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# NIRS-based prediction of mineral content and DCAD status for sustainable livestock nutrition

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**Abstract.** *Isnaini R, Despal, Permana IG, Zahera R, Rosmalia A, Farras MN. 2025. NIRS-based prediction of mineral content and DCAD status for sustainable livestock nutrition. Biodiversitas 26: 3281-3293.* The Dietary Cation-Anion Difference (DCAD)—the balance between positively and negatively charged minerals (e.g., $Na^+$, $K^+$ vs. $Cl^-$, $S^{2-}$)—is a key factor in maintaining acid-base balance, calcium homeostasis, and metabolic health in dairy cows (*Bos taurus* (Linnaeus, 1758)). However, DCAD data for individual feedstuffs, especially those used in tropical regions like Indonesia, are largely unavailable, limiting formulation accuracy. This study analyzed the mineral content (Ca, Na, K, Mg, P, S, Cl) of 289 Indonesian feed samples, all feed samples in each category were derived from one species only (66 grasses, 114 legumes, 47 roughages, and 62 concentrates) using wet chemistry, and calculated their DCAD values. Near-Infrared Reflectance Spectroscopy (NIRS) was used to develop predictive models as a rapid, non-destructive, cost-effective, and multi-parameter alternative to laboratory analysis. DCAD values varied widely: most grasses, roughages, and concentrates were negative, while legumes tended to be positive. This variation is nutritionally relevant, as negative DCAD diets are beneficial during the prepartum phase to reduce the risk of hypocalcemia in transition cows. NIRS models showed strong performance ($R^2>0.8$; RPD>1.4), with successful external validation (SEP/SEL<2, except in concentrates). The resulting DCAD database and NIRS models can be integrated into mobile apps or formulation tools to support precision feeding strategies, particularly for managing transition cow health in tropical dairy systems.

**Keywords:** Dietary cation anion difference, feedstuff mineral, NIRS prediction models, sustainable dairy farming, tropical forages

**Abbreviations:** Ca: Calcium; Cl: Chloride; DCAD: Dietary Cation Anion Difference; K: Pottasium; Mg: Magnesium; n: Total number of observations; Na: Sodium; P: Phosphor; $R^2C$: Coefficient of determination calibration; $R^2V$: Coefficient of determination validation; RPD: Residual Predictive Deviation; S: Sulphur; SD: Standard Deviation; SEC: Standard Error Calibration; SEP: Standard Error Predicted

## INTRODUCTION

Dairy cattle (*Bos taurus* (Linnaeus, 1758)) nutrition relies on a precise nutrient balance to support health and productivity (NRC 2001). Among the critical dietary factors, the Dietary Cation-Anion Difference (DCAD) plays a vital role in regulating acid-base balance, calcium homeostasis, and other physiological functions. DCAD is calculated as the difference between milliequivalents of cationic ($K^+$, $Na^+$) and anionic ($Cl^-$, $S^{2-}$) minerals per kilogram of dry matter (Zachwieja et al. 2022). It significantly affects dairy cow performance, particularly during transition and lactation phases. According to NRC (2001), the predicted DCAD values are >100 mEq $kg^{-1}$ for growing calves and heifers, around -100 mEq $kg^{-1}$ during close-up, and >130 mEq $kg^{-1}$ during lactation. Excessively negative DCAD can cause metabolic acidosis, reducing intake and performance, while overly positive values prepartum may increase the risk of hypocalcemia and milk fever. Adjusting DCAD levels affects milk yield (Li et al. 2022), reduces the risks of metabolic disorders, and enhances feed efficiency (Martinez et al. 2018). Recent studies also show DCAD's role in improving performance under heat stress in tropical climates (Nguyen et al. 2018).

However, applying DCAD-based formulation is challenged by the lack of standardized mineral composition data of local feed ingredients in tropical areas. This limitation hinders accurate DCAD calculation. In Indonesia, ration formulation commonly considers TDN, crude protein, fiber, minerals (Sahroni et al. 2021), fat (Anzhany et al. 2022), RDP:RUP ratio and non-fiber carbohydrate content (Rosmalia et al. 2022), but rarely includes DCAD. As a result, diets may be imbalanced in cation-anion content, increasing risks such as milk fever. This highlights the need to generate reliable DCAD data for Indonesian feedstuffs and develop tools for accurate, phase-specific formulation strategies.

The gap is especially critical during late gestation and early lactation, which involves major physiological changes. The three weeks before and after calving mark a period of high susceptibility to metabolic disorders such as milk fever, ketosis, retained placenta, and displaced abomasum. Feeding strategies that create mild compensated metabolic acidosis through negative DCAD can improve calcium mobilization and reduce hypocalcemia risk (Redfern et al. 2021). Their success, however, depends on accurate DCAD values based on local feed composition. In the tropics, high feed variability and differences in mineral profiles of leguminous forages further complicate formulation. Indonesia's forage biodiversity includes legumes such as *Moringa oleifera* and *Gliricidia sepium*, which reflect diverse soil types, microclimate, and agro-ecological zones. For example, *M. oleifera* is known for its high chloride and sulfur content (Moyo et al. 2011), potentially lower or negative DCAD values, which is uncommon among legumes and may affect their role in ration formulation (Tremblay et al. 2013; Despal et al. 2024).

Near-Infrared Reflectance Spectroscopy (NIRS) offers a promising tool for rapid, non-destructive analysis of mineral content in feed. NIRS databases have been used in nutrient prediction for legumes (Agustiyani et al. 2021), in dairy fiber digestibility (Zahera et al. 2022), diurnal milk composition (Oktavianti et al. 2022), milk fatty acid health index (Despal et al. 2021a), and silage evaluation (Ath-Thifa et al. 2024). In contrast, conventional wet chemistry methods like Atomic Absorption Spectroscopy (AAS) are laborious and slow, limiting their application in routine feed evaluation (Tomar et al. 2021). While NIRS is commonly used to predict protein, fiber, and energy, its use for mineral and DCAD estimation remains limited (Hossain et al. 2024), especially in tropical settings. Developing a NIRS-based model for mineral and DCAD prediction would improve accuracy and efficiency. A well-calibrated NIRS model, when validated with laboratory data, provides a practical bridge from research to application (Despal et al. 2023). This allows integration of local feed resources into precision feeding programs, which are vital for enhancing productivity and sustainability in tropical dairy systems (Despal et al. 2021b).

This study aimed to determine the mineral content and DCAD values of Indonesian dairy feedstuffs, including grasses, legumes, roughages, and concentrates, using both conventional and NIRS methods. It also seeks to develop predictive models for estimating DCAD, supporting more accurate ration formulation. Establishing a comprehensive DCAD feed database will enable precise feeding, strategies to improve cow health, productivity, and farm profitability.

