Prediction Accuracy Improvement of Indonesian Dairy Cattle Fiber Feed Compositions Using Near-Infrared Reflectance Spectroscopy Local Database

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ABSTRACT

The accuracy of near infrared reflectance spectroscopy (NIRS) depends on the database generated from the conventional wet chemistry (CWC). Currently, the local database of fiber-source feeds for tropical dairy cattle are still limited. The study aimed to compare CWC and NIRS initial database (NIRSID) results, to predict CWC from NIRSID, and to improve the accuracy of NIRS prediction using local database (NIRSLD). Five feeds as sources of fiber (Napier grass, natural grass, corn leaves, corn husk, and rice straw) from 4 areas of dairy cattle farming were used (4 farms from each area). For external calibration, 20 independent Napier grass samples were tested. Samples were analyzed using NIRS and CWC to measure dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and silica (Si) to calculate hemicellulose, cellulose, and lignin contents. The results obtained by NIRSID were compared to those obtained by CWC using T-test. Predictions of CWC from the results obtained by NIRSID were attempted using regressions. The NIRSLD was developed by inputting the CWC value to NIRS spectra. Internal calibration and validation as well as external calibration, were run. The results showed that NIRSID has low capacity in determining CWC (R²<0.63). Calibration using local database (NIRSLD) improved CWC prediction accuracy (residual predictive deviation (RPD) > 2 except for DM, EE, CF, ADL, and lignin). External validation showed that CWC and NIRSLD were similar in all parameters (p<0.05). The ratios of the standard error of prediction (SEP) to the standard error of laboratory (SEL) were > 2 for CP, CF, and ADF. It is concluded that the local database of NIRS of fiber-source feeds is necessary to improve the prediction accuracy of local dairy fiber-source feeds values using NIRS.

Keywords: dairy cattle; databases; fiber feed source; NIRS; prediction

INTRODUCTION

Feedstuff compositions are important in formulating ration to fulfill animal requirements (Hall, 2014). The data needed include proximate composition (DM, CP, EE, CF, and nitrogen free extract (NFE)), cell wall fraction (NDF, ADF, ADL, hemi-cellulose, cellulose, lignin, and silica), rumen fermentability, and digestibility. The chemical and utility values of feedstuffs can be analyzed using CWC methods or called chemo-metrics such as proximate analysis (AOAC, 2015) to analyze chemical compositions, van Soest method (Van Soest et al., 1991) to analyze cell wall structures, as well as two stage method (Tilley & Terry, 1963) to analyze rumen fermentability and digestibility.

One alternative method to the analysis is the NIRS method. NIRS method has advantages for its fast, low cost, non-destructive, and no chemical requirements for solvents or reagents (Parrini et al., 2018). The NIRS method has been used in analyzing several chemical parameters (Pierna et al., 2011) for several purposes such as moisture content during the drying process (Phetpan et al., 2019), sugar content in potatoes (Rady & Guyer, 2015), total phenols, condensed tannins, and 3-deoxyanthocyanidins in sorghum grain (Dykes et al., 2014), and the digestibility of feedstuffs (Samadi et al., 2018).

The accuracy of the NIRS method depends on the database used in the calibration process by the NIRS instrument (Hall, 2014). The calibration process involved data acquired from the CWC methods to develop a database (Soldado et al., 2013). Initial NIRS database (NIRSID) that come together with the instrument was not only expensive but also less accurate to be used for local feedstuffs. The database provided by the instrument was developed mostly based on temperate or subtropical feedstuffs that might differ from tropical feedstuffs (Cooke et al., 2020) due to different species used, age at harvesting, and range of nutrient concentrations in the similar feedstuffs. Some unconventional fiber-source feeds used in a dairy farm in a tropical area such as rice straw, corn husk, and corn stover (Lestari et al., 2015) might have different spectrums, out of the
range of the initial database, and difficult to analyze using NIRSID accurately. Some important nutrients information for the formulation of dairy cattle ration, such as cellulose and hemicellulose might not be available because of the initial general purposes of database designs. Therefore, NIRS database calibration for local fiber-source feeds with relevant parameters of dairy cattle needs to be developed.

