

## The Influence of Ingredients, Corn Particle Size, and Sample Preparation on the Predictability of the Near Infrared Reflectance Spectroscopy

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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 relies upon calibration standards to account for variations in material composition and particle shape and size. The purpose of this study was to determine the effect of alternative ingredient inclusion and corn particle size along with sample preparation method on the accuracy of the NIRS technique using standard calibrations provided with the instrument. Treatments were arranged as a  $4 \times 3 \times 3$  factorial with diet type (soybean meal (SBM) + DDGS (SD); SBM + fish meal + DDGS (SFD); SBM + fish meal + wheat bran (SFB); and SBM + wheat bran (SB)); corn particle size (400, 600, and 800  $\mu\text{m}$ ); and method of analysis (laboratory, NIRS-ground, and NIRS-unground). All samples were evaluated for crude protein (CP) content. Laboratory values from wet chemistry analyses were obtained using the Dumas Combustion method for comparison to results from the NIRS. Ground and unground samples for NIRS 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 no diet  $\times$  particle size  $\times$  method interaction on CP; however, there was an interaction ( $P \leq 0.05$ ) between diet and method of analysis. When analyzing diets using laboratory methods there were no differences in CP, but when using the NIRS, grinding samples prior to NIRS analysis improved the results compared to not grinding, though they were still lower than laboratory analysis. There was also an interaction ( $P \leq 0.05$ ) between corn particle size and method of analysis. The CP content of NIRS-ground and laboratory samples were similar within the methods used, and values obtained for the different particle sizes were closer to the expected CP (20%) as compared to the NIRS-unground samples. Results from NIRS-unground samples of diets were significantly different and lower than results from laboratory analysis. However, results from the NIRS-ground samples were intermediate between NIRS-unground and laboratory analysis. Results of this trial indicate the necessity for proper calibration biasing to improve the prediction accuracy of NIRS, especially when diets contain alternative ingredients. Grinding the sample prior to scanning with the NIRS will improve accuracy, though values may still differ from

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laboratory methods when using standard equipment calibrations, further emphasizing the importance of calibration biasing.

## Introduction

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. In contrast to traditional wet chemistry methods, NIRS is an economical approach that facilitates qualitative and quantitative analyses and is non-destructive to samples. The NIRS technique can be used to determine the content of multiple nutrients (crude protein, fat, moisture, fiber, and amino acids) of both complete feeds and individual feedstuffs in a single scan, unlike individual wet chemistry methods. Though efficient, there is concern about the accuracy of NIRS predictions as results may be influenced by both equipment (equations) and sample factors (particle shape and size).

The NIRS works by measuring light absorption of a sample when scanned using wavelengths in the near-infrared region (780 to 2500 nm). The spectrum absorption can then be assessed and quantified using calibrated equations provided by the manufacturer of the equipment. Bias adjustments to the standard calibration equations can be made in an attempt to increase accuracy, but this can be difficult and requires increased knowledge and training on the NIRS equipment. Equation biasing may be of particular importance when the scanned material varies in composition from the database used to generate the standard equations. Limited research is available on the influence of alternative ingredient diet formulations and corn particle size on the accuracy of the NIRS with standard calibrations and whether sample preparation may also be of importance. Thus, the objective of this study was to determine the effect of alternative ingredient inclusion and corn particle size along with sample preparation method on the accuracy of the NIRS technique using standard calibrations provided with the instrument.

## Materials and Methods

Treatments were arranged as a  $4 \times 3 \times 3$  factorial with diet type (Table 1): (soybean meal (SBM) + DDGS (SD); SBM + fish meal + DDGS (SFD); SBM + fish meal + wheat bran (SFB); and SBM + wheat bran (SB)); corn particle size (400, 600, and 800  $\mu\text{m}$ ); and method of analysis (laboratory, NIRS-ground, and NIRS-unground). Diets were formulated to contain 20% CP based on book values for ingredients. Corn was ground using a hammermill (Model 2215, Bliss Industries, LLC, Ponca City, OK) with particle size treatments (400, 600, and 800  $\mu\text{m}$ ) verified according to ASAE S319.2<sup>2</sup> with agitators and agent. Treatments were mixed (Model 2261905, Hayes-Stolz, Burleson, TX) for 6 min and 3 replicates were manufactured for each diet and corn particle size combination. 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. 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). Crude protein content of samples based on the laboratory method were determined using a Leco Nitrogen

<sup>2</sup> ASABE Standards. (1995). S319.2: Method of determining and expressing fineness of feed materials by sieving. St. Joseph, Mich.: ASABE.

Analyzer (TruMac N, Leco Corporation, St Joseph, MI) according to the Dumas combustion method (AOAC 990.03<sup>3</sup>). 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.

### *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 as a  $4 \times 3 \times 3$  factorial with diet type: SD, DFM, FMWB, and WB; corn particle size: 400, 600, and 800  $\mu\text{m}$ ; and method of analysis: laboratory, NIRS-ground, and NIRS-unground. All treatments were replicated 3 times.

