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Moist and dry heating-induced changes in protein molecular structure, protein subfractions, and nutrient profiles in camelina seeds

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ABSTRACT

The objectives of the present study were to investigate the nutritive value of camelina seeds (*Camelina sativa* L. Crantz) in ruminant nutrition and to use molecular spectroscopy as a novel technique to quantify the heat-induced changes in protein molecular structures in relation to protein digestive behavior in the rumen and intestine of dairy cattle. In this study, camelina seeds were used as a model for feed protein. The seeds were kept as raw (control) or heated in an autoclave (moist heating) or in an air-draft oven (dry heating) at 120°C for 60 min. The parameters evaluated were (1) chemical profiles, (2) Cornell Net Protein and Carbohydrate System protein subfractions, (3) nutrient digestibilities and estimated energy values, (4) in situ rumen degradation and intestinal digestibility, and (5) protein molecular structures. Compared with raw seeds, moist heating markedly decreased (52.73 to 20.41%) the content of soluble protein and increased (2.00 to 9.01%) the content of neutral detergent insoluble protein in total crude protein (CP). Subsequently, the rapidly degradable Cornell Net Protein and Carbohydrate System CP fraction markedly decreased (45.06 to 16.69% CP), with a concomitant increase in the intermediately degradable (45.28 to 74.02% CP) and slowly degradable (1.13 to 8.02% CP) fractions, demonstrating a decrease in overall protein degradability in the rumen. The in situ rumen incubation study revealed that moist heating decreased (75.45 to 57.92%) rumen-degradable protein and increased (43.90 to 82.95%) intestinal digestibility of rumen-undegradable protein. The molecular spectroscopy study revealed that moist heating increased the amide I-to-amide II ratio and decreased α-helix and α-helix-to-β-sheet ratio. In contrast, dry heating did not significantly change CP solubility, rumen degradability, intestinal digestibility, and protein molecular structures compared with the raw seeds. Our results indicated that, compared with dry heating, moist heating markedly changed protein chemical profiles, protein subfractions, rumen protein degradability, and intestinal digestibility, which were associated with changes in protein molecular structures (amide I-to-amid II ratio and α-helix-to-β-sheet ratio). Moist heating improved the nutritive value and utilization of protein in camelina seeds compared with dry heating.
Key words: heat processing method, protein molecular structure, nutrient availability, camelina seed

INTRODUCTION

Camelina (*Camelina sativa* L. Crantz), also known as false flax or gold-of-pleasure, is an unexploited ancient oilseed crop in the family Brassicaceae. Recently, the crop has received increasing attention, primarily due to the use of its seeds as a source of high-quality oil and animal feed (Putnam et al., 1993; Kirkhus et al., 2013). As a result, the cultivated area of camelina is estimated to reach 0.607 million hectare in North America by 2013. Camelina seeds contain over 40% oil, and the oil is exceptionally high in n-3 (>37%) and total polyunsaturated (>50%) FA (Peiretti et al., 2007; Kirkhus et al., 2013), making it an attractive alternative source of n-3 FA to flaxseed (Moloney et al., 2012). The beneficial effects of camelina meal/cake or oil inclusion in animal diets on milk yield and composition of dairy cattle and ewes (Halmemies-Beauchet-Filleau et al., 2011; Szumacher-Strabel et al., 2011), circulating PUFA in beef cattle (Cappellozza et al., 2012), and the insulin sensitivity of dairy cattle during late pregnancy (Salin et al., 2012) have been intensively investigated. During the early and mid-lactation period, high-producing dairy cattle experience a state of negative energy balance due to lower DMI and high nutritional demands for milk production. One of the effective resolutions to improve energy balance is feeding of energy-dense, fat-supplemented rations. However, feeding >5% of (unprotected) fat or oil negatively affects cellulolytic microflora in the rumen (Henderson, 1973) and DMI of dairy cows (Ruegsegger and Schultz, 1985). Alterna-

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tively, feeding of intact oilseeds directly to the animal can minimize the negative effects on rumen function. The positive effects of supplementing camelina seeds directly to animal diets have been reported in rabbits (Peiretti et al., 2007) and lambs (Noci et al., 2011; Moloney et al., 2012). However, to our knowledge, the nutritive value of camelina seeds for dairy cattle has not been evaluated.

