



Rapid and Non-Destructive Prediction of Animal Feed Nutritive Parameters Using Near Infrared Spectroscopy and Multivariate Analysis

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ABSTRACT

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This study elucidates the application of near-infrared spectroscopy (NIRS) and multivariate analysis for the rapid, non-destructive determination of animal feed nutritive parameters, specifically pH, NH₃, and volatile fatty acid (VFA) values. NIRS spectra within the 1000-2500 nm range, coupled with reference methods, were employed for parameter determination. To enhance prediction accuracy, spectral pre-processing was undertaken using multiplicative scatter correction (MSC), Savitzky-Golay (SG) smoothing, and first-derivative methods. Simultaneous determination of nutritive parameters was achieved through the establishment of prediction models employing three multivariate analyses: principal component regression (PCR), partial least squares regression (PLSR), and support vector machine regression (SVMR). The results indicated superior pH ($r=0.993$) and NH₃ ($r=0.987$) predictions using SVMR, while PLSR yielded the lowest root mean square error (RMSE) for VFA ($r=0.998$). These findings suggest that with appropriate spectral corrections and regression methodologies, NIRS can be effectively utilized for the rapid, simultaneous determination of animal feed nutritive parameters.

1. INTRODUCTION

Agro-industrial byproducts such as cocoa pod husk, a residue prominent in Indonesia, present potential feedstock for animal consumption. However, the high crude fiber content (28.30%) and low protein concentration (1.36%) limit its nutritional quality [1, 2]. Further investigation reveals the presence of starch (58%), cellulose (23%), hemicellulose (9.2%), and lignin (3.0%) in the starch-extracted residue [3, 4]. The crude fiber and lignin impede digestion in ruminants, resulting in limited nutrient absorption, and inadequate nitrogen content fails to meet livestock protein requirements. To improve these nutritive values, fermentation practices are proposed.

Microbial fermentation provides a promising solution for enhancing the nutritive quality of agricultural residues, transforming low-value ingredients into value-added animal feed. Previous research demonstrated the enhancement of bagasse digestibility and nutrient content through fermentation with white rot fungi [5, 6].

Traditionally, animal feed quality—measured in terms of protein, carbohydrate, fat, and mineral content—is determined through laborious laboratory procedures involving complex chemical processes [7, 8]. To circumvent these challenges, alternative non-destructive, rapid assessment methods have been explored, with near-infrared reflectance spectroscopy (NIRS) emerging as a preferred technology for determining feed product quality [9]. This method can analyze feed quality in a very short period of time, is non-destructive or does not harm the product, and is performed without touching the

product.

The NIRS technique is based on the premise that every biological object has a unique spectrum of optical and electromagnetic characteristics [10]. This object's NIR spectrum can then be analyzed using special mathematical techniques, such as multivariate analysis, in order to determine its chemical composition [11]. This phenomenon has prompted a number of scientists to investigate the possibility of using the NIRS method to predict the quality of organic materials such as fruits [12, 13], meats [14, 15], animal feed [9], and herbal oils that will be used in the production of pharmaceuticals [16-18]. The non-destructive nature of the NIRS technology method, the relative ease of sample preparation, the absence of chemicals in the testing process, and the ability to predict several quality or nutritional components simultaneously have led to extensive research and application in many fields, including agriculture and animal husbandry.

The information contained within the NIR spectrum of each biological substance will vary based on the substance's chemical composition. By utilizing this spectrum data in conjunction with its multivariate analysis method (model calibration), it will be possible to determine the product quality of biological materials rapidly, efficiently, and simultaneously without causing any damage to the material [12, 19, 20]. The objective of this study is to use NIRS-based technology to rapidly and simultaneously determine and predict the nutritive quality levels of animal feed namely pH, ammonia (NH₃) and volatile fatty acid (VFA). In addition, the purpose of this research is to compare three different multivariate analyses

namely principal component regression (PCR), partial least square regression (PLSR) and support vector machine regression (SVMR). Moreover, the impact of these three approaches to the prediction performance is also investigated. The PCR and PLSR are linear regression approaches while the SVMR is a non-linear one. In NIRS practices, both PCR and PLSR were commonly used and till being employed to develop prediction models. In this study, we investigated the use of SVMR as a novelty beside PCR and PLSR and the prediction results were compared. The hypothesis of this study is that SVMR provided a better prediction performance than PCR and PLSR.