## **Results and Discussion**

There was no diet  $\times$  particle size  $\times$  method interaction; therefore, these results were not presented. However, there was an interaction ( $P \leq 0.05$ ) between diet (SD, SFD, SFB, and SB) and method of analysis (laboratory, NIRS-ground, and NIRS-unground; Table 2). The effect of diet formulation depended upon the type of method analysis. When analyzing diets using laboratory methods there were no differences in CP, but when using the NIRS, sample preparation was critical. Grinding samples prior to NIRS analysis improved the results compared to not grinding, though results were still lower than laboratory analysis. Additionally, the NIRS calibrations appeared to be unable to handle the SD and SB diet formulations compared to the other diets, especially when analyzed unground, indicating the importance of internal calibration standards developed based on the typical diets manufactured.

There was also an interaction ( $P \leq 0.05$ ) between particle size (400, 600, and 800  $\mu\text{m}$ ) and method of analysis (laboratory, NIRS-ground, and NIRS-unground; Table 2). The CP content of NIRS-ground and laboratory samples were similar within the methods used, and values obtained for the different particle sizes were closer to the expected CP (20%) as compared to the NIRS-unground samples. Results from NIRS-unground samples of diets were significantly different and lower than results from laboratory analysis. However, results from the NIRS-ground samples were intermediate between NIRS-unground and laboratory analysis. Thus, grinding the sample improved the accuracy of the NIRS when using standard calibrations, likely due to the similar spectra characteristics generated by a more uniform sample particle size. It also further indicates the necessity for proper calibration biasing to improve NIRS prediction accuracy. Both laboratory and NIRS-ground samples were ground through a 0.5 mm screen prior to analysis and were of similar particle size at time of analysis, though the NIRS yielded lower CP in comparison to the laboratory.

For main effects, diet and method of analysis influenced the CP predictability of the NIRS. The SFD and SFB had significantly higher CP content as compared to the SD and SB diets (Table 3). The SD diet recorded the lowest CP content, which may

<sup>3</sup> Association of Official Analytical Chemists (AOAC). (1995). Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis.

have been due to the high variability of the chemical composition of DDGS and the use of book values to formulate diets to 20% CP. Additionally, the diets analyzed in the current study may have been different from the diets used in the instrument calibrations. Ingredients used may have been of different origins, harvesting seasons or processing methods, further illustrating the importance of creating unique facility calibration biases based on the feed being manufactured. The reference value from laboratory analysis (20.43%) was greater than CP content of NIRS-unground samples (18.65%), but the CP content of NIRS-ground samples (19.74%) was intermediate between the two methods (Table 3).

Under the constraints of this trial, factory calibrations loaded on the NIRS did not have a sufficient bias for the ingredients used in the diet formulations. This may be due to differences in ingredient compositions between those used in the study and those used in the development of the manufacturer's calibrations. Additionally, the formulations in this study may be somewhat unconventional for the US industry that relies mostly on corn and soybean meal. This was done purposefully to highlight the need for further calibration biasing when analyzing more complex diet formulations, which may be more typical internationally. Though accuracy of NIRS prediction was improved by grinding, it was still unable to match the laboratory results. Thus, it is important for facilities to create internal biases to help improve the prediction of nutrient content with the NIRS. Nutrient predictions will further be improved by grinding the samples to a uniform particle size prior to scanning. Creating a uniform particle size for both mash and pellets will help to reduce the variation in results, leading to better prediction capability using the NIRS.

*Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.*

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

Ingredient, %	Dietary formulation <sup>1</sup>			
	SD	SFD	SFB	SB
Corn <sup>2</sup>	57.58	66.15	62.92	54.01
Soybean meal	30.50	18.00	17.00	30.10
Fish meal	---	9.50	9.70	---
DDGS	2.00	2.00	---	---
Wheat bran	---	---	6.00	6.00
Soy oil	5.80	2.40	2.40	5.80
L-Threonine	0.30	0.30	0.30	0.30
L-Lysine HCl	0.18	0.16	0.18	0.18
DL-Methionine	0.42	0.29	0.30	0.39
Monocalcium P, 21%	1.82	0.50	0.50	1.82
Limestone	0.75	0.35	0.35	0.75
Salt	0.40	0.10	0.10	0.40
Vitamin TM premix <sup>3</sup>	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein, %	20.10	20.10	20.02	20.02
Crude fat, %	8.40	6.13	6.20	8.43
Crude fiber, %	2.45	2.31	2.64	2.78

<sup>1</sup>Diets manufactured with corn ground to 3 different particle sizes: 400, 600, and 800  $\mu\text{m}$ . SD: soybean meal and DDGS. SFD: soybean meal-fish meal-DDGS. SFB: soybean meal-fish meal-wheat bran. SB: soybean meal-wheat bran.

<sup>2</sup>Diet manufactured with corn ground to 600  $\mu\text{m}$ .

