

## EVALUATING CHEMICAL COMPOSITION OF PIG FAECES BASED ON DIFFERENT DIETS USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

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### ABSTRACT

*The aim of this paper is to demonstrate how NIRS (near-infrared spectroscopy) can be applied in swine faecal evaluation. Preliminary starter calibrations for ash, dry matter (DM), crude protein (CP), crude fibre (CF), crude fat, ether extract (EE), lipids, acid and neutral detergent fibre(ADF and NDF), starch. The preliminary results revealed that NIRS can be used on-farm as a rapid non-invasive tool to estimate proximate composition of faeces based on the diet fed to pigs. However, reliable estimates and prediction values and effectively assessing the model's behaviour in predicting the chemical composition of pigs' faeces based on diets require a repeated experiment with large dataset for validation.*

### Introduction

Feed evaluation is important for livestock rearing and management practices. The major challenges with conventional feed evaluation protocols are issues related to cost, time of analysis, and the use of environmentally unfriendly chemicals (Zhou et al., 2012). These challenges make the use of near-infrared spectroscopy (NIRS) as an important rapid (non-invasive) tool for dietary evaluation. NIRS has enormous potential in many fields including health, agriculture, animal feeding and management (Despal et al., 2020; Melfsen et al., 2012; Núñez-Sánchez et al., 2016). In the application of NIRS in dietary or faecal studies, it is noted that, faecal samples contain undigested dietary ingredients and as such, their composition differs between animals, and rearing strategies, and contributes to variation in most NIR spectroscopy-based predictions (Bastianelli et al., 2015; Schiborra et al., 2015). Error in prediction accuracy of NIRS measurements using undried samples is attributed to moisture content (Griggs et al., 1999). To our knowledge, literature on NIRS application in evaluating the chemical composition of pig faecal samples with respect to feeding strategies is limited. However, few available studies have shown good prediction R<sup>2</sup> values and lower standard errors of analysis in using NIRS for predicting faecal and dietary chemical composition and digestibility of nutrients (Noel et al., 2022; Schiborra et al., 2015).

This paper presents some of the preliminary results from our ongoing study on evaluating the chemical composition of fresh and dry faecal matter of pigs fed diets with different dietary protein levels using visible near-infrared spectroscopy (NIRS).

## Materials and methods

The experimental procedure was approved by the Padua University animal care committee (OPBA). The procedures were conducted under European Union requirements and guidelines on the protection of animals used for scientific and educational purposes provided by the “Organismo preposto per il Benessere Animale, OPBA”, University of Padova (OPBA, approval document #36/2018).

The selected number of pigs,  $n = 84$  (gilts and barrows) arrived at the experimental station at  $149 \pm 3$  d old,  $95 \pm 12.5$  kg BW. There were 3 treatments (LP, MP, HP) x 2 pens x 2 sexes x 7 pigs (28 pigs/treatment). The trial duration = 134 days. Room (stable) temperature was maintained at  $19 \pm 2^\circ\text{C}$ .

## Feeds

Feeds were manufactured by the Progeo Feed Industry (Masone, Reggio Emilia, Italy). The average composition of nutrients in the ingredient used in early diets of 90 to 120 kg BW, and late finishing diets of  $> 120$  kg BW pigs are given in Table 1.

Table 1. Nutrient content (% DM) unless otherwise indicated) of early diets (90 to 120 kg average BW) and late finisher diets (over 120 kg average BW).

Analyzed nutrient composition <sup>b</sup>	Late finishing feeds		
	LP	MP	HP
DM, % in fed	90.4	90.2	90.6
CP (N × 6.25) (%)	11.5	13.2	15.2
Starch (%)	54.2	52.1	53.3
Ether extract (%)	5.31	5.54	5.30
aNDF-NDF (%)	14.8	14.6	13.0
Ash (%)	4.54	4.55	4.64
Calculated energy content			
ME, MJ/kg DM	14.5	14.6	14.8
NE, MJ/kg DM	11.0	11.1	11.1
Digestible CP (DCP) (%)	9.70	11.0	13.3
ME/Digestible CP, MJ/kg DCP	0.15	0.13	0.11

<sup>a</sup> LP: low protein diet MP: medium protein diet, and HP: high protein diet.

<sup>b</sup> Analytical results by averaging data on 4 independent replications.

<sup>c</sup> Computed according to NRC (2012) from the ingredient composition of the feeds (2 batches); SID: standardized ileal digestible amino acid content; Optimum ratios according to NRC (2012).