2. MATERIALS AND METHODS

2.1 Samples

A total of 25 samples of fermented cocoa pod husk feed ingredients were used in this study. The cocoa pods used in this study came from farmers in various locations in Aceh Province. Prior to fermentation, the cocoa pods were sun-dried and milled through a 1 mm sieve. For fermentation, 3% white-rot fungi (*Phanerochaete chrysosporium*) were added to 60% dry matter (DM) of cocoa pod husk by adding water. All samples were stored at room temperature for aerobic fermentation at varied fermentation durations 0, 7, 14, 21 and 28 days of fermentation. Each treatment consisted of 5 repetitions. After harvesting, all samples were dried in an oven at 60°C for two days. For each measurement, 33 grams of dry sample was used for spectral data acquisition and actual laboratory measurements.

2.2 Data collection and pre-processing

Initially, NIR spectral data were collected for all feed samples derived from eight sources of agro-industrial residues by-products from cacao pod husk. Approximately 33 g of a bulk sample of feed was placed centrally on the sample holder [9]. Each bulk sample was placed by hand directly into the incoming hole (1 cm in diameter) of the light source to ensure direct contact and reduce light scattering noise. With 64 co-added scans, an absorbance spectrum in the wavelength range of 1000 to 2500 nm was acquired. To ensure uniformity, the sample was set to rotate during spectral acquisition [21].

The next step, following the acquisition of the near infrared spectrum, is to measure the quality parameters of the animal feed, including the pH, ammonia (NH_3) and volatile fatty acid (VFA) content. The pH value was immediately measured with a pH meter. The micro-diffusion technique was used to determine the amounts of NH_3 [22]. VFA concentrations were examined using gas chromatography [23].

Before developing a prediction model, spectra data were preprocessed and enhanced in order to achieve more accurate and robust prediction results. Several correction methods were available and could be implemented based on sample characteristics, spectral impact, and other pertinent information that must be identified prior to spectral corrections. In NIRS, spectral data preprocessing methods can be combined to improve prediction accuracy [24]. Based on our previous study, we found that a combination multiplicative scatter correction-Savitzky Golay smoothing and first derivative methods (MSC+SG+D1) generated best prediction performance. Hence, these spectral enhancements were employed to the spectral data.

2.3 Nutritive prediction models

The important core of NIRS practices and applications is the construction and development of models used to predict the nutritive or qualitative characteristics of studied samples. By regressing NIR spectra data (X variables) and actual measured nutritive attributes, these quality attributes can be predicted rapidly and simultaneously through a process known as calibration (Y variables). Idealistically, the sample used in the regression stage should be representative of both the current and future prediction samples. It means that in both the calibration and validation sample datasets, all expected sources of variability must be considered [19].

In the majority of NIRS applications, principal component regression (PCR) and partial least square regression (PLSR) are two of the most frequently employed regression methods for developing prediction models. Both of these methods remain as the main choice for regression in NIRS applications. The initial PCR and PLSR models assumed a linear relationship between the modelled nutritive parameters or concentrations and the infrared spectral variations. Increasing the number of latent variables (LVs) included in the PCR and PLSR model can resolve weak nonlinearities. However, fewer LVs is preferable for model efficiency. Beside the two multivariate approaches, we also applied the support vector machine regression (SVMR) method to develop the prediction models. The prediction performance of those three methods were compared.

The common linear regression methods for building these models are partial least squares regression (PLSR) and principal component regression (PCR). Meanwhile, support vector machine is very specific class of algorithm, characterized by usage of kernels. In earlier development, this method was applied for classification problems, but nowadays it also has been used to the case of regression. In kernel-based methods, the calibration is carried out in a space of non-linearly transformed input data (so called feature space) without actually carrying out the transformation. The feature space is defined by the kernel function, a measure of similarity between spectra. The most popular kernel functions are the Gaussian and polynomial functions.

