

# Determining the Influence of Particle Size, Diets, Analytical Methods and Feed Form on the Predictability of the Near Infrared Reflectance Spectroscopy

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## Abstract

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 sample particle size, shape and arrangement. The purpose of this study was to determine the effect of corn particle size, methods of analysis, diets and feed form (mash and pellet) on the accuracy of the NIRS technique using standard calibrations provided with the instrument. In Experiment 1, treatments were arranged in a 4 × 3 × 3 factorial design. The major ingredients in the different diets were i) soybean meal+DDGs, ii) soybean meal+fish meal+DDGs, iii) soybean meal+fish meal+wheat bran, and iv) soybean meal+wheat bran. These were manufactured using different corn particle sizes (400, 600 and 800 µm) to contain a calculated protein content of 20% and subsequently analyzed using three different methods (laboratory, NIRS-ground and NIRS-unground). Experiment 2 was a 3 × 2 factorial with three methods of analysis (laboratory, NIRS-ground and NIRS-unground) and two feed forms (mash and pellet). Diets were pelleted and cooled in a counter-flow cooler for 10 minutes. Prior to NIRS analysis, subsample of mash and pellets was ground through a 0.5 mm sieve for the ground treatment. Ground and unground mash and pellet samples for Experiment 1 and 2 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. Laboratory values from wet chemistry analyses were obtained using the Dumas Combustion method and these were compared to results from the NIRS. In Experiment 1, there was no three way interaction. However, a two-way interaction ( $P \leq 0.05$ ) was observed for diets x method of analysis and particle size x method of analysis. Crude Protein (CP) content of samples varied when analyzed with NIRS-unground but similar CP was observed for those analyzed with either NIRS-ground or laboratory method. A difference ( $P \leq 0.05$ ) in CP content was observed for diets, method of analysis but not for particle sizes. Results of NIRS-ground samples were greater and closer to the expected CP (20%) than NIRS-unground samples. In Experiment 2, an interaction was observed between feed form and method of analysis. The CP content of unground feed samples varied for the feed forms but grinding samples yielded similar results for both NIRS and laboratory analyses. Analyzing unground feed samples using standard calibrations yielded less accurate results compared to samples ground prior to analysis using either NIRS or laboratory methods.

**Keywords:** Chemical composition; Feed form; Near infrared spectroscopy; Prediction; Unground; Wavelength

## Introduction

Nutritionist must know the nutrient composition of feed stuffs in order to properly formulate diets that meet the nutrient requirements of livestock, [1,2]. However, traditional methods of analyses for ingredients and feed are expensive, time consuming and require specialized training. The NIRS technique on the other hand 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. Samples analyzed with NIRS require no prior preparation with chemicals and therefore eliminate chemical and disposal costs. This technique can be used to determine multiple nutrients (crude protein, fat, moisture, fiber and amino acid) content of feeds and feed stuffs in a single scan, unlike wet chemistry analysis

where most nutrients are analyzed separately with different 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. The chemical bonds make it possible to identify specific regions of the spectrum associated with sample constituents such as starch, crude protein or fiber [3].

Although the NIRS instrument is highly efficient, variations in sample composition can be observed in results [4]. These variations could be due to factors such as technician, cross-contamination, analysis of a sample that is not present in the installed calibrations, physical form of feed (mash or pellet), particle size, shape, distribution of sample sand also spaces between particles [5,6]. The

NIRS technique is more accurate in predicting the nutrient content of ingredients compared to nutrients in compound feeds. This is because compound feeds are usually made from a wide range of ingredients of different particle sizes from different sources (cereal grains, animal and plant by-products). Additionally, different quantities of ingredients may yield different spectral properties (absorption bands and wavelength) even for diets with similar nutrient contents [7]. Therefore, it is important to routinely make bias adjustments to standard calibrations. Smith KF, et al. [8] suggested that calibrations must be checked when new samples are added to the population (samples containing ingredients from new suppliers, geographic regions and diets formulations).

Since the development of the NIRS, several researchers have demonstrated its ability to predict the chemical composition of feedstuffs and feeds [9-11], but limited research is available in feeds with different particle sizes, feed forms (mash and pellet), diets and physical sample form (ground and unground). Most of the studies done indicated that all samples were ground before NIRS analysis [12,13] for more accurate results. However, NIRS equipment suppliers indicate that samples require limited or no preparation prior to analysis of feed. Therefore, the objective of this study was to determine the accuracy of the NIRS in predicting the crude protein content of compound feeds with different corn particle sizes, different ingredients, methods of analysis (laboratory, NIRS-ground and NIRS-unground) and feed form (mash and pellets) using standard calibrations provided with the instrument.