## MATERIALS AND METHODS

### Collection and preparation of samples

A total of 66 grass samples (3 species), 114 legume samples (13 species), 47 roughage samples (7 species), and 62 concentrate samples (16 species) were collected, each representing the edible portion of the plant at maturity (Table 1). Sampling was conducted during both the dry and rainy seasons to capture seasonal variation. Feed samples were obtained from lowland areas—Padang, Pariaman, Pasaman, and Dharmasraya (West Sumatra) and Bogor District and Bogor City (West Java)—as well as highland areas—Padang Panjang, Solok, and Tanah Datar (West Sumatra) and West Bandung, Bandung, and Sumedang (West Java). These regions represent diverse agroecological zones with varying topography, climate, and soil types, including volcanic Andisols and mineral-rich Inceptisols. The sampling strategy aimed to capture the breadth of forage diversity and environmental conditions across Indonesia, supporting the development of a robust and broadly applicable NIRS calibration model. The study was conducted at the Laboratory of Dairy Nutrition and the Laboratory of Animal Logistics, Faculty of Animal Science, IPB University. Sample collection and analysis were carried out between August 2023 and December 2023, covering both the dry and rainy seasons in Indonesia. Forages (3 kg/sample) and concentrates (200 g/sample) were collected, with larger forage weights required due to their high moisture content (<30% DM) compared to concentrates (>85% DM), ensuring adequate dry matter for analysis. All dried samples were then ground and passed through a 1 mm sieve in preparation for mineral analysis. Mineral preparation was performed using two methods: wet ashing and dry ashing. Wet ashing was used for elements sensitive to volatilization, such as sulfur, while dry ashing was applied for more stable minerals like calcium and magnesium to ensure optimal recovery and accuracy.

### Analysis of mineral content

For wet ashing, 2 g of dried were digested using a mixture of concentrated nitric and perchloric acids, followed by heating until a clear solution was obtained, which was then diluted for mineral analysis using AAS, following the protocol outlined by Ernawati and Abdullah (2022). The concentrations of calcium (Ca), sodium (Na), potassium (K), phosphorus (P), and magnesium (Mg) were determined using an AAS, model AA-6680 (Shimadzu, Japan), with element-specific wavelengths (e.g., Ca: 422.7 nm, Na: 589.0 nm, K: 766.5 nm, Mg: 285.2 nm) and calibrated using certified standard solutions. AAS was calibrated using a series of standard solutions with known concentrations, along with blank and sample readings, to ensure accuracy and reliability of mineral quantification. Mineral concentrations were calculated from absorbance values using the formula provided by AOAC (2000):

$$\text{Mineral content (mg 100g}^{-1}\text{)} = \frac{(\text{C} \times \text{V} \times \text{F.P.})}{\text{W}}$$

Where:
C : Concentration read on AAS (mg 1000 mL$^{-1}$)
V : Volume of the working flask (mL)
F.P. : Dilution factor of the final sample solution
W : Sample weight (g)

Mineral content was initially calculated in mg/100 g dry matter using the standard AOAC formula. These values were then converted into percentage (%) and further into milliequivalents per kilogram of dry matter (mEq/kg DM) using appropriate atomic weight and valence-based conversion factors, as required for DCAD calculation.

A 2 g dried sample was used for dry ashing, following the AOAC protocol (AOAC 2005). Samples were ashed in a muffle furnace at 550°C for 4 hours until a white residue was obtained. This method was employed to determine the concentrations of sulfur (S), as described by AOAC (2000), and chlorine (Cl), as outlined by Tausskyu et al. (1953).

### Estimation of DCAD

The measured percentages of sodium (Na), potassium (K), chloride (Cl), and sulfur (S) were converted into milliequivalents per kilogram of dry matter (mEq $kg^{-1}$ DM) to calculate the DCAD value for each sample, with all results expressed on a 100% dry matter basis. The conversion factors applied were 435 for Na, 256 for K, 282 for Cl, and 624 for S (Tucker et al. 1992). Feedstuffs with DCAD values below 0 mEq $kg^{-1}$ DM are considered anionic, while those with values above 0 are classified as cationic. This classification helps guide their suitability for different physiological phases, such as using anionic feeds in prepartum rations to reduce the risk of hypocalcemia. DCAD values were calculated using the following formula (Tucker et al. 1992):

$$\text{DCAD (mEq kg}^{-1}\text{ DM)} = (Na^{+}+K^{+}) - (Cl^{-}+S^{2-})$$

### Calibration and validation of NIRS

The mineral content prediction was conducted using the Büchi NIR-Flex N-500 Solids Cell (Switzerland), following a procedure similar to that described by Zahera et al. (2022). Spectral scanning was performed in the range of 800-2500 nm, and each sample was scanned in triplicate to obtain an averaged spectrum for analysis. For each sample, three spectra were collected, with two-thirds randomly assigned for calibration and one-third for validation, resulting in a total of 360 spectra for the study. Chemometric analyses were performed using the NIRS Management Console, and calibration and validation models were developed using Partial Least Squares (PLS) regression via NIR-Cal V5.6, as PLS is well-suited for handling multicollinear spectral data and extracting relevant information from complex datasets. The software automatically optimized the number of factors, spectral pre-treatments, and wavenumber ranges for each model. Spectral pre-treatment methods applied in the FT-NIRS Büchi N-500 included Standard Normal Variate (SNV), first derivative, and second derivative transformations. These pre-treatments were selected automatically by the NIRCal software to minimize baseline variation and enhance relevant spectral features for accurate prediction of mineral content and DCAD values. External validation was performed using ten independent samples set that were not included in the original calibration set, comparing predicted values with laboratory-determined mineral contents and DCAD values.

### Data analysis

The calibration and validation of NIRS prediction models were conducted using Partial Least Squares (PLS) regression in NIRCal V5.6. The software automatically generated performance indicators, including the coefficient of determination ($R^2$), which was used to evaluate the relationship between predicted and reference values. These correlation values were not calculated through independent statistical tests, but are integrated outputs of the PLS modeling process.

To compare NIRS predictions with reference laboratory values, a paired t-test was performed using SPSS (version 25) with significance set at $p<0.05$. Normality of the data was assessed using the Kolmogorov-Smirnov test, which is automatically applied by SPSS for sample sizes above 100 (n = 114). The data met the normality assumption ($p>0.05$), supporting the use of the t-test.

## RESULTS AND DISCUSSION

### Mineral content of feedstuffs

Table 1 presents the mineral content of grasses, legumes, roughages, and concentrates, revealing considerable variation across species. Legumes exhibited the widest range in potassium (K) content (0.67-2.73% DM), which, as a major cation, contributes significantly to increasing DCAD values. Conversely, grasses had the highest sulfur (S) levels (0.49-0.61% DM), an anionic component that lowers DCAD values. *Moringa oleifera*, despite being a legume, contained unusually high levels of both S and Cl, resulting in a negative DCAD value.