Smallholder dairy farms in Indonesia used locally available fiber-source feeds such as Napier grass, natural grass, rice straw, corn stover, and cornhusk as the main ration components. The fiber-source feeds used in daily ration changes rapidly according to its availability which affects milk production and persistence because their interchanges are not reformulated correctly. Daily milk production measured in a similar farm drop rapidly from 22-20 L at 2nd-4th month in milk (Hasanah et al., 2017) to 18-16 L at the 3rd-5th month in milk (Zahera et al., 2015), and 12-10 L at the 6th-7th month in milk (Nugroho et al., 2015). With the fast-detection properties of NIRS, the rapid changing in fiber-source feeds can be measured and reformulated.

This research was aimed at comparing the qualities of dairy fiber-source feeds resulted from CWC analysis and NIRS prediction using an initial database (NIRSID), making a prediction of CWC from NIRSID results to adjust the prediction accuracy, and to develop NIRS local dairy fiber-source feeds database (NIRSD) for higher accuracy and complete measurements of nutritive values of tropical dairy fiber-source feeds.

MATERIALS AND METHODS

Sample Preparations

Five main fiber-source feeds used in dairy cattle farms in Indonesia were used. They were Napier grass, natural grass, rice straw, corn stover, and cornhusk. The fiber-source feeds were collected from four dairy cattle main areas in West Java Province of Indonesia (Pangalengan District of Bandung Regency, Lembang District of West Bandung Regency, Parung Kuda District of Sukabumi Regency, and Cibungbulang District of Bogor Regency). For each area, four dairy farms were sampled for fiber-source feeds used in the farm. For external validation, 20 complete independent sets of Napier grass samples from different locations in Bogor City and Regency were used. In total, 100 fiber-source feeds samples were used in this research.

Wet Chemical Analysis

Two kilograms of fiber-source feeds used in each farm were sampled, dried in Eyela NDO 400 (made in Japan) oven at 60°C for 48 hours, ground using laboratory blender at medium speed, and then filtered to pass a 1 mm screen. The powder samples were stored in a separate polyethylene plastic bag for each sample to be used later. Wet chemical analyses were conducted (AOAC, 2015) to measure proximate compositions (DM, Ash, CP, EE, and CF). The proximate analysis used the Eyela Oven to determine dry matter (DM) content. Soxhlet and Kjeldahl systems from Gerhart Instruments (made in Germany) were used to determine EE and CP, respectively.

Concentrations of NDF were measured after digestion in neutral detergent solution (NDS) followed by heat-stable α-amylase and sodium sulfite using the batch procedures (Method 15) recommended for an Ankom200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Subsequently, NDF residues were continued with digestion with acid detergent solution (ADS) to measure ADF using Method 14 recommended for the Ankom200. The ADF residues were further dissolved in sulfuric acid for 3 h to determine ADL (Method 8) and then corrected for residual ash by combustion in a muffle furnace at 500°C for 6 h. Procedures for calculations of NDF, ADF, ADL, and silica followed the formulas of Van Soest et al. (1991). Crude fiber determination was conducted by following AOCs Approved Procedure Ba 6a-05 (AOCS, 2005) recommended for crude fiber analysis in feeds by filter bag technique (Ankom200), including digestion in acid and alkaline solutions.

Measurement Feed Quality Using NIRS

The modular FT-NIR Spectrometer Solids Cell (BUCHI; NIFlex N-500 made in Switzerland) was used to analyze proximate and cell-wall compositions. Before being used, the NIRS was warmed up for approximately 15 minutes. Once the instrument was warmed up, the NIRS was automatically run the system suitability test (SST) to verify the NIRS performance periodically. After completing SST, external and internal references were run using the application of NIRSware operator. Before running the reference tests, an external reference (provided by BUCHI) had been inserted into the external-reference holder. NIRS was ready to be used after completing the reference measurement.