Heat treatments have been widely used to decrease protein degradation in the rumen and optimize protein utilization in ruminants (Faldet et al., 1992; Chouinard et al., 1997; Wright et al., 2005). Heat processing alters the protein digestive behavior by changing the inherent molecular structure of protein (Yu, 2007), and recent investigations have shown that quantifying the heat-induced changes in the molecular structure of the whole protein is vital to determine optimal heating conditions and understand the changes in protein nutritional quality, digestive behavior, and utilization in animals (Doiron et al., 2009; Samadi and Yu, 2011). However, studies on the heat-induced changes in protein intrinsic molecular structures in relation to protein nutritive value and digestive behavior are extremely rare, partly due to the lack of appropriate analytical techniques. Conventional wet chemical analysis rely heavily on harsh chemicals for extraction and derivatization, which can destroy the native structure of protein, and fail to detect protein molecular structure or chemical makeup (Budevska, 2002). Recent research has shown that Fourier transform/infrared-attenuated total reflectance (**FT/IR-ATR**) molecular spectroscopy can be used as a rapid, direct, nondestructive, and noninvasive bioanalytical technique to detect protein molecular structures in the intact feed tissues (Yu, 2007; Doiron et al., 2009; Samadi and Yu, 2011).

Therefore, the present study was designed to use FT/IR-ATR-based molecular spectroscopy to reveal protein molecular structures and characterize the nutritive value of whole camelina seeds in ruminant nutrition. The second objective was to correlate dry and moist heat-induced changes in protein molecular structures to the changes in protein nutritional value, and digestive behavior in the rumen and intestine of the dairy cattle. The parameters evaluated were (1) chemical profiles, (2) protein subfraction profiles, (3) nutrient digestibilities and estimated energy values, (4) in situ rumen degradation and intestinal digestibility, and (5) protein molecular structures in terms of amide I and amide II areas, α-helices and β-sheets, as well as their ratio. We hypothesized that different heat processing methods would result in different changes in protein inherent molecular structure and that these changes are associated with protein digestive behaviors and availability in dairy cattle.

MATERIALS AND METHODS

Heat Treatment and Processing

Seeds from 2 camelina varieties (Blaine Creek and Celine), harvested in 2 different years (2010 and 2011) were obtained from Feeds Innovation Institute, University of Saskatchewan (Saskatoon, SK, Canada). For heat processing, a 1-kg sample of each camelina variety was spread in aluminum pans (33 × 23 cm and 5 cm high), and moist heated by autoclave (Amsco Eagle SG-3031; Steris Corp., Mentor, OH) at 1.05 kg/cm^2 pressure or dry heated (roasted) by air-draft oven for 60 min at 120°C. The heat processing was carried out in a single batch and harvest years were used as replicate. The heated samples were subsequently cooled at room temperature (20–22°C) for 30 min and stored at 4°C for further processing. Raw seeds were used as the control.

The seeds were ground through 0.25-mm screen (Retsch ZM-1; Brinkmann Instruments Ltd., Mississauga, ON, Canada) for molecular spectral analysis. For chemical analysis, the seeds were ground through a 1-mm screen, whereas for in situ incubation, the seeds were ground through a 2-mm screen. During grinding, the samples were kept cool and slowly fed to the grinder to minimize sticking and clumping and to ensure that the seeds were cracked and not extruded.

Molecular Spectroscopy

The molecular spectroscopic analyses were performed at the molecular spectroscopy laboratory of the department of Animal and Poultry Science, University of Saskatchewan. The molecular spectral profiles were collected using a JASCO FT/IR-ATR-4200 spectrometer (Jasco Inc., Easton, MD). The FT/IR spectrometer was equipped with a ceramic IR light source and a deuterated L-alanine doped triglycine sulfate detector, and a MIRacle ATR accessory module and a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI). The spectra were generated in the mid-IR (ca. 4,000–800 cm^{-1}; Figure 1) and fingerprint (ca. 1,800–800 cm^{-1}; Figure 2) regions, with 128 co-added scans and a spectral resolution of 4 cm^{-1}, in transmission mode. Each sample was run 5 times. The spectra were collected with Jasco Spectra Manager II software (Jasco Inc.) and corrected against air as the background. The molecular structural features of protein from the FT/IR spectra were quantified by OMNIC 7.3 software (Spectra-Tech Inc., Madison, WI). The spectral bands associated with protein functional groups were identified and assigned using information reported by Theodoridou and Yu (2013b). Briefly, the primary molecular structure of protein was characterized through the unique func-