Bergsma, R., Lei, H., Rodas-Gonzales, A. (2026) Does drying matter? Evaluating NIR accuracy for protein digestibility in pigs, *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 24045: https://doi.org/10.31274/wcgalp.24045
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Does drying matter? Evaluating NIR accuracy for protein digestibility in pigs

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Introduction

Protein efficiency in pig production is gaining importance due to increasing feed costs and stricter regulations on nitrogen (N) emissions. At the same time, there is growing recognition among commercial companies of their responsibility to improve protein utilization through innovation. Protein-rich feed ingredients are a major cost factor, while N losses from pig production contribute to environmental pollution. Improving the utilization of dietary protein is therefore essential to enhance both economic and environmental sustainability.

Genetic selection has markedly increased growth rate and lean tissue deposition in modern pig genotypes, resulting in higher requirements for standardized ileal digestible (SID) lysine (Knap, 2025). While nutritional strategies can partially address these demands, genetic improvement of protein utilization efficiency represents an additional opportunity. Apparent total tract digestibility of crude protein (ATTD_CP) is a relevant trait in this context, as it directly affects fecal N excretion and overall protein efficiency (Le Goff & Noblet, 2001). However, selection for ATTD_CP is limited by the lack of cost-effective, large-scale phenotyping methods suitable for breeding programs.

Near-infrared reflectance spectroscopy (NIR) offers a rapid and low-cost alternative to conventional chemical analyses and has been successfully applied to predict nutrient composition and digestibility-related traits in pig feed and feces (Bastianelli *et al*., 2010). To our knowledge, all existing studies rely on dried fecal samples to reduce variability associated with moisture content, but drying is labor-intensive and limits high-throughput implementation.

For practical applications in pig breeding programs, the use of fresh fecal samples would substantially reduce costs and processing time. Therefore, the objective of this study was to quantify differences between NIR predictions obtained from fresh and dried fecal samples and to evaluate the suitability of fresh feces for estimating ATTD_CP in the context of genetic selection.

Materials & Methods

All procedures were approved by the University of Manitoba Animal Care Committee (F20-026) in accordance with CCAC guidelines (2009).

Sample collection. At 11 weeks, purebred male Large White pigs from TN sire and dam line nucleus farms were transferred to the TN Canada testing facility and housed in pens of 12 pigs (eight pens/week) for 80 days. Feed intake and body weight were recorded individually via automatic feeding stations. Pigs were fed a corn–wheat–canola diet with ad libitum access to feed and water.

On the day of computed tomography (CT) scanning, fecal samples were collected from pigs in individual pens using sterile bags. NIR spectra were obtained in duplicate. Samples were shipped on ice to the University of Manitoba and stored at −20 °C. In total, 306 fecal samples

were collected from animals preselected for low, intermediate, and high efficiency based on genomic estimated breeding values (GEBV) for feed-to-gain ratio.
Feces and feed were analyzed for dry matter (AOAC 934.01), acid-insoluble ash (AIA; McCarthy *et al*., 1974), and crude protein (AOAC 990.03). Apparent total tract digestibility (ATTD, %) was calculated as:

$$\text{ATTD (\%)} = 100 - [100 \times (\text{CP_feces} / \text{CP_feed}) \times (\text{AIA_feed} / \text{AIA_feces})]. \quad (1)$$

Calibration curves. NIR spectra were obtained using a FoodScan™ 2 instrument (FOSS, Denmark) across 400–1100 nm at 0.5 nm intervals. Spectral data were standardized (mean = 0, SD = 1), and outliers removed using PCA (three components, >99% variance explained). Water-related wavelengths were excluded from fresh samples. Optimal wavelength ranges were selected separately for fresh and dried samples based on Pearson correlations with chemical data. PLS regression models (1–50 components) were evaluated using a leave-one-animal-out approach; final models were applied to all 306 samples to obtain predicted values. Phenotypes for genetic analyses were defined as the mean of duplicate measurements.

Estimation of variance components. Genetic parameters were estimated via REML using ASReml (Gilmour *et al*., 2002) with a standard animal model:

$$y = Xb + Za + e, \quad (2)$$

where y is the vector of observations; X and Z are known incidence matrices; b is the vector of fixed effects; and a is the vector of random additive genetic effects (breeding values), which were assumed to follow a normal distribution, $a \sim N(0, A\sigma_A^2)$. Three generations were included in the pedigree, comprising a total of 1,534 animals.

Results

The chemical properties of feces are presented in Table 1. Typical ATTD of crude protein in practical diets is roughly 75-90% meaning 81.4% is within the expected range.