## Material and Methods

### Experiment 1

Treatments were arranged as a 4 × 3 × 3 factorial. The factors were; 1) diet at four levels; i) Soybean Meal+DDGS (SD), ii) Soybean Meal+Fish Meal+DDGS (SFD), iii) Soybean Meal+Fish Meal+Wheat Bran (SFB), and iv) Soybean Meal+Wheat Bran (SB), 2) corn particle size at three levels (400, 600 and 800 μm) and, 3) method of analysis at three levels (laboratory, NIRS-ground and NIRS-unground) (Table 1).

Corn was ground using a hammer mill (Model 2215, Bliss industries, LLC, Ponca City, OK) to obtain the different corn particle sizes. All diets were mixed for six minutes using a Hayes-Stolz mixer (Model 2261905, Burleson, TX). Three replicates were manufactured per diet and four samples per replicate. Samples were collected from the replicates of each diet after mixer discharge using a sampling probe. Four samples were collected from each replicate of each diet manufactured. Thus, there were 12 samples per diet.

Each sample was divided and ground with a centrifugal mill (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871 Haan, Germany) through a 0.5 mm sieve. Unground and ground samples were analyzed with the NIRS and compared with laboratory results from wet chemistry analysis using the Dumas combustion method.

### Experiment 2

Treatments were arranged in a 2 × 3 factorial design with method of analysis and feed form as factors. A corn-SBM wheat bran diet was formulated to contain 20% crude protein and manufactured using 600 μm corn particle size (Table 2). The diet was manufactured the same way as in Experiment 1. Mash samples were collected after mixer discharge using a sampling probe. The diet was pelleted in the Feed Safety Research Center at the O.H. Kruse Feed Technology and Innovation Center using a CPM pellet mill (Model CL-5, California Pellet Co., Crawfordsville, IN). Diet was conditioned to 85°C in a 13 cm × 91 cm conditioner and pelleted using a die with an L/D ratio

**Table 1:** Composition of experiment 1 diets<sup>1</sup>.

Ingredient, %	Diet <sup>2</sup>			
	SD	SFD	SFB	SB
Corn	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
Monocal 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
<b>Calculated analysis</b>				
CP, %	20.01	20.10	20.02	20.02
Crude fat, %	8.40	6.13	6.20	8.43
Fiber, %	2.45	2.31	2.64	2.78
<b>Lysine, %</b>	<b>1.25</b>	<b>1.25</b>	<b>1.25</b>	<b>1.25</b>
Calcium, %	0.89	0.87	0.88	0.88
Phosphorus (Total), %	0.79	0.74	0.78	0.83
Sodium, %	0.20	0.18	0.16	0.19

<sup>1</sup>Diets were manufactured for three different particle sizes, 400, 600, and 800 μm.

<sup>2</sup>SD: Soybean Meal and DDGS, SFD: Soybean meal-Fish meal-DDGS; SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran.

<sup>3</sup>Supplied 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; I, 500 mg; and Se, 60 mg.

of 5.55 (diameter=4.0 mm, effective thickness=22 mm.). Conditioner retention time was 45 seconds for all diets pelleted. Samples of each treatment were collected and cooled in an experimental counter-flow cooler for 10 minutes. Pellet samples were collected after cooling. Samples of the mash and pellets were ground in the same way as in Experiment 1. Ground and unground mash and pellet samples were analyzed in the same way as in Experiment 1.

### NIRS analysis

Ground and unground samples of the mash and pellets were scanned with a NIRS (Model, DS2500 Monochromator, Foss NIR Systems, Laurel, MD) using a large ring cup. All near infrared spectra were collected at wavelength between 400 and 2,500 nm registering absorbance values log (1/R) (where, r=reflectance) at 2 nm interval for each sample. Samples were analyzed for crude protein using the factory calibrations provided with the instrument.

