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Summary

The near infrared reflectance spectroscopy (NIRS) technique is a rapid and non-destructive technique used to evaluate the chemical composition of complete feed and ingredients. The accuracy of its prediction is not only affected by instrument calibrations but also by sample particle size, shape, and arrangement. The purpose of this study was to determine the effect sample preparation method and feed form (mash and pellet) have on the accuracy of the NIRS technique using standard calibrations provided with the instrument. The experiment was designed as a 3×2 factorial with three methods of analysis (laboratory, NIRS-ground, and NIRS-unground) and two feed forms (mash and pellet). All samples were evaluated for crude protein (CP) content. Prior to analysis, subsamples were ground through a 0.5 mm sieve for analysis by laboratory and NIRS-ground methodologies. Laboratory values from wet chemistry analyses were obtained using the Dumas Combustion method for comparison to results from the NIRS. Ground and unground samples were scanned on a Foss NIRS D2500 machine with a wavelength range of 400 to 2,500 nm at a reflectance of $\log(1/R)$ at 2 nm intervals for each sample. There was an interaction ($P \leq 0.05$) observed between feed form and method of analysis. The CP content of unground feed samples varied for the feed forms, but the grinding samples yielded similar results for both NIRS and laboratory analyses. Analyzing unground feed samples using standard calibrations yielded less accurate results compared to the samples ground prior to analysis using either NIRS or laboratory methods.

Introduction

Nutritionists must know the nutrient composition of feedstuffs in order to properly formulate diets that meet an animal's nutrient requirements. However, traditional methods of analyses are expensive, time consuming, and can require specialized training. The near infrared reflectance spectroscopy (NIRS) technique provides rapid and accurate information from high resolution spectra for solid and liquid samples with minimal sample preparation. The technique is economical, facilitates qualitative and quantitative analyses, and is non-destructive to samples. This technique can be used to determine nutrient (crude protein, fat, moisture, fiber, and amino acids) content of feeds and

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feedstuffs in a single scan, in contrast to individual wet chemistry methods. The NIRS technique measures light absorption of a feed or ingredient sample when scanned using wavelengths in the near-infrared region (780 to 2500 nm). Spectrum absorption in the NIR region depends on the chemical bonds (C-H, N-H, S-H, or O-H) as well as the physical and structural characteristics of the sample.

Although the NIRS instrument is highly efficient, variations in accuracy may occur due to factors such as equipment calibrations, physical form of feed (mash or pellet), particle size, shape, distribution, and spacing. Thus, making routine bias adjustments to the standard calibrations may help to increase accuracy when scanning a wide variety of sample types. However, this can be difficult and requires increased knowledge and training on the NIRS equipment. Limited research is available on the accuracy of the NIRS with standard calibrations on the predictability of nutrients in complete feeds manufactured with different particle sizes of corn. Additionally, little is known about how the scanned feed form, whether ground or unground, and mash or pellet, affects the results. Therefore, the objective of this study was to determine the accuracy of the NIRS in predicting the crude protein (CP) content of compound feeds with different methods of sample preparation, sample analysis, and feed form using standard calibrations provided with the instrument.

Materials and Methods

Treatments were arranged in a 2×3 factorial design with feed form (mash and pellet) and method of analysis (laboratory, NIRS-ground, and NIRS-unground). A corn, soybean meal, and wheat bran diet was formulated to contain 20% CP and manufactured using corn with a particle size of 600 μm (Table 1). Treatments were mixed (Model 2261905, Hayes & Stolz, Burleson, TX) for 6 min with 3 replicates manufactured for each diet. After discharge from the mixer, a representative sample of each replicate was obtained using an open-handled grain probe. Samples were then riffle divided to yield 3 aliquots of each replicate, 1 for laboratory analysis and 2 for NIRS analysis. For the pelleted treatment, the diet was conditioned at 185°F for approximately 30 s and pelleted (Model CL-5, California Pellet Mill Co., Crawfordsville, IN) using a 0.16 \times 0.88 in. vertical die. Pellets were collected and cooled in an experimental counterflow cooler for 10 minutes. Samples of the mash and pellets were each riffle divided into 3 aliquots for analysis. Subsamples analyzed by laboratory and NIRS-ground methods were pre-ground to pass through a 0.5 mm screen using a centrifugal mill (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871 Haan, Germany). The CP content of samples based on the laboratory method were determined using a Leco Nitrogen Analyzer (TruMac N, Leco Corporation, St Joseph, MI) according to the Dumas combustion method.² Ground and unground samples analyzed by NIRS for CP were scanned (Model, DS2500 Monochromator, Foss NIR Systems, Laurel, MD) using a large ring cup and the factory calibrations provided with the instrument. All near infrared spectra were collected at wavelengths between 400 and 2,500 nm registering absorbance values $\log(1/R)$ (where r = reflectance) at 2 nm interval for each sample.