Table 1. Descriptive statistics of the chemical analyses of feces (n=310).

	Mean	Std
DM fresh samples (%)	26.9	2.3
DM after oven drying (%)	96.7	1.5
AIA in feces (%)	1.67	0.29
CP in feces (%)	16.7	2.2
ATTD CP (%)[1]	81.4	5.5

[1] The CP in the diet was established at 16.0%; and the AIA at 0.28%

The largest peak in the fresh samples at 965.5 nm as shown in Figure 1a, corresponds to a water absorption band. In the dried samples, the largest peak was observed at 435.5 nm, which lies within the visible light range (380–800 nm). The specific organic compounds responsible for absorption at this wavelength are unclear. Although the magnitude of the correlations differed between fresh and dried samples, the overall pattern of peaks was rather similar.

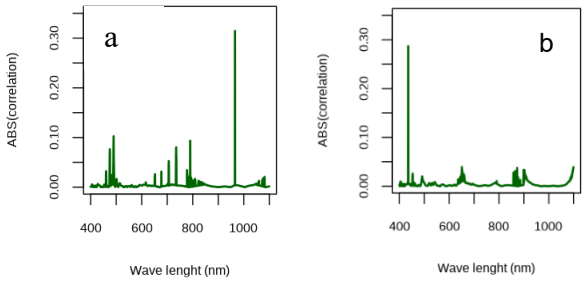

Figure 1. Wavelength association with CP content in fresh (a) and dried fecal samples (b).

Table 2 shows that NIR on dried samples generally predicts phenotypes more accurately, with higher correlations, slopes closer to one, and lower prediction errors.
How these differences translate into the accuracy of estimated breeding values is presented in Table 3. The heritability of ATTD_CP, based on chemically analyzed samples, is higher than the heritability estimates derived from NIR predictions for both dried and fresh samples. Although the predictive performance of the NIR calibration for crude protein is better for dried samples than for fresh samples, the heritability estimates show the opposite trend. However, it is important to note that none of the heritability estimates differ significantly from each other.

Table 2. Predictive value of NIR-calibration curves for CP in fresh and dried fecal samples, using the Leave One Animal Out-technique.

Sample type	R	Slope	Residual SE
Dried	0.86	0.78	0.94
Fresh	0.76	0.66	1.17

Table 3. Genetic parameters ATTD_CP measured in fresh and dried fecal samples.

Sample type	σ_A^2	σ_E^2	h^2
Chemical	4.6	5.6	0.45 ± 0.22
Dried	2.6	8.0	0.25 ± 0.19
Fresh	3.2	6.6	0.33 ± 0.20

The model used to estimate variance components initially included LINE (sire/dam line), BIRTH-FARM (five classes), and SAMPLING-WEEK (36 classes). LINE and BIRTH-FARM were removed from the model because their effects were not statistically significant.

Discussion

When calculating ATTD_CP for both fresh and dried samples, chemically determined AIA values were used for feed and feces. Consequently, the predictive performance may be overestimated, as the use of NIR-predicted AIA would introduce additional bias. Because AIA is inorganic and cannot be measured directly by NIR spectroscopy, chemical analyses were retained and the evaluation was restricted to crude protein. The use of lignin as an alternative organic marker is currently under investigation but was beyond the scope of this study.
Prediction accuracy may be further improved, as major absorption bands associated with crude protein occur at higher wavelengths (1500–1700 nm; Bruker, 2025) that were not covered by the instrument used. Calibration performance for fresh samples was lower than for dried samples. Although wavelength regions associated with water absorption were excluded, residual spectral interference likely remained. A constant feed composition was assumed when estimating ATTD_CP. Potential temporal variation in crude protein or AIA was partially accounted for by including SAMPLING-WEEK in the variance component analyses.

The dataset used for calibration development was relatively large, while the dataset for variance component estimation was more limited, with no significant differences detected. Our results align with a previous study on dried samples, where a calibration curve applied to fecal samples from 1,890 pigs yielded a heritability of 0.20 (Hov Martinsen *et al*., 2023). NIR-based estimation of ATTD_CP reduced the additive genetic standard deviation by 17% and 25% for fresh and dried samples, respectively, compared to chemical analysis, and despite introducing some bias, substantial additive genetic variation remained detectable.
Using fresh samples in a genomic breeding value context could potentially reduce the disadvantage of lower prediction accuracy, as the phenotype of an individual animal has less influence on its EBV compared to pedigree-based breeding value estimation.

Conclusions

Using fresh fecal samples for NIR-based determination of CP content is a practical and feasible approach for genetic selection on ATTD_CP.

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