The prediction performances were evaluated using the following statistical parameters: the coefficient of correlation (r) and determination (R^2) between predicted and measured nutritive parameters or quality attributes, the prediction error, which is defined as the root mean square error (RMSE), and the residual predictive deviation (RPD), which is defined as the ratio between the standard deviation (SD) of the population's actual value of animal feed nutritive parameters and the prediction RMSE. Literature suggests that a good NIRS model should have coefficients of r and R^2 above 0.75 and RPD index above 2.5. The greater the RPD value, the greater the likelihood that models will accurately and robustly predict the nutritive parameters or chemical concentrations of the sample [1, 10, 25-27].

3. RESULTS AND DISCUSSION

The spectral data corrections used affected the accuracy of the prediction model. In our previous study, a combination of multiplicative scatter correction-Savitzky Golay smoothing and the first derivative (MSC+SG+D1) methods generated the best prediction performance for predicting quality attributes of

cocoa pod husk feedstuffs, namely dry matter (DM), crude protein (CP), crude fiber (CF) and extract ether (EE), using the PLSR approach. The NIR spectrum of cocoa pod husks corrected by a combination of MSC+SG+D1 methods is shown in Figure 1.

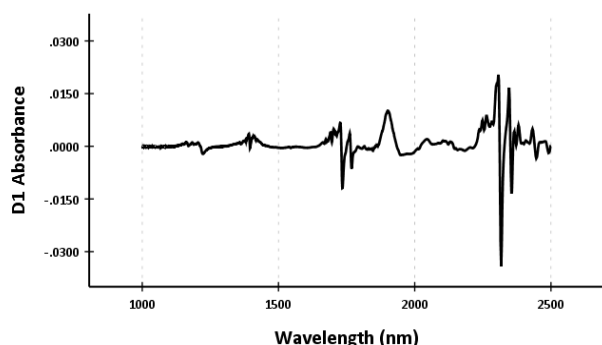


Figure 1. Typical NIR spectra of cocoa pod husks in form of derivative absorbance (D1)

As can be seen in Figure 1, NIR spectra could record multi- and co-frequency information about the chemical bond responses (C–H, C–O, N–H and O–H), which indicate the presence of organic materials such as moisture, fat, protein and carbohydrates. The primary products of microbial fermentation are volatile fatty acid (VFA) and ammonia (NH₃), and their production represents diet degradation in the rumen. Soluble carbohydrates and protein content in the feed are the main factors influencing VFA and NH₃ concentrations in the rumen [28, 29]. Therefore, in this study, we employed the MSC+SG+D1 spectra correction technique to build VFA, NH₃ and pH calibration models for predicting the level of feed degradation. Further, different multivariate analyses were also employed to compare the precision of calibration models.

Initially, the linear regression approach was performed on the entire range to examine the NIR model. To correlate NIR spectral data with actual data, a principal component regression (PCR) approach was contracted and justified using full cross-validation. The calibrated results for nutritive parameters using the PCR approach are shown in Table 1.

Table 1. Calibration performances of various regression approaches for pH, NH₃ and VFA parameters

| Nutritive Parameters | Method | LVs | r | R ² | RMSE | RPD |
|----------------------|--------|-----|-------|----------------|-------|-------|
| pH | PCR | 5 | 0.928 | 0.812 | 0.05 | 1.75 |
| | PLSR | 9 | 0.939 | 0.969 | 0.02 | 4.14 |
| | SVMR | 21 | 0.993 | 0.997 | 0.01 | 6.46 |
| NH ₃ (mM) | PCR | 5 | 0.785 | 0.728 | 0.70 | 1.49 |
| | PLSR | 9 | 0.842 | 0.918 | 0.41 | 2.57 |
| | SVMR | 19 | 0.987 | 0.994 | 0.18 | 5.68 |
| VFA (mM) | PCR | 5 | 0.767 | 0.725 | 14.48 | 1.48 |
| | PLSR | 9 | 0.998 | 0.999 | 0.98 | 21.97 |
| | SVMR | 24 | 0.991 | 0.996 | 3.45 | 6.21 |

Note: VFA: volatile fatty acid, PCR: principal component regression, PLSR: partial least squares regression, SVMR: support vector machine regression, LVs: latent variables, r: coefficient of correlation, R²: coefficient of determination, RMSE: root mean square error, RPD: ratio of prediction to deviation.