### ***Chemical analysis of faecal samples***

Dried grounded faecal samples were analysed for DM, Ash, CP, EE, CF, Starch, NDF, ADF, ADL-with-AIA, AIA, ADL-without-AIA, Acid-insoluble-ash using 3 independent replications for dry matter (DM: # 934.01; AOAC, 2003), N (# 976.05; AOAC, 2003), ether extract (EE: # 920.29; AOAC, 2003), ash (# 942.05, AOAC, 2003) and neutral detergent fibre with amylase treatment and expressed inclusive of residual ash (aNDF-NDF) contents (Van Soest et al., 1991).

### ***Acquisition of faecal spectra***

All faecal samples were scanned at room temperature (25 °C) using a LabSpec 2500 spectrophotometer (ASD Inc./ Malvern Panalytical (Malvern/UK) equipped with a visible and infrared wavelength of 350 nm to 1830 nm at 1 nm intervals. The spectrometer was calibrated against a standard white barium sulphate plate before spectra acquisition (Patel et al., 2021). Three successive spectra were recorded for each sample in different positions at each scanning. Thus, one sample was represented by 6 spectra in total, from 3 fresh and 3 dried ground faecal samples. The total number of scanned samples was  $n = 37$ , and the total number of stored spectra was  $n = 111$  both for fresh and dried. The spectra were collected in three replicates taken in reflection mode from three different positions on the surface of each fresh and dry faecal sample. The detector was in direct full contact with surface of the petri dishes for the fresh faecal spectra acquisitions. For dried-grounded faecal samples, the detector was immersed into the samples and the surface of the detector was cleaned before next sample is scanned.

### ***Spectral analysis***

The Unscrambler 9.7 (CAMO Software AS, Oslo, Norway) was applied for data processing and analysis. Low energy light at longer wavelengths could not pass through the samples, meaning absorbance spectra above 1850 nm showed stray light. Wavelength region at 350–1000 nm presented undefined noise during basic spectral evaluation. Therefore, the 1100–1830 nm range was selected for evaluation of NIR data. Spectra data were mathematically treated with different transformations (Norris, 2001). PCA was used to explain the multidimensional characteristics of the NIR data (Bázár et al., 2016). PLSR was applied as Y-variables of the predictable constituents and optimizes the latent variables of the model to describe as much amount of the variance of X-(spectral data) and Y-variables (reference data) while the precision ( $R^2$ ) and accuracy of the chemometric models were evaluated by the determination coefficient  $R^2_{cv}$  and the root mean square error (RMSE) of calibration and cross-validation ( $RMSE_{cv}$ ) as described in (Bázár et al., 2016).

### **Results and discussion**

Figure 1 below shows the PCA score plot of the NIR data of the faecal samples with grouping based on the dietary source. In both (fresh and dry) PCAs, there exist group dependent variations based on protein levels low (LP), medium (MP) and high (HP).

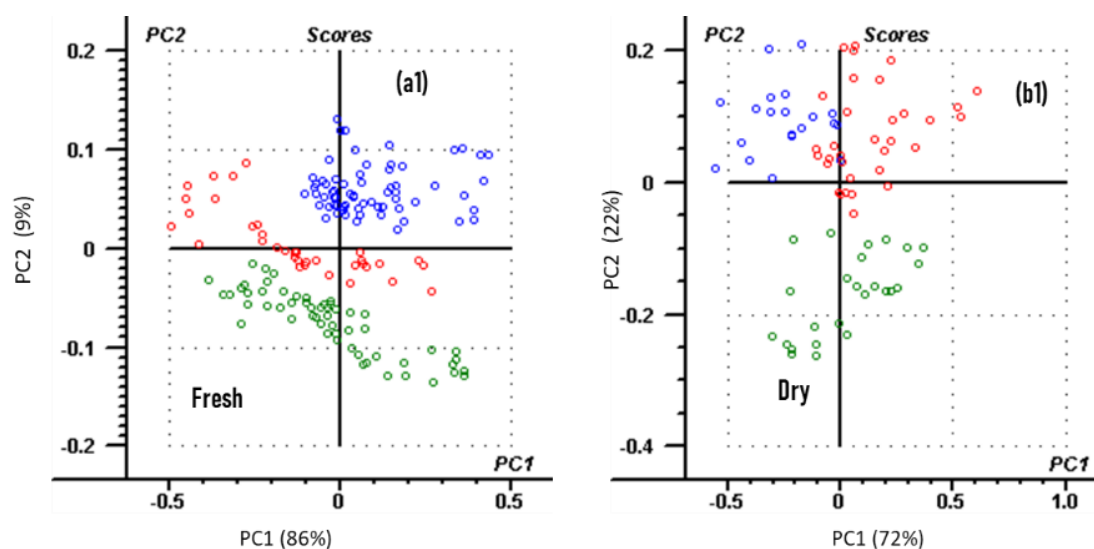


Figure 1. PCA score plots of MSC treatment of fresh (a1) and Dry (b1) faeces acquired within 1100 - 1800 nm coloured by diet groups (Blue: LP, Red: MP, Green: HP160).