**Table 2:** Composition of experiment 2 diet<sup>1</sup>.

Ingredient	%
Corn	54.01
Soybean meal	30.10
Wheat bran	6.00
Soy oil	5.80
L-Threonine	0.30
L-Lysine HCl	0.18
DL-Methionine	0.39
Monocal P, 21%	1.82
Limestone	0.75
Salt	0.40
Vitamin TM premix <sup>2</sup>	0.25
Total	100.00
<b>Calculated analysis</b>	
CP, %	20.02
CF, %	8.43
Fiber, %	2.78
Lysine	1.25
Calcium	0.88
Phosphorus	0.83
Sodium	0.19

<sup>1</sup>Diet contained corn of particle size 600 µm.

<sup>2</sup>Supplied 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; I, 500 mg; and Se, 60 mg.

### Particle size method

Particle sizes were analyzed using the 13-sieve method (ANSI/ASAE S319.4) [14]. A 100 ± 5 g of representative sample of ground corn was obtained after collecting and splitting sample. Sieve agitators were added to the sieve stack and 0.5 g of flow agent (Model SSA-58, Gilson Company, Inc., Lewis Center, OH) was weighed and mixed with the 100 ± 5 g sample according to Kalivoda JR, et al. [15]. The mixture was then placed on top of the sieve stack and the sieve stack was placed in the Ro-tap sieve shaker (Model RX-29, W. S. Tyler Industrial Group, Mentor, OH) and run for ten minutes. The amount of material on each sieve was used to calculate the mean (d<sub>gw</sub>) particle size.

### Chemical analysis

Portions of the ground feed samples were analyzed for crude protein using wet chemical reference methods. A 0.5 g of each sample was weighed into a tared crucible and placed on the carousel in the machine. Protein was determined using a Leco Nitrogen Analyzer (TruMac N, Leco Corporation, St Joseph, MI) according to the Dumas combustion method (AOAC 990.03) [16].

### Statistical analysis

Diets and feed form (mash and pellet) was the experimental unit in Experiment 1 and 2 respectively. Data from the study were analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) and significant statements were based on  $P \leq 0.05$ . Differences between means were separated using Tukey's studentized pair wise analysis. In Experiment 1 particle size *versus* methods of

analysis *versus* diets were compared, while in Experiment 2 methods of analysis vs feed form were compared.

## Results

### Experiment 1

There was no particle size × diet × method interaction; therefore, these results were not presented. However, significant interactions ( $P \leq 0.05$ ) were recorded between diet (SD, SFD, SFB and SB) × methods of analysis (laboratory, NIRS-ground and NIRS-unground) (Table 3) and, particle size (400, 600 and 800 µm) × methods of analysis (laboratory, NIRS-ground and NIRS-unground) (Table 4). The effects of diets and particle size on sample CP content depended on the type of method of analysis used.

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 (Table 3). 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 (Table 4).

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 5). The SD diet recorded the lowest CP content. Reference value from laboratory analysis (20.43%)

**Table 3:** Interaction between diets and method of crude protein analysis<sup>1</sup>.

Diet <sup>2</sup>	Method of analysis <sup>3</sup>	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>cd</sup>
SFD	NIRS-ground	20.05 <sup>abd</sup>
SFB	NIRS-ground	19.68 <sup>bcd</sup>
SB	NIRS-ground	19.78 <sup>bcd</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>4</sup>		0.139
P-value		<0.0001

<sup>1</sup>Treatments were arranged as a 3 × 3 × 4 factorial design and contained 20% crude protein. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.

<sup>2</sup>Diet; SD: Soybean meal-DDGS, SFD: Soybean meal-Fish meal-DGGS, SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran.

<sup>3</sup>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.

<sup>4</sup>SEM: Standard error of means of interaction.

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

**Table 4:** Interaction between particle size and method of crude protein analysis<sup>1</sup>.

Particle size	Method of analysis <sup>2</sup>	Crude protein (%)
400	Laboratory	20.48 <sup>a</sup>
600	Laboratory	20.42 <sup>a</sup>
800	Laboratory	20.38 <sup>a</sup>
400	NIRS-ground	19.75 <sup>b</sup>
600	NIRS-ground	19.73 <sup>b</sup>
800	NIRS-ground	19.73 <sup>b</sup>
400	NIRS-unground	18.41 <sup>d</sup>
600	NIRS-unground	18.50 <sup>d</sup>
800	NIRS-unground	19.06 <sup>c</sup>
SEM <sup>3</sup>		0.120
P-value		0.0152

<sup>1</sup>Treatments were arranged as a 3 × 3 × 4 factorial design and contained 20% crude protein. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.