Sodium (Na) content was lowest in legumes, ranging from 0.001% DM in *Medicago sativa* to 0.033% DM in *Acacia mangium*. This low Na content is nutritionally significant because Na plays a critical role in maintaining osmotic pressure, nerve function, and nutrient transport in ruminants. From a DCAD perspective, Na is a key cation, and its low concentration in legumes reduces their contribution to the overall DCAD value. This can lead to insufficient cationic load in lactating cow rations if not balanced with other Na-rich ingredients, potentially affecting feed intake, rumen buffering capacity, and milk yield.

In contrast, roughages had the highest Na content, ranging from 0.101% DM in corn stover to 0.239% DM in rice straw. This contributes positively to DCAD values by increasing the cationic component of the balance. This higher Na content can shift the feed's DCAD status toward a more positive value, which may be beneficial during lactation phases but less suitable for transition cows requiring a more anionic diet.

Legumes also exhibited the highest levels of other key minerals: potassium (0.67% DM in *Paraserianthes falcataria* to 2.73% DM in *Peperomia pellucida*, chloride (0.13% DM in *Calliandra calothyrsus* to 0.88% DM in *Moringa oleifera*), calcium (0.41% DM in *Paraserianthes falcataria* to 2.31% DM in *Gliricidia sepium*), and magnesium (0.48% DM in *Medicago sativa* to 0.91% DM in *Sesbania grandiflora* and *Peperomia pellucida*). In comparison, roughages had the lowest levels of these minerals. The lower concentrations of Na, K, and Mg in roughages contribute to their predominantly negative DCAD values. This mineral profile pattern aligns with supports the observation that roughages in our dataset consistently exhibited anionic DCAD status (ranging from -423.89 to -73.91 mEq/kg DM), which can be advantageous in transition diets but must be managed carefully during lactation to avoid metabolic imbalances.

Potassium contents ranged from 0.14% DM in carrot leaf (*Daucus carota*) to 0.23% DM in corn stover (*Zea mays*); Cl from 0.18% DM in corn leaf (*Z. mays*) to 0.44% DM in vegetable waste; Ca from 0.72% DM in corn husk (*Z. mays*) and corn straw (*Z. mays*) to 0.88% DM in carrot leaf (*Z. mays*); and Mg from 0.11% DM in corn leaf (*Z. mays*) to 0.24% DM in vegetable waste.

Grasses had higher sulfur (S) content than other feed types, ranging from 0.49% DM in elephant grass (*Pennisetum purpureum*) to 0.61% DM in natural grass. This elevated sulfur level, as observed in grasses (up to 0.61% DM), contributes negatively to DCAD values due to sulfur being a strong anion. This can lower the overall DCAD of the ration, increasing the risk of metabolic acidosis if not balanced appropriately. While a slightly negative DCAD is beneficial during the transition phase to support calcium mobilization, excessive sulfur may lead to uncompensated acidosis.

In contrast, legumes exhibited the lowest S content, ranging from 0.06% DM in *Paraserianthes falcataria* to 1.44% DM in *M. oleifera*. Phosphorus (P) was highest in concentrates, ranging from 0.22% DM in brewery waste to 0.79% DM in palm kernel meal, while legumes had the lowest P levels. Although P is not directly involved in DCAD calculation, it is crucial for energy metabolism, bone development, and rumen microbial activity (NRC 2001). Adequate P levels are essential to support optimal nutrient utilization and animal productivity. Inadequate phosphorus in feedstuffs—such as in some legumes observed in this study—may limit overall performance even if DCAD is properly balanced.

### DCAD status of feedstuffs

The results of DCAD value calculations are presented in Table 2. Overall, most grasses, roughages, and concentrates exhibited anionic (negative) DCAD values, whereas legumes generally showed cationic (positive) DCAD values. Exceptions included a few legumes and concentrates with negative or positive DCAD values, respectively. Each feed type exhibited a wide range of DCAD values, allowing flexible diet formulation to support physiological needs, such as preventing metabolic disorders during dairy cows' transition period: grasses ranged from -344.77 to -230.26 mEq $kg^{-1}$, legumes from -467.90 to 340.30 mEq $kg^{-1}$, roughages from -423.89 to -73.91 mEq $kg^{-1}$, and concentrates from -458.07 to 193.68 mEq $kg^{-1}$. Most Indonesian feedstuffs—particularly grasses, roughages, and concentrates—exhibited anionic DCAD values, which can be beneficial during the transition period by helping prevent metabolic alkalosis and improving calcium metabolism in dairy cows. In contrast, nearly all legumes showed cationic DCAD values, with the exceptions of *Sesbania grandiflora* (-11.66 mEq $kg^{-1}$) and *Moringa oleifera* (-467.90 mEq $kg^{-1}$), which were anionic. Moringa's negative DCAD may result from its relatively high levels of acidic minerals such as sulfur and chloride, which lower the overall DCAD despite its classification as a legume. Several concentrate feedstuffs also had positive DCAD values, including pollard, tofu waste, copra meal, rice bran, brewery waste, cassava, and cassava waste.

### Predicting mineral and DCAD status of feedstuffs using NIRS

The NIRS calibration and validation results for mineral content and DCAD are summarized in Table 3. The best models ($R^2>0.9$ and RPD>3.0) were observed for Na and DCAD in grasses, P and S in legumes, K, Cl, and DCAD in roughages, and P and K in concentrates. The calibration models demonstrated strong performance ($R^2>0.8$ and RPD>2.0) for most parameters. Calibration models with coefficients of determination ($R^2$) greater than 0.80 were achieved for Na, Mg, Cl, S, and DCAD in grasses; Ca, Mg, P, S, and DCAD in legumes; Na, Mg, P, K, Cl, S, and DCAD in roughages; and Ca, Na, Mg, P, K, Cl, S, and DCAD in concentrates. Moderate calibration performance ($0.70<R^2<0.80$ and 1.4<RPD<2) was observed for Ca, and P in grasses; Na and K in legumes; and Ca in roughages. Notably, no model produced an $R^2$ below 0.70, except for K in grasses. According to the threshold categories defined by Saha et al. (2017), these results fall into Category B (moderately reliable) and Category A (highly reliable), further supporting the robustness of the calibration models.