Table 2. Calibration and cross-validation statistics of the PLS models for faecal constituents

Math treatment	LV	Constituents	R <sup>2</sup> C	RMSEC	R <sup>2</sup> CV	RMECV
RAW	11	DM	0.70	0.261	0.54	0.326
RAW	10	CP	0.84	0.892	0.75	1.124
RAW	9	CF	0.74	0.704	0.66	0.812
RAW	10	EE	0.72	0.304	0.58	0.3778
RAW	11	Starch	0.77	0.092	0.62	0.118
RAW	7	NDF	0.83	1.622	0.80	1.770
RAW	10	ADF	0.80	0.773	0.70	0.942
RAW	10	Ash	0.68	0.619	0.56	0.771
RAW	9	AIA	0.79	0.106	0.70	0.129
RAW	8	ADL-with-AIA	0.69	0.564	0.62	0.637
RAW	9	ADL-without-AIA	0.78	0.382	0.73	0.435
SNV	9	DM	0.65	0.27	0.54	0.312
SNV	10	CP	0.84	0.892	0.76	1.090
SNV	10	CF	0.77	0.668	0.67	0.799
SNV	11	EE	0.77	0.273	0.63	0.353
SNV	10	Starch	0.67	0.108	0.52	0.131
SNV	7	NDF	0.83	1.622	0.81	1.758
SNV	11	ADF	0.84	0.672	0.75	0.846
SNV	10	Ash	0.68	0.605	0.56	0.719
SNV	10	AIA	0.72	0.125	0.56	0.158
SNV	8	ADL-with-AIA	0.71	0.546	0.61	0.639
SNV	8	ADL-without-AIA	0.67	0.471	0.56	0.553
MSC	10	DM	0.69	0.271	0.54	0.332
MSC	8	CP	0.73	1.155	0.65	1.320
MSC	4	CF	0.81	0.599	0.64	0.852
MSC	11	EE	0.79	0.265	0.65	0.346
MSC	11	Starch	0.83	0.075	0.71	0.098
MSC	8	NDF	0.85	1.544	0.81	1.761
MSC	10	ADF	0.77	0.844	0.63	1.074
MSC	10	Ash	0.70	0.571	0.56	0.704
MSC	10	AIA	0.75	0.113	0.62	0.142
MSC	10	ADL-with-AIA	0.80	0.442	0.68	0.561
MSC	10	ADL-without-AIA	0.80	0.360	0.70	0.444
2D5G5S	6	DM	0.83	0.208	0.54	0.345
2D5G5S	4	CP	0.68	1.243	0.56	1.475
2D5G5S	4	CF	0.65	0.815	0.52	0.966
2D5G5S	5	EE	0.60	0.386	0.30	0.511
2D5G5S	6	Starch	0.77	0.086	0.54	0.014

2D5G5S	5	NDF	0.82	1.655	0.71	2.151
2D5G5S	4	ADF	0.66	0.999	0.52	1.204
2D5G5S	3	Ash	0.56	0.666	0.51	0.701
2D5G5S	6	AIA	0.85	0.089	0.64	0.239
2D5G5S	6	ADL-with-AIA	0.81	0.393	0.65	0.590
2D5G5S	6	ADL-without-AIA	0.84	0.393	0.63	0.607

CP: crude protein, EE: ether extracts (crude fat), CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, SNV: standard normal variate, MSC: multiplicative scatter correction, 2D5G5S5 second derivative with 5-point gap and 5-point segment, RMSEC: root mean square error of calibration, RMSE<sub>CV</sub>: root mean square error of cross-validation, R<sup>2</sup><sub>C</sub>: determination coefficient of calibration, R<sup>2</sup><sub>CV</sub>: determination coefficient cross-validation.

