with the greatest changes were PB1, which showed a dramatic reduction, and PB2, showing a dramatic increase; demonstrating a decrease in the overall protein degradability. In situ experiments showed a reduction in rumen-degradable

DM and RDP without significant differences between the treatments. Intestinal digestibility, as estimated by the 3-step procedure, showed no changes to the protein. The intestinally absorbable protein supply (feed DVE value) using the DVE/OEB systems showed increases and there were no differences between the treatments. The degraded protein balance (feed OEB value) was decreased. Our results showed that heating changed the chemical profile and protein sub-fractions, generally decreased ruminal degradability of protein and increased potential nutrient supply. The protein secondary structure α -helix to β -sheet ratio had positive correlation with total intestinally absorbed protein supply (feed DVE value) and negative correlation with degraded protein balance (feed OEB value).

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