The NIRS Local Database (NIRSLD) effectively predicted mineral content and DCAD, potential use in real-time feed analysis and on-farm decision-making, allowing rapid, non-destructive prediction of mineral content and DCAD values. This supports more responsive and phase-specific ration formulation, particularly valuable for managing dairy cow health and performance in tropical production systems. This is supported by Residual Predictive Deviation (RPD) values greater than 2.0 for most parameters, including Na, Mg, Cl, S, and DCAD in grasses; Ca, Mg, P, S, and DCAD in legumes; Na, Mg, P, K, Cl, S, and DCAD in roughages; and Ca, Na, Mg, P, K, Cl, S, and DCAD in concentrates. Models for Ca, P, and K in grasses; Na in legumes; and Ca in roughages had RPD values between 1.4 and 2.0, indicating acceptable predictability. Among these, the model for K in legumes showed the lowest performance, aligning with earlier observations. Potassium (K) in legumes showed the lowest model performance, with $R^2$ and RPD values falling below the threshold for strong predictive reliability. This aligns with earlier observations and may be due to the spectral overlap or variability in K concentration across legume species. Identifying this limitation helps clarify the scope and boundaries of the NIRS calibration models presented.

As shown in Table 4, the external validation (n = 10) results indicated no significant differences (p>0.05) between Wet Chemical Analysis (WCA) and NIRSLD predictions for Ca, Mg, P, K, S, and DCAD in grasses; Ca, Na, Mg, P, Cl, and S in legumes; Na, P, K, and Cl in roughages; and Na, Mg, P, K, S, and DCAD in concentrates. However, significant differences (p<0.05) were observed for Na and Cl in grasses; K and DCAD in legumes; Ca, Mg, S, and DCAD in roughages; and Ca and Cl in concentrates. These discrepancies may be attributed to factors such as limited calibration ranges, weak NIR absorbance bands for certain minerals, or sample heterogeneity.

The predictive models demonstrated acceptable accuracy (SEP/SEL<2) for Ca, Na, Mg, Cl, and S in grasses; all parameters in legumes; Ca, Na, Mg, P, and S in roughages; and Na and K in concentrates. Nonetheless, further refinement is required for parameters with SEP/SEL>2 to improve model reliability—particularly for P, K, and DCAD in grasses; K, Cl, and DCAD in roughages; and Ca, Mg, S, and DCAD in concentrates. The SEP/SEL ratio compares the Standard Error of Prediction (SEP) to the Standard Error of the Laboratory method (SEL), indicating good model accuracy when prediction error is less than twice the lab error.

In this study, the calibration data from spectral curves of various tropical forages showed low variation in spectral shape across parameters, despite clear differences in chemical composition (Figure 1)—a condition favorable for NIRS calibration. The low variation refers specifically to spectral shape, not to chemical composition. This uniformity is likely due to consistent sample preparation and the dominance of key chemical bonds—such as O-H, C-H, and N-H—that absorb strongly in the NIR region. These bonds generate characteristic absorption patterns that remain relatively stable across samples. Additionally, the homogeneous physical properties of the ground samples contribute to the consistent spectral profiles. Together, these factors enhance model robustness and reduce spectral noise, supporting the reliability of NIRS-based predictions despite compositional variability.

Figure 2 presents plots comparing NIRSLD predicted values with WCA values for Ca, Cl, DCAD, and S reference in feedstuffs. The relationship between NIRSLD predicted values and WCA values for DCAD in roughage showed the strongest correlation compared to the other reference values. Conversely, the Ca reference in grasses exhibited the weakest correlation. This weak correlation for Ca may be attributed to its relatively low concentration in the grass samples or possible interactions with the organic matrix that limit its bioavailability or detectability in the analysis.