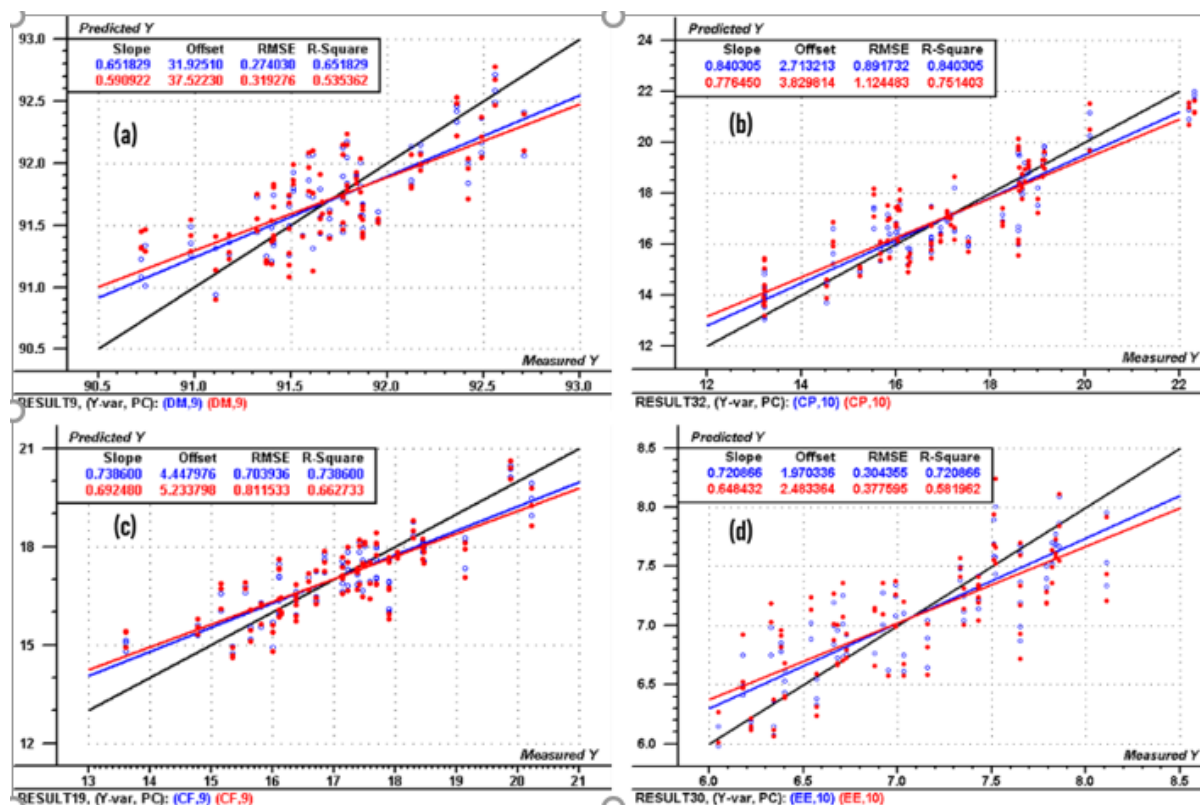


Figure 2. The optimum Y-fit (black diagonal) and the Y-fits of the best calibrations (blue) and cross-validations (red) for (a) dry-matter (DM), (b) crude protein (CP), (c) crude fiber (CF), (d) ether extract (EE).

## Discussion

The qualitative grouping of LP, MP and HP faecal samples show the potential of NIR to differentiate faecal samples based on dietary source (Baker et al., 1994; Harris et al., 2018; Le Cocq et al., 2022).

The calibration and cross-validation statistics in Table 2 show promising outcome in the starter calibration models generated. In both the calibration and cross-validation results, the R<sup>2</sup><sub>C</sub> and R<sup>2</sup><sub>CV</sub> achieved 0.5-0.8 values, with most of the models showing lower errors. This is shown in Figure 2, where the calibration and cross-validations lines do not deviate significantly from the regression line (optimal Y-fit) (Yakubu et al., 2021). Results are in agreement to other studies by Evangelista et al., 2021; Melfsen et al., 2012; Park et al., 1999; Van Milgen et al., 2015.

## Conclusions

From the study, preliminary results show the possibility of predicting faecal constituent based on dietary source (low, medium and high protein sources) using NIR spectroscopy. This research was funded by the European Union Rural Development program 2014-2020, Reg. (CE) 1305/2013 - PSR Veneto DGR n. 2175 – December 23, 2016, interventions 16.1.1 and 16.2.2, code 3682902. The University of Padua also funded with Institutional funds (DOR2059255/20, DOR1990028/19, DOR1845849/18) and with a three-year PhD grant for Isaac Malgwi.

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