Before sample measurements, a database used should be selected from internal applications of NIRSware operator (NIRSID). Sample measurement was conducted by putting the sample (50 g dried fiber-feed mash) in a petri dish add-on 100 mm diameter of glass dish for solid sample (powder dried fiber-source feed). The sample should be distributed evenly and cover all the dishes. The dish should be put into the petri dish holder to measure the spectrum. The near infra-red light was sent into the sample and measured its absorbance at various wavelengths (800–2500 nm or 12500–4000 cm⁻¹) allowing for sample identification by penetrating the sample up to several millimeters deep. The scanning was done three times for each sample. The results will be automatically calculated as an average. The software marked the outrange sample automatically and, therefore, should be excluded from the results.

Database development was done using a similar procedure as sample measurement. However, in this step, no database was selected. The collected spectra were input with chemo-metric results with the help of NIRSware Management Console. Using NIRCal V5.6, calibration and validation of the database were
conducted. The collected spectra were automatically divided into 2/3 for calibration and 1/3 for validation using block-wise methods. Calibration models used partial least square regression while validation models used a validation set. The calibration and validation processes produced a comparison between chemo-metric and NIRS prediction values. The database resulted from the calibration and validation processes can be used as standard references for subsequent measurement after external validation. The best models generated were selected based on the smallest standard error of calibration (SEC) and standard error of prediction (SEP) with the highest calibration coefficients ($R^2$) and residual predictive deviation (RPD). RPD is a ratio between the standard deviation (SD) to SEP. External validation was conducted by measuring samples using a new database (NIRSLD) and the results were validated with chemometrics results. The comparison values of the standard error of prediction to the standard error of laboratory (SEP/SEL) were calculated.

**Research Design and Data Analysis**

This study used field explorative and laboratory research. Data analysis was conducted using T-test to compare CWC and NIRSID or NIRSLD data. Prediction of CWC values from NIRSID were done using regression after correlation analysis to include the correlated parameters into the estimated model. Calibration and validation of databases were conducted using partial least squares from NIRCal V5.6.

**RESULTS**

**Comparison between NIRSID and CWC**

Two general types of feed assay, chemical (sometimes called “wet chemistry”) and near infrared (NIR) reflectance were compared in this study. Table 1 showed that the values of fiber-source feeds obtained from NIRSID were similar to CWC values for DM, Ash, and CF, ADL, silica, hemicellulose, and cellulose parameters. However, the values were different for CP, EE, NDF, and ADF parameters.

**Prediction CWC Value from NIRSID**

An effort to improve the accuracy of measurement by adjustment of NIRSID values was made by using a prediction model regression from the correlated parameters. The results of correlation tests between CWC and NIRSID data are shown in Table 1. The correlation coefficients between related parameters were high ($R>0.7$) for Ash, NDF, and ADF, but were low ($R<0.7$) for DM, CP, EE, and CF. Adjustment of CWC from NIRSID data were made for the high correlation coefficient parameters, as were shown in Table 2. The highest regression value was found in ash ($R^2=0.764$), which resulted in more than 23% unpredicted factors.

**Development of Local NIRS Dairy Fiber-Source Feeds Database**

Spectra produced from the inputting local fiber-source feeds database (NIRSLD) are shown in Figure 1. The spectra showed a range of 4000 to 10000 cm$^{-1}$. This spectrum contained physical and chemical information about molecules. However, the information cannot be extracted directly because NIR spectra consists of a number of overlap bands (multicollinearity), the poor signal to noise (SN) ratios, and baseline fluctuations (Ozaki et al., 2007). The various peaks shown in this figure came from overlapping absorptions, which related mainly to overtones and combinations of vibrational modes involving some useful chemical bonds (C-H, O-H, N-H, and S-H). These absorption bands are indications of major unique constituents in the samples.