**Table 1.** Descriptive statistics of feedstuff mineral content

| **Feedstuffs** | **n** | **Mineral content (% DM)** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Na** | **K** | **Cl** | **S** | **P** | **Ca** | **Mg** |
| Grasses | | | | | | | | |
| Elephant grass (*Pennisetum purpureum* Schumach.) | 40 | 0.268±0.051 | 0.21±0.04 | 0.32±0.06 | 0.49±0.12 | 0.44±0.09 | 0.84±0.14 | 0.19±0.02 |
| Natural grass | 25 | 0.162±0.044 | 0.26±0.05 | 0.37±0.08 | 0.61±0.18 | 0.29±0.06 | 0.88±0.14 | 0.17±0.02 |
| Odot grass (*Pennisetum purpureum* cv. Mott) | 1 | 0.239 | 0.30 | 0.36 | 0.58 | 0.28 | 0.94 | 0.28 |
| Range | | 0.162-0.268 | 0.21-0.30 | 0.32-0.37 | 0.49-0.61 | 0.28-0.44 | 0.84-0.94 | 0.17-0.28 |
| Average | | 0.223±0.055 | 0.26±0.05 | 0.35±0.02 | 0.56±0.06 | 0.34±0.09 | 0.89±0.05 | 0.21±0.06 |
| Legumes | | | | | | | | |
| *Pterocarpus indicus* Willd. | 3 | 0.005±0.001 | 1.62±0.92 | 0.25±0.04 | 0.12±0.00 | 0.21±0.02 | 0.51±0.10 | 0.63±0.12 |
| *Bauhinia ×blakeana* Dunn | 7 | 0.004±0.001 | 0.74±0.19 | 0.29±0.06 | 0.11±0.02 | 0.15±0.05 | 1.75±0.81 | 0.87±0.28 |
| *Calopogonium mucunoides* Desv. | 2 | 0.010±0.004 | 0.72±0.01 | 0.30±0.07 | 0.19±0.07 | 0.13±0.03 | 1.49±0.22 | 0.83±0.04 |
| *Gliricidia sepium* (Jacq.) Kunth | 28 | 0.009±0.005 | 1.46±0.37 | 0.52±0.21 | 0.14±0.06 | 0.20±0.02 | 2.31±0.57 | 0.76±0.25 |
| *Indigofera zollingeriana* Miq. | 2 | 0.002±0.000 | 1.00±0.11 | 0.55±0.06 | 0.12±0.02 | 0.24±0.00 | 1.99±0.29 | 0.90±0.04 |
| *Calliandra calothyrsus* Meisn. | 32 | 0.004±0.002 | 1.19±0.83 | 0.13±0.11 | 0.15±0.05 | 0.12±0.06 | 1.22±0.61 | 0.69±0.14 |
| *Leucaena leucocephala* (Lam.) de Wit | 30 | 0.007±0.005 | 1.19±0.56 | 0.68±0.20 | 0.20±0.05 | 0.13±0.05 | 2.26±0.89 | 0.82±0.22 |
| *Acacia mangium* Willd. | 1 | 0.033 | 1.59 | 0.49 | 0.19 | 0.09 | 0.71 | 0.58 |
| *Medicago sativa* L. | 1 | 0.001 | 1.83 | 0.39 | 0.10 | 0.19 | 1.75 | 0.48 |
| *Paraserianthes falcataria* (L.) I.C.Nielsen | 1 | 0.008 | 0.67 | 0.34 | 0.06 | 0.11 | 0.41 | 0.72 |
| *Sesbania grandiflora* (L.) Poir. | 2 | 0.004±0.001 | 1.04±0.26 | 0.56±0.48 | 0.19±0.02 | 0.32±0.08 | 0.88±0.00 | 0.91±0.09 |
| *Peperomia pellucida* (L.) Kunth** | 2 | 0.012±0.011 | 2.73±1.35 | 0.83±0.18 | 0.21±0.07 | 0.29±0.11 | 0.87±0.08 | 0.91±0.87 |
| *Moringa oleifera* Lam.** | 3 | 0.015±0.001 | 2.62±1.26 | 0.88±0.77 | 1.44±0.36 | 0.29±0.05 | 1.53±0.63 | 0.88±0.06 |
| Range | | 0.001-0.033 | 0.67-2.73 | 0.13-0.88 | 0.06-1.44 | 0.09-0.32 | 0.41-2.31 | 0.48-0.91 |
| Average | | 0.009±0.008 | 1.42±0.67 | 0.48±0.22 | 0.25±0.36 | 0.19±0.08 | 1.36±0.64 | 0.77±0.14 |
| Roughages | | | | | | | | |
| Rice straw (*Oryza sativa* L.) | 25 | 0.239±0.059 | 0.20±0.10 | 0.18±0.04 | 0.67±0.14 | 0.28±0.08 | 0.79±0.10 | 0.20±0.04 |
| Corn husk (*Zea mays* L.) | 9 | 0.117±0.010 | 0.18±0.02 | 0.24±0.05 | 0.71±0.06 | 0.26±0.03 | 0.72±0.11 | 0.14±0.02 |
| Corn stover (*Zea mays* L.) | 2 | 0.101±0.001 | 0.23±0.00 | 0.20±0.00 | 0.30±0.00 | 0.25±0.00 | 0.79±0.01 | 0.15±0.00 |
| Corn leaf (*Zea mays* L.) | 2 | 0.113±0.002 | 0.16±0.00 | 0.18±0.00 | 0.34±0.01 | 0.20±0.02 | 0.82±0.02 | 0.11±0.00 |
| Carrot leaf (*Daucus carota* L.) | 2 | 0.182±0.022 | 0.14±0.01 | 0.35±0.04 | 0.14±0.00 | 0.28±0.034 | 0.88±0.05 | 0.22±0.00 |
| Vegetable waste | 6 | 0.202±0.027 | 0.21±0.02 | 0.44±0.09 | 0.25±0.05 | 0.24±0.02 | 0.79±0.08 | 0.24±0.01 |
| Corn straw (*Zea mays* L.) | 1 | 0.111 | 0.19 | 0.202 | 0.29 | 0.32 | 0.72 | 0.13 |
| Range | | 0.101-0.239 | 0.14-0.23 | 0.18-0.44 | 0.14-0.71 | 0.20-0.32 | 0.72-0.88 | 0.11-0.24 |
| Average | | 0.258±0.304 | 0.19±0.03 | 0.26±0.10 | 0.39±0.22 | 0.26±0.04 | 0.79±0.06 | 0.17±0.05 |
| Concentrates | | | | | | | | |
| Corn | 2 | 0.215±0.008 | 0.38±0.00 | 0.34±0.01 | 0.67±0.03 | 0.35±0.02 | 0.69±0.01 | 0.20±0.00 |
| Pollard | 1 | 0.244 | 0.57 | 0.11 | 0.22 | 0.73 | 0.97 | 0.24 |
| Soybean | 3 | 0.157±0.007 | 0.36±0.02 | 0.28±0.02 | 0.53±0.04 | 0.52±0.03 | 0.68±0.06 | 0.17±0.00 |
| Roasted soybean | 2 | 0.164±0.018 | 0.37±0.01 | 0.25±0.00 | 0.56±0.05 | 0.59±0.00 | 0.65±0.06 | 0.16±0.00 |
| Soybean meal | 2 | 0.156±0.015 | 0.26±0.01 | 0.18±0.01 | 0.24±0.01 | 0.32±0.00 | 0.84±0.00 | 0.24±0.01 |
| Onggok | 6 | 0.228±0.004 | 0.26±0.01 | 0.40±0.02 | 0.82±0.04 | 0.68±0.03 | 0.94±0.02 | 0.14±0.00 |
| Tofu waste | 20 | 0.248±0.059 | 1.35±0.18 | 0.33±0.04 | 0.42±0.06 | 0.76±0.06 | 1.29±0.22 | 0.18±0.02 |
| Palm kernel meal | 2 | 0.141±0.001 | 1.21±0.00 | 0.16±0.01 | 0.71±0.00 | 0.79±0.00 | 0.94±0.07 | 0.26±0.01 |
| Copra meal | 2 | 0.097±0.001 | 1.37±0.03 | 0.16±0.02 | 0.32±0.01 | 0.77±0.00 | 0.94±0.13 | 0.23±0.00 |
| Rice bran | 3 | 0.182±0.003 | 1.12±0.04 | 0.18±0.00 | 0.24±0.02 | 0.40±0.02 | 1.00±0.104 | 0.25±0.03 |
| Dried cassava | 2 | 0.116±0.000 | 0.35±0.037 | 0.19±0.01 | 0.37±0.01 | 0.38±0.01 | 0.79±0.013 | 0.16±0.01 |
| Brewery waste | 1 | 0.285 | 1.05 | 0.28 | 0.42 | 0.22 | 1.12 | 0.11 |
| Corn waste | 3 | 0.144±0.014 | 0.66±0.15 | 0.17±0.02 | 0.36±0.03 | 0.62±0.04 | 0.91±0.04 | 0.12±0.00 |
| Tempe waste | 2 | 0.294±0.000 | 0.26±0.01 | 0.46±0.02 | 0.27±0.00 | 0.344±0.01 | 0.71±0.02 | 0.24±0.01 |
| Cassava | 1 | 0.458 | 1.20 | 0.37 | 0.34 | 0.53 | 0.87 | 0.16 |
| Cassava waste | 10 | 0.247±0.045 | 1.31±0.06 | 0.34±0.04 | 0.42±0.11 | 0.76±0.02 | 1.27±0.16 | 0.19±0.03 |
| Range | | 0.097-0.458 | 0.26-1.37 | 0.11-0.46 | 0.22-0.82 | 0.22-0.79 | 0.65-1.29 | 0.11-0.26 |
| Average | | 0.211±0.089 | 0.75±0.45 | 0.26±0.10 | 0.43±0.18 | 0.55±0.19 | 0.91±0.19 | 0.19±0.05 |

Note: n: Total number of observations, Na: Sodium, K: Potassium, Cl: Chloride, S: Sulphur, P: Phosphor, Ca: Calcium, Mg: Magnesium, **: Non legumes

