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Estimation of feed crude protein concentration and rumen degradability by Fourier-transform infrared spectroscopy

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ABSTRACT

Currently, rapid methods are needed for feed analysis. This study examined the potential of Fourier-transform infrared (FTIR) spectroscopy to predict the nutritional value of a wide range of feeds for ruminants, as an alternative to the in situ technique. Moreover, we investigated whether universal equations could be developed that would allow the low-cost determination of crude protein (CP) concentrations and their kinetics of degradation into the rumen. Protein nutritional values of 663 samples comprising 80 different feed types were determined in terms of concentrations of CP, water-soluble CP (CP_{WS}), total-tract mobile bag CP digestibility (CP_{TTD}), and in situ CP degradability, including the rumen soluble fraction (CP_A), the degradable but not soluble fraction (CP_B), rate of CP_B degradation (CP_C), effective degradability (CP_{ED}), and potential degradability (CP_{PD}). Infrared spectra of dry samples were collected by attenuated total reflectance from 4000 to 600 cm^{-1}. Models were developed by partial least squares (PLS) regression in a randomly selected subset of samples, and the precision of the equations was confirmed by using an external validation set. Analysis by FTIR spectroscopy was sufficiently sensitive to allow the accurate prediction of sample CP concentration ($R^2 = 0.92$) and to classify feeds according to their CP_{WS} concentrations using universal models ($R^2 = 0.78$) that included all sample types. Moreover, substantial improvements in predictions were observed when samples were subdivided in groups. Models for forages led to accurate predictions of CP_{WS} and fractions CP_A and CP_B ($R^2 > 0.83$), whereas models for CP_{TTD} and CP_{ED} could be used for screening purposes ($R^2 > 0.67$). This study showed that models for protein-rich concentrates alone could also be used for screening according to the feed concentrations of CP_{WS}, CP_{TTD}, CP_{ED}, CP_A, and CP_B, but models for energy-rich concentrates gave relatively poor predictions. The general difficulty observed in predicting CP_C is because of a low correlation between FTIR spectra and the kinetics of CP degradation, which may be the result of large variation in the reference method (i.e., in situ degradation studies) and perhaps also because of the presence of compounds that can modify the CP degradation pattern in the rumen. In conclusion, FTIR spectroscopy should be considered as a low-cost alternative in the feed evaluation industry.
Key words: crude protein, Fourier-transform infrared spectroscopy, feed evaluation, rumen degradability

INTRODUCTION

Due to the presence of symbiotic microbial populations within the rumen, ruminants are the only livestock capable of using cellulose and nonprotein N efficiently. This evolutionary adaptation substantially increases the diversity of feeds and feed by-products that can be consumed by ruminants, and decreases feed competition with monogastric livestock and humans. The efficiency of dietary N use for retention in meat, milk, and wool is not optimal, however, and improvements in the feeding evaluation systems for ruminants are required. Traditionally, nutritionists have relied on static models, which take account of the chemical composition of the feeds, to balance rations for ruminants (ARC, 1984). Most modern feed evaluation systems are, however, based on dynamic models in which kinetic parameters of individual nutrients are used for ration balancing (Danfaer et al., 2006). These latter approaches account for the dynamic process of CP degradability in the rumen, which directly influences the quantities of N available for rumen microbial use and the passage of undegraded CP through the rumen to the small intestine. However, successful implementation of advanced ration formulation regimens requires the ability to measure or predict the degree of the feed's CP degradation and its digestibility in the intestinal tract.

To determine the characteristics of feed CP degradability in the rumen, the in situ method was used because it is a well-established procedure in which the degradation profile is determined as the progressive feed disappearance from a nylon bag incubated in the ru-

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men. Similarly, the mobile bag technique is recurrently used to determine total-tract digestibility (Hvelplund, 1985). These procedures are chosen as reference methods in several feed evaluation systems for ruminants (Verité and Peyraud, 1989; Madsen et al., 1995; NRC, 2001; Van Duinkerken et al., 2011). Nevertheless, these feed evaluation techniques have several limitations that make them impractical for on-farm conditions because rumen-cannulated animals and substantial labor inputs are required. The availability of a robust, cost-effective technique to evaluate the protein nutritional value in feeds would help farmers and their advisors improve diet formulations under farm conditions.