Calibration and validation of the local dairy fiber-source feeds database is shown in Table 3 for proximate compositions and cell wall structures. The table showed...
that the local database produced a better statistical value with high $R^2_C$ (> 0.7) and low SEC (< 2.153). The highest $R^2_C$ was found in ash prediction ($R^2_C = 0.929$). The $R^2_C$s for CP, NDF, ADF, hemicellulose, cellulose, and silica were > 0.8. Calibration made using the local database of fiber-source feeds improved coefficient of determination ($R^2_C$) from 0.524 to 0.828 for CP, from 0.672 to 0.891 for NDF, and from 0.683 to 0.804 for ADF.

External validation of database (NIRSLD) using 20 samples of Napier grass from different locations are presented in Table 4. The results showed that NIRSLD values were not significantly different from CWC for

Table 2. Prediction of wet chemical proximate and cell wall compositions from NIRSID

<table>
<thead>
<tr>
<th>Y</th>
<th>Constant</th>
<th>X</th>
<th>X2</th>
<th>X3</th>
<th>X4</th>
<th>X5</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>63.99</td>
<td>0.2936</td>
<td>2.6583</td>
<td>-0.2145</td>
<td>0.0079</td>
<td>-0.0001</td>
<td>0.7637*</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>30.457</td>
<td>-13.868</td>
<td>3.4264</td>
<td>-0.0207</td>
<td>-0.0199</td>
<td>0.0009</td>
<td>0.5238*</td>
</tr>
<tr>
<td>CP (%)</td>
<td>65.825</td>
<td>-26.613</td>
<td>3.4264</td>
<td>-0.0717</td>
<td>0.0404</td>
<td>-0.1376</td>
<td>0.5135*</td>
</tr>
<tr>
<td>EE (%)</td>
<td>2.6639</td>
<td>-0.9144</td>
<td>0.0819</td>
<td>0.4404</td>
<td>-0.0124</td>
<td>0.0124</td>
<td>0.6727*</td>
</tr>
<tr>
<td>CF (%)</td>
<td>-4690.3</td>
<td>913.54</td>
<td>-69.91</td>
<td>2.6433</td>
<td>-0.0494</td>
<td>0.0004</td>
<td>0.3298*</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>82.353</td>
<td>-3.8474</td>
<td>0.086</td>
<td>-0.0005</td>
<td>-0.0015</td>
<td>-0.0015</td>
<td>0.6832*</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>-0.0651</td>
<td>-0.2124</td>
<td>0.0916</td>
<td>-0.0015</td>
<td>-0.0015</td>
<td>-0.0015</td>
<td>0.6832*</td>
</tr>
</tbody>
</table>

Note: NIRSID= near infrared reflectance spectroscopy initial database, DM= dry matter, CP= crude protein, EE= ether extract, CF= crude fiber, NDF= neutral detergent fiber, ADF= acid detergent fiber, Y= conventional wet chemical value, X= near infrared reflectance spectroscopy initial database value, $R^2_C$= coefficient determination, * = p<0.05.

Table 3. Calibration and validation statistics in NIRSLD models for estimation of nutrient content (proximate) of tropical fiber-source feeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calibration</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>DM (%)</td>
<td>160</td>
<td>90.23</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>160</td>
<td>11.55</td>
</tr>
<tr>
<td>CP (%)</td>
<td>160</td>
<td>8.36</td>
</tr>
<tr>
<td>EE (%)</td>
<td>160</td>
<td>2.36</td>
</tr>
<tr>
<td>CF (%)</td>
<td>160</td>
<td>28.66</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>160</td>
<td>47.62</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>160</td>
<td>33.53</td>
</tr>
<tr>
<td>ADL (%)</td>
<td>160</td>
<td>4.53</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>160</td>
<td>28.00</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>160</td>
<td>28.82</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>160</td>
<td>3.32</td>
</tr>
<tr>
<td>Silica (%)</td>
<td>160</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Note: NIRSID= near infrared reflectance spectroscopy initial database, n= total number of observation, DM= dry matter, CP= crude protein, EE= ether extract, CF= crude fiber, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, SD= standard deviation, SEC= standard error of calibration, $R^2_C$= coefficient determination of calibration, RPD= residual predictive deviation, SEP= standard error of prediction, $R^2_V$= coefficient determination of validation, * = p<0.05.
all parameters measured. The coefficient correlation between CWC and NIRSLD was high (R=0.5), higher than the coefficient correlations between CWC and NIRSID (Table 1) for DM, ash, CF, and ADF. Comparison or ratios of SEP/SEL were <2 for DM, Ash, and NDF, while for CP, CF, and ADF the ratios were >2.