**Table 2.** Descriptive statistics of feedstuff DCAD status

| **Feedstuffs** | **n** | **DCAD status (mEq kg$^{-1}$ DM)** | | | | |
|---|---|---|---|---|---|---|
| | | **Na** | **K** | **Cl** | **S** | **DCAD** |
| Grasses | | | | | | |
| Elephant grass | 40 | 115.61±21.48 | 53.88±9.74 | 91.88±17.45 | 307.86±73.6 | **-230.26±86.79** |
| Natural grass | 25 | 69.60±19.12 | 67.81±12.37 | 105.49±23.07 | 376.69±110.27 | **-344.77±106.67** |
| Odot grass | 1 | 103.79 | 76.97 | 101.17 | 360.22 | **-280.64** |
| Range | | 69.60-115.61 | 53.88-76.97 | 91.88-105.49 | 307.86-376.69 | -344.77- (-230.26) |
| Average | | 96.33±23.89 | 66.22±11.63 | 99.51±6.95 | 348.26±35.94 | -285.22±57.39 |
| Legumes | | | | | | |
| *Pterocarpus indicus* Willd. | 3 | 2.16±0.25 | 413.98±235.69 | 70.05±13.15 | 75.19±6.05 | 270.90±240.36 |
| *Bauhinia ×blakeana* Dunn | 7 | 2.00±0.63 | 204.43±32.92 | 78.84±21.96 | 74.21±16.53 | 53.38±33.09 |
| *Calopogonium mucunoides* Desv. | 2 | 6.22±0.24 | 216.14±44.88 | 90.22±12.25 | 94.84±4.46 | 37.30±52.90 |
| *Gliricidia sepium* (Jacq.) Kunth | 28 | 4.18±2.55 | 380.75±88.36 | 146.24±58.96 | 91.10±40.24 | 147.59±91.01 |
| *Indigofera zollingeriana* Miq. | 2 | 0.97±0.12 | 257.67±27.24 | 155.48±15.74 | 78.17±15.41 | 24.99±27.69 |
| *Calliandra calothyrsus* Meisn. | 32 | 2.25±1.34 | 337.22±282.31 | 37.62±53.14 | 97.79±43.09 | 204.07±249.54 |
| *Leucaena leucocephala* (Lam.) de Wit | 30 | 3.24±2.25 | 321.70±152.81 | 189.09±58.05 | 125.43±33.54 | 10.42±153.38 |
| *Acacia mangium* Willd. | 1 | 14.17 | 407.06 | 140.44 | 122.33 | 158.47 |
| *Medicago sativa* L. | 1 | 0.64 | 468.87 | 108.83 | 62.70 | 297.97 |
| *Paraserianthes falcataria* (L.) I.C.Nielsen | 1 | 3.58 | 171.04 | 97.38 | 41.02 | 36.22 |
| *Sesbania grandiflora* (L.) Poir. | 2 | 2.04±0.74 | 266.54±66.72 | 159.58±136.89 | 120.67±11.33 | **-11.66±191.55** |
| *Peperomia pellucida* (L.) Kunth** | 2 | 5.42±4.81 | 698.20±345.11 | 234.60±52.78 | 128.72±42.34 | **340.31±339.47** |
| *Moringa oleifera* Lam.** | 3 | 6.84±1.17 | 670.60±321.99 | 247.57±217.82 | 897.76±226.66 | **-467.90±344.67** |
| Range | | 0.64-14.17 | 171.04-698.20 | 37.62-247.57 | 41.02-897.76 | -467.90-340.30 |
| Average | | 4.13±3.58 | 370.32±165.50 | 135.07±63.02 | 154.61±224.87 | 84.77±203.80 |
| Roughages | | | | | | |
| Rice straw (*Oryza sativa* L.) | 25 | 101.86±23.41 | 52.15±26.57 | 50.04±12.71 | 428.15±79.14 | **-324.17±77.73** |
| Corn husk (*Zea mays* L.) | 9 | 51.08±4.93 | 45.06±4.888 | 67.00±13.92 | 453.03±31.79 | **-423.89±41.55** |
| Corn stover (*Zea mays* L.) | 2 | 44.32±0.07 | 60.14±0.385 | 56.56±0.43 | 190.50±1.54 | **-142.60±1.52** |
| Corn leaf (*Zea mays* L.) | 2 | 49.22±0.97 | 40.78±0.619 | 50.8±0.00 | 210.96±6.40 | **-171.77±4.82** |
| Carrot leaf (*Daucus carota* L.) | 2 | 79.4±9.78 | 35.46±3.578 | 99.25±11.67 | 89.52±2.98 | **-73.91±4.67** |
| Vegetable waste | 6 | 89.38±12.6 | 53.44±6.454 | 126.76±29 | 158.16±37.80 | **-142.11±40.16** |
| Corn straw | 1 | 48.37 | 48.72 | 56.83 | 184.78 | **-144.52** |
| Range | | 44.32-101.86 | 35.46-60.14 | 50.04±126.76 | 89.52±453.03 | -423.89-(-73.91) |
| Average | | 66.23±23.44 | 47.96±8.29 | 72.46±29.34 | 245.01±139.22 | -203.28±123.75 |
| Concentrates | | | | | | |
| Corn | 2 | 93.57±3.50 | 98.19±0.76 | 96.23±1.88 | 417.05±18.75 | **-321.52±19.61** |
| Pollard | 1 | 106.30 | 145.54 | 31.10 | 139.03 | 81.72 |
| Soybean | 3 | 69.62±3.93 | 92.11±4.64 | 76.31±7.44 | 340.84±29.94 | **-255.44±20.09** |
| Roasted soybean | 2 | 71.69±8.24 | 93.91±1.76 | 70.08±1.16 | 346.83±33.18 | **-251.31±22.02** |
| Soybean meal | 2 | 68.15±6.94 | 65.70±1.30 | 51.74±1.98 | 146.78±4.36 | **-64.67±1.90** |
| Onggok | 6 | 99.16±1.74 | 65.86±2.91 | 114.41±6.78 | 508.68±24.84 | **-458.07±26.94** |
| Tofu waste | 20 | 107.31±26.35 | 346.95±48.18 | 92.97±10.75 | 264.01±37.03 | 97.27±58.71 |
| Palm kernel meal | 2 | 61.47±0.46 | 310.34±1.18 | 44.93±3.91 | 445.03±0.42 | **-118.16±2.77** |
| Copra meal | 2 | 42.52±0.78 | 351.79±7.56 | 44.32±4.47 | 197.67±4.26 | 152.32±8.12 |
| Rice bran | 3 | 79.57±1.32 | 285.88±9.73 | 50.35±0.19 | 150.15±10.28 | 164.95±11.86 |
| Dried cassava | 2 | 50.61±0.41 | 89.16±9.56 | 52.8±2.16 | 233.22±8.84 | **-146.25±2.48** |
| Brewery waste | 1 | 124.34± | 268.99 | 78.60 | 259.88 | 54.85 |
| Corn waste | 3 | 62.33±9.01 | 188.88±21.49 | 48.95±7.07 | 215.97±26.56 | **-13.71±11.01** |
| Tempe waste | 2 | 127.97±0.35 | 68.94±3.37 | 126.27±6.48 | 167.98±1.93 | **-97.33±7.57** |
| Cassava | 1 | 199.40 | 307.83 | 103.91 | 209.64 | 193.68 |
| Cassava waste | 10 | 106.63±20.82 | 335.83±15.11 | 97.18±11.49 | 253.03±67.78 | 92.25±56.47 |
| Range | | 42.52-199.39 | 65.70-351.78 | 31.09-126.26 | 139.03-508.68 | -458.07-193.68 |
| Average | | 91.91±38.46 | 194.74±115.59 | 73.76±28.74 | 268.49±112.78 | -55.59±192.69 |