Infrared (**IR**) spectroscopy is a technique based on the principle that the type of atoms and atomic bonds within molecules determine the wavenumbers in which the electromagnetic vibrations happen when molecules are excited with a laser. Therefore, analysis of feeds by IR spectroscopy provides chemo-structural information that can be applied by nutritionists and plant breeders using chemometric approaches as an alternative method to traditional ("wet") chemistry. These IR techniques require only relatively small amounts of material and generally allow rapid, low-cost, and robust predictions. To this end, near-IR reflectance spectroscopy (**NIRS**) has been successfully used to predict feed composition and the kinetics of nutrient degradation in the rumen (Herrero et al., 1997; Andres et al., 2005a; Ohlsson et al., 2007).

Mid-IR spectroscopy scans a wider spectral range than NIRS (600 to 4000 cm^{-1} vs. 800 to 2500 cm^{-1}, respectively). Moreover, mid-IR spectroscopy, in contrast to NIRS, yields information on fundamental molecular vibrations, rather than on harmonic and overtone absorptions, and gives better insight into the molecular bonds present in a sample. This means that the mid-IR spectrum of a sample should be directly related to its chemical composition. Over recent years, mid-IR spectroscopy has been revolutionized by the development of Fourier-transform infrared (**FTIR**) spectrometers, which allow greater sensitivity and rapid rates of data acquisition. However, the potential abilities of this technique to predict feed nutritional composition need further investigation.

The objective of the present study was to determine the ability of FTIR spectroscopy to predict the nutritional value of several feedstuffs. We hypothesized that FTIR spectra could be used to develop a universal model that enables the determination of feed CP concentration and the kinetics of rumen CP degradation in feeds. In addition, we investigated the potential advantages of developing specific equations for forages (**FOR**), energy-rich concentrates (**ERC**), and protein-rich concentrates (**PRC**).

MATERIALS AND METHODS

Sample Composition and Rumen Degradability

A total of 663 samples, comprising 80 different feed types, were collected over the course of a 10-yr period and used in this study. These samples represent many of the feed types frequently used in ruminant nutrition, such as conserved forages, concentrates, and by-product feeds. Table 1 shows the samples analyzed indicating their botanical or industrial origin. All samples were freeze-dried, milled to pass through a 1.5-mm screen (Pulverisette 15, Fritsch, Idar-Oberstein, Germany), and stored at room temperature in airtight containers until further analysis.

All freeze-dried samples were analyzed for CP and in situ rumen CP degradability. Total N concentration was determined using an automated Foss-Kjeldahl apparatus (Fisher Scientific, Pittsburgh, PA), and CP concentration was calculated as N × 6.25 (AOAC International, 2005). Estimation of the feed rumen CP degradation profiles was determined using the in situ method using 3 Danish Holstein nonlactating cows cannulated at the rumen and duodenum (Hvelplund and Weisbjerg, 2000). The procedure complied with the guidelines of Danish Ministry of Justice law number 382 (June 10, 1987) act number 726 (September 9, 1993) concerning experiments with animals and the care of experimental animals. Cows were fed at maintenance twice daily with a diet consisting of 67% hay and 33% concentrate on a DM basis. The CP concentration of the diet was always greater than 13.7% of the DM and comprised at least 3 different protein sources. Polyester (Dacron) mesh bags (11 × 8.5 cm and 38-μm pore size, Saatifil PES 38/31, Saatitec S.p.A, Veniano, Italy) containing between 1 and 2 g of sample DM were presoaked in tap water at 39°C for 20 min before incubation in the cows' rumens. Samples were incubated in the rumen ventral sac for 0, 2, 4, 8, 16, 24, 48, and 96 h. Samples from the 0-h time point were not incubated in the rumen and were used for the estimation of soluble CP (see above). After incubation, bags were washed in a washing machine at 25°C without soap or spinning. For forage samples, bags were mixed using a stomacher (Seward, Worthing, UK) with 60 mL of water and then washed to reduce microbial contamination attached to the feed residue. Thereafter, incubation residues were dried overnight at 100°C before analysis of total N concentrations.

Degradation profile parameters based on in situ incubation were estimated by nonlinear regression according to the equation described by Ørskov and McDonald (1979):

$$\text{CP degraded } (t) = \text{CP}_\text{A} + \text{CP}_\text{B} \times (1 - e^{-\text{CPc} \times t}), \quad [1]$$