² Association of Official Analytical Chemists (AOAC). (1995). Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis.

Statistical analysis

Data were analyzed using the GLIMMIX procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC) and significance were based on $P \leq 0.05$. Treatments were arranged in a 2×3 factorial with feed form (mash and pellet) and method of analysis (laboratory, NIRS-ground, and NIRS-unground). All treatments were replicated 3 times.

Results and Discussion

There was an interaction ($P \leq 0.05$) between feed form (mash and pellet) and methods of analysis (laboratory, NIRS-ground, and NIRS-unground; Table 2). For the interactions observed, mash and pellet samples analyzed as unground using the NIRS had decreased CP compared to those analyzed using the laboratory method. Additionally, ground samples of both mash and pellets analyzed with the NIRS technique were similar to results from laboratory method. Thus, grinding mash and pellet samples to similar particle size, 0.5 mm, eliminated the differences in feed form when analyzed with either wet chemistry method (laboratory) or with the NIRS. These results indicate that mash and pellet samples analyzed as unground samples may adversely affect the accuracy of CP predictability, but grinding samples prior to analysis will improve results from the NIRS.

Table 1. Diet composition (as-fed basis)¹

Ingredient	%
Corn	54.01
Soybean meal (46.5% CP)	30.10
Wheat bran	6.00
Soy oil	5.80
L-Threonine	0.30
L-Lysine HCl	0.18
DL-Methionine	0.39
Monocalcium P, 21%	1.82
Limestone	0.75
Salt	0.40
Vitamin trace mineral premix ²	0.25
Total	100.00
Calculated analysis	
Crude protein, %	20.02
Crude fat, %	8.43
Crude fiber, %	2.78

¹Diet manufactured with corn ground to 600 μm and fed in mash or pelleted form.

²Supplied the following minimum supplements per kilogram of diet: vitamin A, 635,600 IU; vitamin D₃, 22,7000 ICU; vitamin E, 1,362 IU; menadione, 68.1 mg; riboflavin, 544.8 mg; thiamine, 90.8 mg; d-pantothenic acid, 544.8 mg; niacin 2.270 mg; vitamin B₆, 113.5 mg; folic acid, 56.75 mg; choline, 31,780 mg; biotin, 3.632 mg; Mn, 40,000 mg; Zn, 40,000 mg; Fe, 20,000 mg; Cu, 4,500 mg; iodine, 500 mg; and Se, 60 mg.

Table 2. Interaction of feed form and method of analysis on crude protein analysis¹

Feed form	Method of analysis ²	Crude protein, %
Interaction		
Mash	Laboratory	20.26 ^a
Mash	NIRS-ground	19.93 ^a
Mash	NIRS-unground	18.81 ^b
Pellet	Laboratory	20.32 ^a
Pellet	NIRS-ground	19.20 ^{ba}
Pellet	NIRS-unground	16.88 ^c
SEM ³		0.279
Source of variation		Probability, <i>P</i> <
Form × method		0.008

¹Treatments were arranged in a 2 × 3 factorial design with feed form (mash and pellet) and method of analysis (laboratory, NIRS-ground, and NIRS-unground). There were 3 replicates per treatments and diets were formulated to contain 20% crude protein. NIRS = near infrared reflectance spectroscopy.

²Method, Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (Association of Official Analytical Chemists (AOAC). (1995). Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis.) NIRS-ground and NIRS-unground: NIRS.

³SEM = standard error of mean.

^{a-c}Means within a column with different superscript are significantly different based on *P* ≤ 0.05.