<sup>2</sup>Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09), NIRS-ground and NIRS-unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm.

<sup>3</sup>SEM: Standard error of means of interaction.

<sup>a-d</sup> Means with different superscripts within a column are significantly different based on P ≤ 0.05.

**Table 5:** Main effects of particle size, diets and method on crude protein analysis<sup>1</sup>.

Particle size	Diet <sup>2</sup>	Method of analysis <sup>3</sup>	Crude protein (%)
400			19.54
600			19.56
800			19.72
SEM <sup>4</sup>			0.069
	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>a</sup>
		NIRS-ground	19.74 <sup>b</sup>
		NIRS-unground	18.65 <sup>c</sup>
SEM <sup>6</sup>			0.070
P-value			
Particle size			0.124
Diet			<0.0001
Method			<0.0001

<sup>1</sup>Treatments were arranged as a 3 × 3 × 4 factorial design and contained 20% crude protein. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.

<sup>2</sup>Diet: SD: Soybean meal-DDGS, SFD: Soybean meal-Fish meal-Wheat bran, SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran

<sup>3</sup>Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); NIRS-ground and NIRS-unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm.

<sup>4-6</sup>SEM: Standard error of means of main effects.

<sup>a-c</sup>Means with different superscripts within a column is significantly different based on P ≤ 0.05.

was significantly higher than CP content of NIRS-unground samples (18.65%) but NIRS-ground samples (19.74%) was intermediate between the two methods (Table 5).

Average CP content of NIRS-ground samples (19.74%) were about 0.7% lower whereas NIRS-unground samples (18.65%) were about 1.8% less than laboratory results (20.43%). Overall, correlation between CP content of laboratory and NIRS-ground samples was better (R=0.30) than the correlation observed between laboratory and NIRS-unground samples (R=0.19) (Figure 1). However, both NIRS-ground and NIRS-unground CP results were not well correlated with laboratory results.

## Experiment 2

There was an interaction (P ≤ 0.05) between feed form and methods of analysis (Table 6). For the interactions observed, mash and pellet samples analyzed as unground using the NIRS was significantly lower than samples analyzed using the laboratory method. However, CP content of NIRS-ground mash sample was similar to NIRS-unground mash sample. Additionally, ground samples of both mash and pellet analyzed with the NIRS technique was 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.

## Discussion

### Experiment 1

The interactions observed between particle size and method as well as diets and method were as a result of the methods of analysis used. Thus, effect of diets and particle size on CP predictability will only be evident if different methods are used. Analyzing samples as NIRS-unground yielded different and lower CP content but when analyzed as NIRS-ground will yield lower but similar results. The impact of diets on the CP predictability may have been due to variations in particle sizes of the ingredients in the diets. This differences in particle sizes may have caused variation in the surface area, which in turn affected the amounts of light transmitted, absorbed or reflected [17] leading to differences in spectral characteristics (absorbance bands, wavelength,

**Table 6:** Interaction between feed form and method on crude protein analysis<sup>1</sup>.

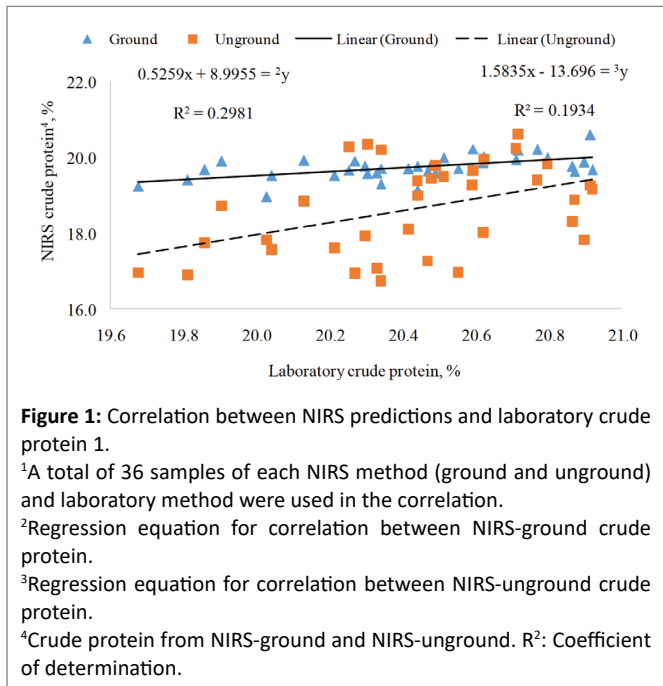
Feed form	Method of analysis <sup>2</sup>	Crude protein (%)
Mash	Laboratory	20.26 <sup>a</sup>
Mash	NIRS-ground	19.93 <sup>a</sup>
Mash	NIRS-unground	18.81 <sup>b</sup>
Pellet	Laboratory	20.32 <sup>a</sup>
Pellet	NIRS-ground	19.20 <sup>ba</sup>
Pellet	NIRS-unground	16.88 <sup>c</sup>
SEM <sup>3</sup>		0.2792
P-value		<0.0079