**DISCUSSION**

**Comparison between NIRSID and CWC**

The significantly different values between NIRSID and CWC were especially found for the complex organic compounds of high molecular weight (Lozano, 2015). The differences were due to the different ranges of database used in NIRS for local fiber-source feeds for dairy cattle in Indonesia. Dried ruminant fiber-source feeds database used in Buchi NIRflex N-500 Solids Cell (NIRSID) might be developed mostly using temperate forage, which was very different from tropical forages both species and nutrient contents. The different nutrient contents of tropical and subtropical forages were might also due to the different light intensities and day-lengths (Cooke et al., 2020), different forage species used and different generative stages of the plants (Sriagtula et al., 2017), and different ages at the harvesting of the plants (Puteri et al., 2015).

The CP, NDF, and ADF values were common measures of protein and fiber contents of feed used in ruminant (van Soest et al., 1991), especially in dairy nutrition (Hammond et al., 2016) which can be used in determination of forage relative value (RFV) system (Olafadehan & Okunade, 2018). Accurate measurements of these parameters will determine the ration quality and affect feed intake (Riaz et al., 2014), digestibility (Stergiadis et al., 2015), and dairy performance (Krämer-Schmid et al., 2016). High CP in ration will increase milk production (Mutsvanga et al., 2016), while high HDF and ADF are related to a low digestibility (Stergiadis et al., 2015) and are considered as two important limited factors for the estimation of the nutritive qualities of feed and forage (Wolfrum et al., 2009). Because CP, NDF, and ADF are important for dairy nutrition as indicators of protein and fiber availabilities for the animal and milk synthesis, the accuracies of these parameters’ measurements are very important.

**Prediction CWC Value from NIRSID**

Models to predict CP, EE, NDF, and ADF values had lower R² (<0.68), which showed that adjustments of CWC values from NIRSID were inaccurate. An effort to improve the accuracies of NIRSID predictions of CP, NDF, and ADF could not be done by adjusting the NIRSID value through the regression model. The adjustment of NIRSID values to achieve CWC values can be made by using regression if the values have high correlations (Yin, 2020). Low correlations of NIRSID values to CWC values found in this study resulted in the low NIRSID prediction capacity of CWC values. The accuracy of NIR in predicting CWC was higher by using a single forage species (Yang et al., 2017) or by developing NIRSID calibration equations for individual species or groups of similar forage species (Fairbrother & Brink, 1990). Lower prediction accuracies using NIR for NDF and ADF were also reported by Hoffman et al. (1999). Therefore, it needs a new pre-calibrated database with local CWC data before it’s used in NIRS.