Note: DCAD: Dietary Cation Anion Difference, n: Total number of observations, Na: Sodium, K: Potassium, Cl: Chloride, S: Sulphur, P: Phosphor, Ca: Calcium, Mg: Magnesium, **: Non legumes. Feedstuffs with negative DCAD values (value in bold) are more suitable for prepartum cows, while those with positive DCAD values are recommended for lactating cows to support calcium balance and productivity

Among the tested parameters, DCAD showed the highest correlation ($R^2 = 0.9586$), indicating strong agreement between NIRS prediction and wet chemistry. Conversely, sulfur content in concentrates had the lowest correlation ($R^2 = 0.624$), possibly due to its lower concentration or potential interference from the organic matrix in the NIR spectrum.

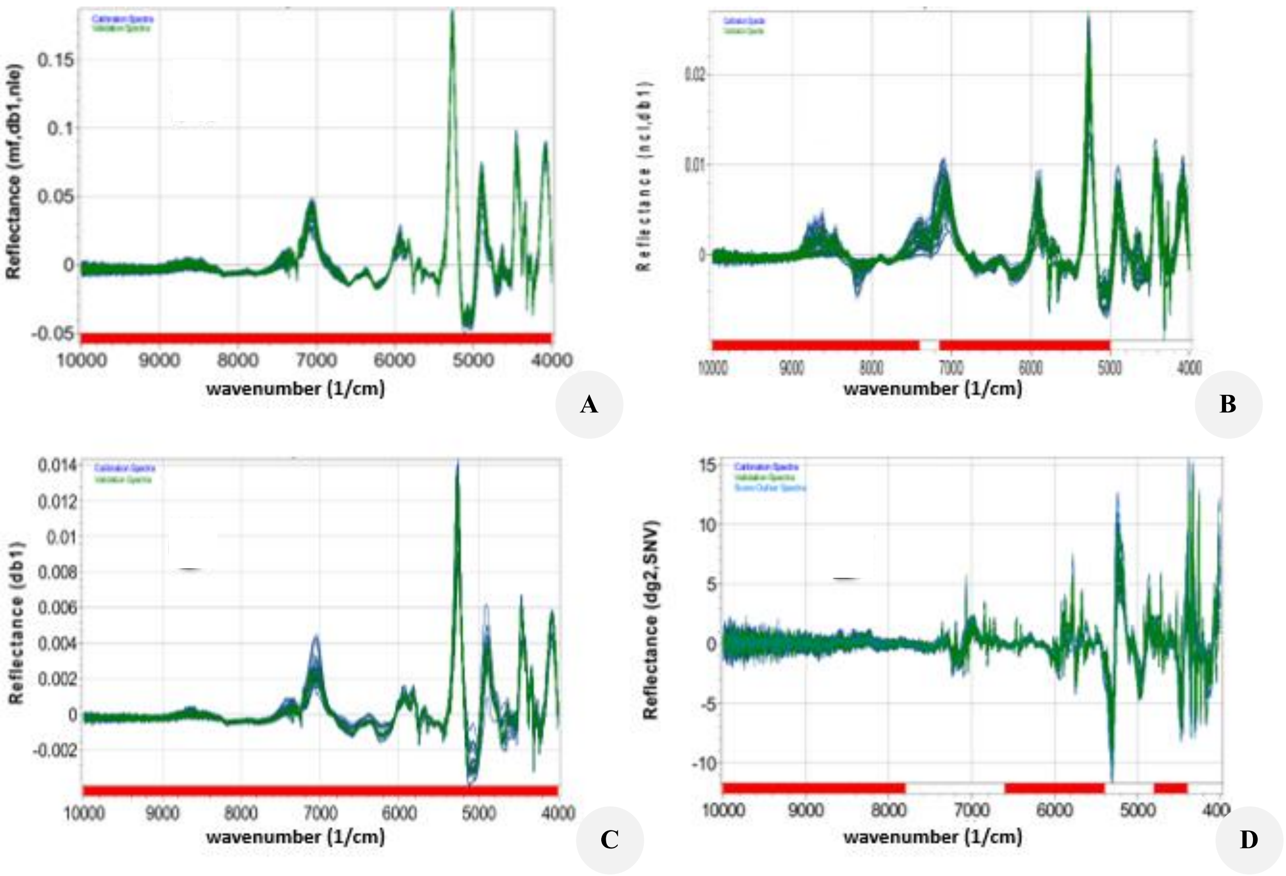

**Figure 1.** Spectra resulted from external validation (the relationship between wavenumber (cm$^{-1}$) and NIRS prediction) for reference: A. Ca of grasses, B. Mg of legumes, C. P of roughages, and D. K of concentrates. Each subplot includes three replicates, shaded areas represent spectral variation. Axis units for both wavenumber (x-axis, in cm$^{-1}$) and reflectance (y-axis, in log(1/R)) are shown

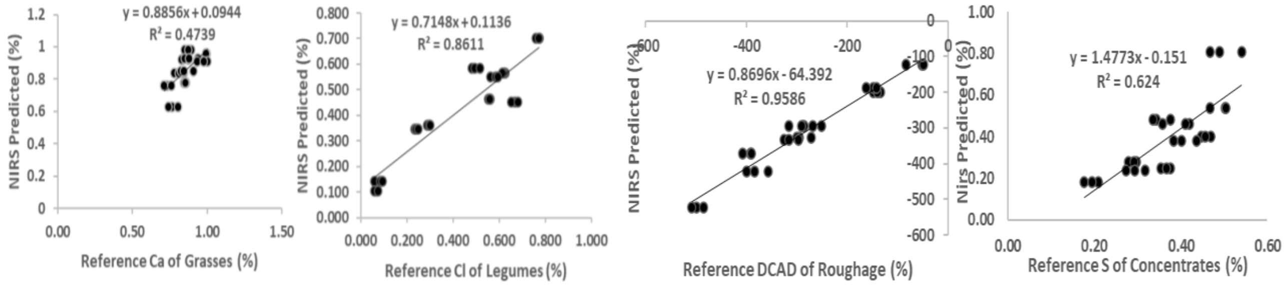

**Figure 2.** Scatter plots of NIRS Local Database (NIRSLD) predicted values versus Wet Chemical Analysis (WCA) reference values for external validation sets of some selected parameters of feedstuffs (grasses, legumes, roughage, and concentrates)