<sup>1</sup>Treatments were analyzed as a 3 × 2 factorial, with method (laboratory, NIRS-ground and NIRS-unground), and feed form (mash and pellet).

<sup>2</sup>Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); NIRS-ground and NIRS-unground: NIRS DS2500 Foss analyzer. Means of each treatment was obtained from four samples.

<sup>3</sup>SEM: Standard error of mean of interaction.

<sup>a-c</sup>Means within a column with different superscript are significantly based on P ≤ 0.05.



etc.) [18] and CP predictions from the NIRS. The surface area of unground samples is rather rough as compared to that of ground samples. Particle size affects the distance light travels inside a sample before it is scattered or reflected to the surface. The larger the particle size, the smaller the amount of light that reappears from within the sample causing higher absorption and *vice versa*. This affects spectra resulting in differences in chemical composition of samples of variable particle sizes [19]. The difference observed was eliminated when samples were ground and analyzed with either NIRS or wet chemistry. This is because grinding samples to similar particle sizes resulted in similar surface area and therefore similar spectra characteristics.

The lower CP content of the SD diet could be due to the DDGs present. The chemical composition of DDGs is highly variable and this variability may be observed even for products from the same grain [20,21]. This variability is likely to occur due to the heat treatment used in processing [20]. In heat processing, there is the likelihood of Maillard reaction occurrence [22] and this reduce amino acids content, particularly lysine. In the SFD, the variability may have been eliminated or reduced by the addition of fish meal which is a rich source of lysine.

The findings of this study agreed with the report of Abram (1989) who suggested that it was important to analyze mixed feed samples as ground rather than unground samples due to variability associated with unground samples when using the NIRS. Differences between NIRS-ground, NIRS-unground and laboratory values of samples were expected because bias adjustments were not performed on standard calibrations for the sample set. The diets analyzed in the current study may have been different from the diets used in the instrument calibrations. Ingredients used may have also been of different origins, harvesting seasons or processing methods. Results from this study were less accurate as compared to the findings of [12,23,24] who accurately predicted the CP content of finished feeds using the NIRS. This was primarily due to calibrations that were specifically developed for their sample sets before analyses as compared to the current study where standard calibrations installed on the NIRS were used. This indicated

that it is important to verify the ability of standard calibrations to accurately predict CP content of new samples prior to analysis.

## Experiment 2

Analyzing unground samples of mash and pellets with the NIRS produced different CP content but grinding samples resulted in similar CP content, for both laboratory and NIRS methods. The higher CP observed for mash as compared to that of pellets could be due to differences in spaces between particles and shape of particles [18]. Compared to mash, the particle size of pellets were much larger causing spaces between particles to be larger than those between mash particles. The differences in spaces between particles might have influenced light transmission, absorption and reflection, affecting spectral response and predicted CP results. Nonetheless, grinding feed samples to similar finer particle sizes eliminated differences in spaces between particles for mash and pellet samples and yielded improved but similar results for both laboratory and NIRS-ground. These results indicated that mash and pellet analyzed as unground samples may adversely affect the accuracy of CP predictability but grinding samples prior to analysis will improve results from the NIRS.

## Conclusion

In conclusion, analyses of unground mash or pellet samples may result in greater variation in NIRS results but grinding samples prior to analysis may help improve results. Additionally, samples should be routinely analyzed with reference methods and the calibrations adjusted based on the feed manufactured at the feed mill.