<sup>3</sup>Supplied the following minimum supplements per kilogram of diet; vitamin A, 635,600 IU; vitamin D<sub>3</sub>, 227,000 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 B<sub>6</sub>, 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; I, 500 mg; and Se, 60 mg.

**Table 2. Interactions between diet × method of analysis and method of analysis × corn particle size on crude protein analysis<sup>1</sup>**

Diet <sup>2</sup>	Method of analysis <sup>3</sup>	Particle size, <sup>4</sup> $\mu\text{m}$	Crude protein, %
SD	Laboratory		20.14 <sup>a</sup>
SFD	Laboratory		20.69 <sup>a</sup>
SFB	Laboratory		20.57 <sup>a</sup>
SB	Laboratory		20.32 <sup>a</sup>
SD	NIRS-ground		19.45 <sup>dc</sup>
SFD	NIRS-ground		20.05 <sup>bda</sup>
SFB	NIRS-ground		19.68 <sup>bdc</sup>
SB	NIRS-ground		19.78 <sup>bdc</sup>
SD	NIRS-unground		17.23 <sup>f</sup>
SFD	NIRS-unground		19.50 <sup>c</sup>
SFB	NIRS-unground		19.62 <sup>c</sup>
SB	NIRS-unground		18.26 <sup>e</sup>
SEM <sup>5</sup>			0.139
	Laboratory	400	20.48 <sup>w</sup>
	Laboratory	600	20.42 <sup>w</sup>
	Laboratory	800	20.38 <sup>w</sup>
	NIRS-ground	400	19.75 <sup>x</sup>
	NIRS-ground	600	19.73 <sup>x</sup>
	NIRS-ground	800	19.73 <sup>x</sup>
	NIRS-unground	400	18.41 <sup>z</sup>
	NIRS-unground	600	18.50 <sup>z</sup>
	NIRS-unground	800	19.06 <sup>y</sup>
	SEM <sup>5</sup>		0.120
Source of variation			Probability, $P <$
Diet × method <sup>a-f</sup>			0.001
Method × particle size <sup>w-z</sup>			0.015

<sup>1</sup>Treatments were arranged in a 4 × 3 × 3 factorial design of diet formulation, method of analysis, and ground corn particle size. There were 3 replicates per treatments, and diets were formulated to contain 20% crude protein.

<sup>2</sup>Diet = SD: SBM + DDGS. SFD: SBM + fish meal + DDGS. SFB: SBM + fish meal + wheat bran. SB: SBM + wheat bran.

<sup>3</sup>Method, Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); ground and unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm. (Association of Official Analytical Chemists (AOAC). (1995). Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis.)

<sup>4</sup>Particle size of ground corn according to ASAE S319.2 with agitators and dispersing agent.

<sup>5</sup>SEM: Standard error of the mean. (ASABE Standards. (1995). S319.2: Method of determining and expressing fineness of feed materials by sieving. St. Joseph, Mich.: ASABE.)

<sup>a-f; w-z</sup> Means with different superscripts within a column are significantly different based on  $P \leq 0.05$ .

**Table 3. Main effects of diet, method of analysis, and corn particle size on crude protein analysis<sup>1</sup>**

Diet <sup>2</sup>	Method of analysis <sup>3</sup>	Particle size, <sup>4</sup> $\mu\text{m}$	Crude protein, %
SD			18.94 <sup>c</sup>
SFD			20.08 <sup>a</sup>
SFB			19.96 <sup>a</sup>
SB			19.45 <sup>b</sup>
SEM <sup>5</sup>			0.080
	Laboratory		20.43 <sup>x</sup>
	NIRS-ground		19.74 <sup>y</sup>
	NIRS-unground		18.65 <sup>z</sup>
	SEM <sup>5</sup>		0.070
		400	19.54
		600	19.56
		800	19.72
		SEM <sup>5</sup>	0.069
Source of variation			Probability, $P <$
Diet <sup>a-c</sup>			<0.001
Method <sup>x-z</sup>			<0.001
Particle size			0.124

<sup>1</sup>Treatments were arranged in a  $4 \times 3 \times 3$  factorial design of diet formulation, method of analysis, and ground corn particle size. There were 3 replicates per treatments and diets were formulated to contain 20% crude protein.

<sup>2</sup>Diet = SD: SBM + DDGS. SFD: SBM + fish meal + DDGS. SFB: SBM + fish meal + wheat bran; SB: SBM + wheat bran.

<sup>3</sup>Method = Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); ground and unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm. (Association of Official Analytical Chemists (AOAC). (1995). Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis).

<sup>4</sup>Particle size of ground corn according to ASAE S319.2 with agitators and dispersing agent.

<sup>5</sup>SEM: Standard error of the mean. (ASABE Standards. (1995). S319.2: Method of determining and expressing fineness of feed materials by sieving. St. Joseph, Mich.: ASABE.)

<sup>a-c, x-z</sup> Means with different superscripts within a column are significantly different based on  $P \leq 0.05$ .