According to the table, the parameters of NH₃ and VFA can only be determined sufficiently and coarsely with coefficient correlations (r) of 0.785 and 0.767, respectively. However, the PCR model yields a high value of correlation (0.928) for the

pH parameter, indicating excellent predictive accuracy. The partial least square regression (PLSR) was the subsequent regression approach used to establish the NIR model. PLSR was applied using the Kernel algorithm, and full cross-validation was used for validation. The PLSR model for pH, NH₃ and VFA determination shown in Table 1 had r and root mean square error (RMSE) values between 0.842-0.998 and 0.02-0.09, respectively. From the table, it can be seen that the PLSR model produced a significant improvement in terms of calibration performance (larger r and smaller RMSE) compared to the PCR model. This is based on the fact that PLSR attempts to find the best correlation between NIR spectra and reference data during the transformation to latent variables (LV) in the regression process. PCR approach, on the other hand, only converts the NIR spectra data into LV without involving the reference data [30]. Based on residual predictive deviation (RPD) values (Table 1), the PLSR model is categorized as an excellent prediction model (except for the NH₃ parameter, which is almost 3). Nonetheless, the model with an RPD value between 2.5 and 3 has good predictability [31, 32]. The latent variables required to establish this prediction model are composed of nine factors, and they accounted for 99% of the variations in the data set. It is generally known that PCR or PLSR prediction requires some variable adjustment to reduce dimensionality, remove the impacts of outliers, or increase linearity.

The pH, NH₃ and VFA levels of cocoa pod husk were also predicted using a non-linear regression approach. This technique is used in addition to linear regression because there isn't always a linear relationship between spectra data and the components to be modeled. Intrinsic non-linear sources between spectra and components can vary widely and are difficult to discover by classical linear regression like PLSR [33]. Hence, a special non-linear approach is also required besides methods of spectral correction to obtain more accurate and robust results.

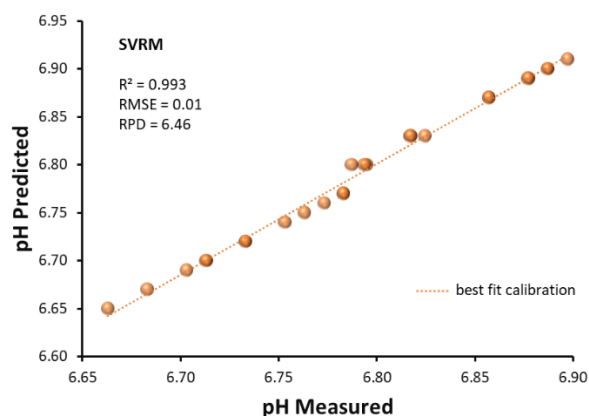


Figure 2. Scatter plots of the measured and NIR-predicted pH parameters derived from SVMR approach

Lastly, we applied the support vector machine regression (SVMR) method to develop the prediction models. A radial basis function and 10-fold cross-validation were used to assess and validate the models. Grid search was also run to optimize the C, ϵ , and γ function values in order to achieve a model with a high R² and low RMSE. The values of the turning function in this study are as follows: C=1, ϵ =0.1, and γ =0.01 for the pH model; and C=4.6, ϵ =0.1, and γ =0.01 for the NH₃ and VFA models. The obtained calibration results for nutritive

parameters of cocoa pod husk using the SVMR approach are presented in Table 1. Overall, the SVMR provide the models with excellent performance ($r > 0.9$ and $RPD > 3$). Based on r and RPD values (Table 1), different multivariate methods provide calibration results ranging from good to excellent prediction models, and most of them are robust. Compared to all methods, the SVMR performed better than the PCR and PLSR in predicting the pH and NH_3 concentrations. Figures 2 and 3 illustrate scatter plots produced from the SVMR model for the pH and NH_3 parameters.