## References

1. United States Department of Agriculture (USDA) (2012) Animal diets and feed management. Natural Resources Conservation Services. Nutr Manag Tech Note.
2. Goedhart PW (1990) Comparison of multivariate calibration methods for prediction of feeding value by Near Infrared Reflectance Spectroscopy. Neth J Agric Sci 38: 449-460.
3. Mould FL (2003) Predicting feed quality-chemical analysis and *in vitro* evaluation. Field Crops Res 84: 31-44.
4. Undersander D (2006) Uses and abuses of NIR for feed analysis. Florida Ruminant Nutrition Symposium, Best Western Gateway Grand, Gainesville FL.
5. Norris KH, Hruschka WR, Bean MM, Slaughter DC (1989) A definition of wheat hardness using near infrared reflectance spectroscopy. Cereal Foods World 34: 696-705.
6. Williams PC, Sobering DC (1986) Attempts at standardization of hardness testing of wheat. II. The near-infrared reflectance method. Cereal Foods world 31: 417-420.
7. Givens DI, Deaville ER (1999) The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. Aust J Agric Res 50: 1131-1145.
8. Smith KF, Flinn PC (1991) Monitoring the performance of a broad-based calibration for measuring the nutritive value of two independent populations of pasture using Near Infrared Reflectance (NIR) Spectroscopy. Aust J Exp Agric 31: 205-210.
9. Garrido-Varo A, Pérez-Marín MD, Gómez-Cabrera A, Guerrero JE, De Paz F, et al. (2002) Near infrared reflectance spectroscopy as an essential tool in food safety programmes: predicting ingredients in commercial compound feed. Near Infrared Spectroscopy: Proceedings of the 10<sup>th</sup> International Conference.

10. Tillmann P, Horst H, Danier J, Dieterle P, Philipps P (2002) Analysis of mixed feeds and their components using near infrared spectroscopy. *Near Infrared Spectroscopy: Proceedings of the 10<sup>th</sup> International Conference*.
11. Xiccato G, Trocino A, De Boever JL, Maertens L, Carabaño R, et al. (2003) Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Anim Feed Sci Technol* 104: 153-168.
12. Swart E, Brand TS, Engelbrecht J (2012) The use of near infrared spectroscopy (NIRS) to predict the chemical composition of feed samples used in ostrich total mixed rations. *S Afr J Anim Sci* 42: 550-554.
13. Abram SM (1989) Future applications for NIRS: mixed feeds. *Near Infrared Reflectance Spectroscopy (NIRS) Analysis of Forage Quality*. USDA-ARS Agriculture Handbook 643: 55-56.
14. American Society of Agricultural Engineers (1983) Method of determining and expressing fineness of feed materials by sieving. ASAE Standard S319, Agriculture Engineers Yearbook of Standards.
15. Kalivoda JR, Jones CK, Stark CR (2017) Impact of varying analytical methodologies on grain particle size determination. *J Anim Sci* 95: 113-119.
16. Association of Official Analytical Chemists (AOAC) (2006) Protein (crude) in animal feed. Combustion method (990.03). Official methods of analysis.
17. Tamburini E, Vincenzi F, Costa S, Mantovi P, Pedrini P, et al. (2017) Effects of Moisture and Particle Size on Quantitative Determination of Total Organic Carbon (TOC) in Soils Using Near-Infrared Spectroscopy. *Sensors* 17: 2366.
18. Wetzel DL (1983) Near-Infrared Reflectance analysis. *Anal Chem* 55: 1165A-1176A.
19. Spragg R, Seer Green (2013) Reflection Measurements in IR Spectroscopy. Perkin Elmer.
20. Stein HH (2006) DDGS: Energy and Nutrient Content and Digestibility. *Midwest Swine Nutrition Conference* 58-65.
21. Spiels MJ, Whitney MH, Shurson GC (2002) Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J Anim Sci* 80: 2639-2645.
22. Cromwell GL, Herkelman KL, Stahly TS (1993) Physical, chemical and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *J Anim Sci* 71: 679-686.
23. Pérez-Marín DC, Garrido-Varo A, Guerrero-Ginel JE, Gómez-Cabrera A (2004) Near-infrared reflectance spectroscopy (NIRS) for the mandatory labelling of compound feedingstuffs: chemical composition and open-declaration. *Anim Feed Sci Technol* 116: 333-349.
24. González-Martín I, Álvarez-García N, Hernández-Andaluz JL (2006) Instantaneous determination of crude proteins, fat and fiber in animal feeds using Near Infrared Reflectance Spectroscopy Technology and a remote reflectance fiber-optic probe. *Anim Feed Sci Technol* 128: 165-171.