**Development of Local NIRS Dairy Fiber-Source Feeds Database**

The near-infrared spectrum was located from 2500 nm to 800 nm or from 4000 cm⁻¹ to 12,500 cm⁻¹ (Ozaki et al., 2007). However, the region of 4000 to 9000 cm⁻¹ were the most prominent bands to absorb polymers originating from OH, NH, CH, and SH overtone stretching vibrations and stretching-bending combinations. Yang et al. (2017) found the critical wavelengths to construct optimal NIRS models for Italian ryegrass were located at 4247-6102, 4247-5450, and 5446-6102 cm⁻¹ for CP, NDF, and ADF contents, respectively. The precise and proper spectral analysis of NIR spectra allows us to get useful information from NIR spectra, whereas improper spectral analysis may lead to the wrong information (Ozaki et al., 2007). In this study, adjustment made in the range of 4200-6200 cm⁻¹ did not improve CP, NDF, and ADF statistical parameters significantly. The difficulties in determining what wavelength or region accurately in the near-infrared spectrum carrying the most quantitative information about the contents of natural compounds were also found by Saha et al. (2017). This problem might be caused by the wider range of substrate available in the local dairy fiber-source feeds in comparing to Italian ryegrass found by Yang et al. (2017). Several
species of natural grass found such as *Panicum repens*, *Cynodon dactylon* Pers, *Leersia hexandria*, *Brachiaria mutica*, *Cyperus rotundus* L, and *Trichola enarosa* (Despal et al., 2017) might contain an abundance of some specific functional groups of various organic compounds (Saha et al., 2017).

The high determination coefficient (R^2^C>0.8) found in this study indicated a good prediction of calibration models. Moreover, R^2^C= 0.929 for the ash parameter indicated an excellent prediction of the calibration model (Williams & Sobering 1993). Even though some mineral absorptions might be absent in the near-infrared region, it was still possible to estimate ash contents accurately. This condition was probably due to complexes of ash with organic compounds (Parrini et al., 2018).

The ratio of prediction to the deviation (RPD) after partial least square regression found was higher than 2 for CP (RPD= 2.487), NDF (RPD= 2.862), and ADF (RPD= 2.025), hemicellulose, cellulose, and silica. While, the RPD < 2 were found in DM, EE, CF, ADL, and lignin. Internal validation of the data resulted in almost similar statistics parameters. The RPD is the ratio of the SD to SEP. This value represented the ability of the NIRS model to predict a substance (Williams & Sobering, 1993). According to Baillères et al. (2002), SEP data alone may be misleading, therefore, the RPD value is needed. An RPD value of more than 2 was categorized as a relevant prediction of NIRS. If the SEP value closed to the SD value (RPD<2), the NIRS calibration process was not efficient in predicting chemo-metric value. In this study, NIRS local dairy fiber-source feeds database developed (NIRSLD) was relevant in predicting CWC because RPD value found for CP, NDF, ADF, hemicellulose, cellulose, and silica were higher than 2. However, Lobos et al. (2013) gave a higher category (RPD>2.5) as a valid measurement ability of a NIRS model to predict a constituent. In this case, the ADF database produced needs to be improved. The low RPD found in the prediction of ADL and lignin (RPD=2) in this study might be caused by the presence of polyphenolic compounds that can alter the lignin absorption bands located in the same spectral zones (Baillères et al., 2002). Williams (2004) suggested five categories of prediction accuracy based on RPD values, i.e., 1) The RPD<1.5 indicated an unusable; 2) The 1.5<RPD<2.0 grouped as the ability of prediction to distinguish between high and low values; 3) The 2.0<RPD<2.5 produced an “approximate” quantitative prediction; 4) The 2.5<RPD<3.0 reflected a “good” quantitative prediction; and 5) The RPD>3.0 indicated an “excellent” quantitative prediction.

Improvement of prediction value of Napier grass CWC from NIRSLD is caused by the similarities in database input into the spectrum. The prediction error relative (PRL) was needed for further evaluation of the model accuracy. The PRL was the ratio of the SEP to the SEL values (Yang et al., 2017). In this study, it was found that PRL for CP and ADF were more than 2; therefore, they need to be improved. The SEP should closely match the SEL. If SEP was much more than the SEC, then it could be an indication of too many wavelengths in the models that do not represent the substrate being modeled (Ozaki et al., 2007).

**CONCLUSION**

Low prediction accuracy of dairy cattle fiber feed using initial NIRS database can be overcome by development local database. The improvements are mainly in prediction of protein and fiber fractions. More chemometric samples of single species fiber feed are needed to be able to further improve the prediction accuracy.

**CONFLICT OF INTEREST**

Luki Abdullah serves as an editor of the Tropical Animal Science Journal, but has no role in the decision to publish this article. The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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