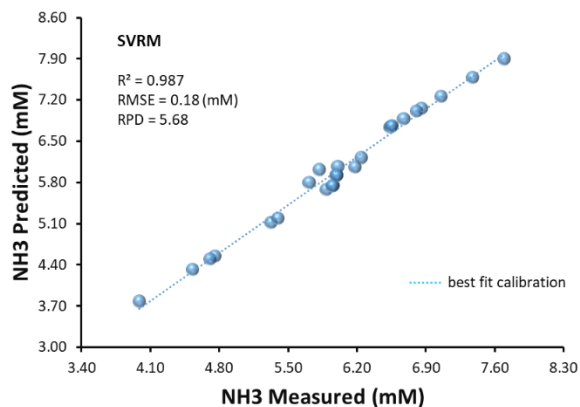


Figure 3. Scatter plots of the measured and NIR-predicted NH_3 parameters derived from SVMR approach

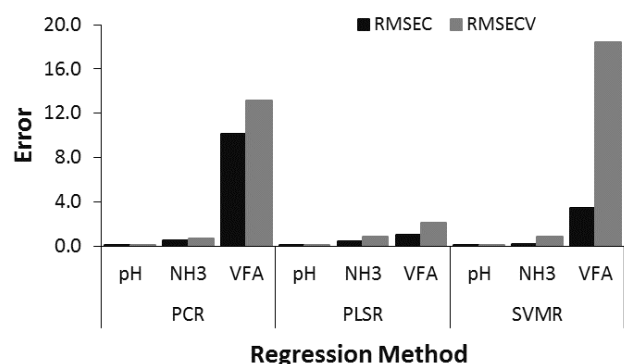


Figure 4. Summary of nutritive parameter errors (RMSEC and RMSECV) using different regression approaches

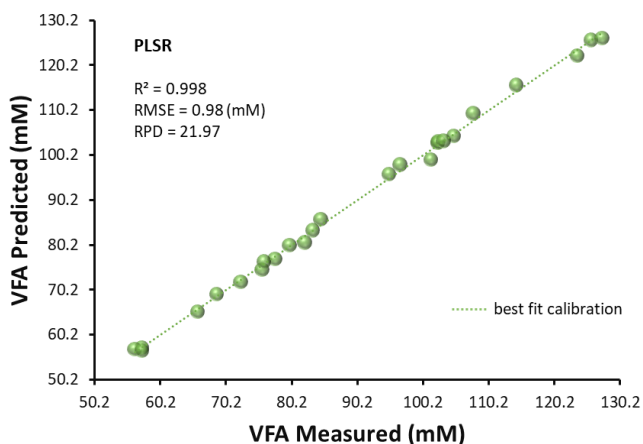


Figure 5. Scatter plots of the measured and NIR-predicted VFA parameters derived from PLSR approach

These results indicate that the SVMR method is an emerging alternative to the NIR calibration technique for quantifying the pH and NH_3 levels in fermented cocoa pod husks. This method may offer some advantages when there is a nonlinearity between the spectral data and the desired quantitative information [34]. Specifically for the VFA parameter, the PLSR model outperformed the SVMR because the SVMR method provided the smallest error index (RMSEC and RMSECV), as summarized in Figure 4. Scatter plots derived from the PLSR model for the VFA parameters is shown in Figure 5.

As can be seen in Figure 5, the PLSR model generates RMSEC and RMSECV values of 0.98 and 2.12, respectively. The RMSEC index denotes the modeling error or calibration variance. The RMSECV index reveals the prediction error or validation variance, which represents the regression model's quality [34, 35]. In terms of this consideration, the PLSR technique gives better results for VFA concentrations in cocoa pod husk samples. The advantage of PLSR is that it can accommodate multi-collinearity and is used to fit models when there is more spectral data than samples [36]. The SVMR algorithm provided the most accurate and robust performance compared to PCR and PLSR, since SVMR is more adaptive to the spectral data and can follow the trend of the data.

4. CONCLUSIONS

The potential of NIRS to predict nutritive parameters in fermented cocoa pod husks was evaluated. The obtained results in this study show that NIRS combined with different regression approaches can be successfully used in the determination of pH, NH_3 and VFA concentrations. The modeling result of SVMR performed better than the PCR and PLSR models for pH and NH_3 prediction. In terms of VFA prediction, the PLSR model outperformed other models, with higher correlations (r) index and lower error (RMSE) values. This implies that NIRS is applicable for use as a rapid and non-destructive tool for measuring feed degradation quality. Further study with more samples and broader validations is required to enhance the robustness of the prediction model. The only limitation in this work is that the number of samples is too small for developing calibration models used for real time prediction. This study is more suitable for a feasibility study. Hence, further works can be performed to enhance the achieved prediction performance by adding more samples and varied fermentation levels.

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