**Table 3.** Calibration statistics and validation of the single-species models for mineral content and DCAD status prediction

| Parameter | n | Mean | Range | | SD | SEC | $R^2$C | RPD |
|---|---|---|---|---|---|---|---|---|
| | | | Min | Max | | | | |
| Grasses calibration | | | | | | | | |
| Ca | 84 | 0.868 | 0.520 | 1.052 | 0.092 | 0.056 | 0.727 | 1.631 |
| Na | 84 | 0.249 | 0.108 | 0.417 | 0.072 | 0.023 | **0.906** | **3.100** |
| Mg | 76 | 0.188 | 0.130 | 0.259 | 0.026 | 0.013 | 0.810 | 2.068 |
| P | 76 | 0.398 | 0.175 | 0.677 | 0.094 | 0.050 | 0.781 | 1.889 |
| K | 68 | 0.224 | 0.151 | 0.322 | 0.039 | 0.029 | 0.648 | 1.356 |
| Cl | 66 | 0.334 | 0.241 | 0.462 | 0.038 | 0.015 | 0.865 | 2.535 |
| S | 68 | 0.527 | 0.357 | 0.785 | 0.123 | 0.030 | **0.943** | **4.063** |
| DCAD | 78 | -255.818 | -433.743 | -106.499 | 91.834 | 28.875 | **0.910** | **3.180** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Legumes calibration | | | | | | | | |
| Ca | 146 | 1.921 | 0.495 | 3.962 | 0.699 | 0.343 | 0.806 | 2.036 |
| Na | 136 | 0.008 | 0.001 | 0.021 | 0.005 | 0.003 | 0.757 | 1.763 |
| Mg | 114 | 0.769 | 0.508 | 1.184 | 0.153 | 0.073 | 0.813 | 2.088 |
| P | 134 | 0.140 | 0.017 | 0.271 | 0.068 | 0.021 | **0.913** | **3.248** |
| K | 100 | 1.316 | 0.357 | 2.543 | 0.532 | 0.319 | 0.736 | 1.668 |
| Cl | 116 | 0.531 | 0.053 | 1.089 | 0.252 | 0.095 | 0.875 | 2.644 |
| S | 150 | 0.231 | 0.054 | 1.581 | 0.295 | 0.070 | **0.947** | **4.214** |
| DCAD | 96 | 102.175 | -784.164 | 836.676 | 242.200 | 70.335 | **0.922** | **3.444** |
| Roughages calibration | | | | | | | | |
| Ca | 60 | 0.806 | 0.620 | 0.980 | 0.090 | 0.047 | 0.787 | 1.919 |
| Na | 60 | 0.190 | 0.078 | 0.311 | 0.068 | 0.021 | **0.909** | **3.151** |
| Mg | 64 | 0.186 | 0.101 | 0.302 | 0.046 | 0.016 | 0.891 | 2.861 |
| P | 60 | 0.270 | 0.176 | 0.535 | 0.076 | 0.038 | 0.800 | 2.003 |
| K | 68 | 0.203 | 0.080 | 0.463 | 0.066 | 0.029 | 0.834 | 2.239 |
| Cl | 78 | 0.228 | 0.141 | 0.542 | 0.095 | 0.032 | **0.900** | **2.992** |
| S | 66 | 0.578 | 0.140 | 0.830 | 0.201 | 0.055 | **0.924** | **3.631** |
| DCAD | 60 | -291.194 | -525.652 | -73.089 | 130.658 | 32.216 | **0.943** | **4.056** |
| Concentrates calibration | | | | | | | | |
| Ca | 122 | 0.954 | 0.644 | 1.658 | 0.240 | 0.082 | 0.895 | 2.925 |
| Na | 116 | 0.261 | 0.115 | 0.343 | 0.063 | 0.021 | **0.902** | **3.030** |
| Mg | 110 | 0.205 | 0.148 | 0.296 | 0.043 | 0.012 | **0.927** | **3.575** |
| P | 114 | 0.582 | 0.240 | 0.827 | 0.188 | 0.039 | **0.959** | **4.816** |
| K | 124 | 1.133 | 0.218 | 1.518 | 0.336 | 0.101 | **0.918** | **3.334** |
| Cl | 134 | 0.270 | 0.103 | 0.431 | 0.107 | 0.035 | **0.905** | **3.080** |
| S | 138 | 0.371 | 0.128 | 0.828 | 0.128 | 0.051 | 0.862 | 2.501 |
| DCAD | 150 | 106.487 | -318.829 | 360.371 | 163.399 | 68.138 | 0.852 | 2.398 |
| Grasses validation | | | | | | | | |
| Ca | 42 | 0.875 | 0.581 | 1.023 | 0.084 | 0.063 | 0.661 | 1.325 |
| Na | 42 | 0.249 | 0.108 | 0.406 | 0.072 | 0.025 | 0.895 | 2.928 |
| Mg | 38 | 0.188 | 0.133 | 0.253 | 0.026 | 0.013 | 0.804 | 1.983 |
| P | 38 | 0.398 | 0.192 | 0.657 | 0.087 | 0.052 | 0.768 | 1.657 |
| K | 34 | 0.224 | 0.155 | 0.307 | 0.038 | 0.030 | 0.638 | 1.265 |
| Cl | 33 | 0.336 | 0.257 | 0.455 | 0.035 | 0.017 | 0.832 | 2.083 |
| S | 34 | 0.522 | 0.367 | 0.788 | 0.122 | 0.040 | **0.902** | **3.066** |
| DCAD | 39 | -253.347 | -407.344 | -93.581 | 85.800 | 31.683 | 0.897 | 2.708 |
| Legumes validation | | | | | | | | |
| Ca | 73 | 1.919 | 0.608 | 3.949 | 0.693 | 0.357 | 0.791 | 1.940 |
| Na | 68 | 0.009 | 0.001 | 0.018 | 0.004 | 0.003 | 0.647 | 1.331 |
| Mg | 57 | 0.781 | 0.529 | 1.163 | 0.146 | 0.079 | 0.786 | 1.861 |
| P | 67 | 0.140 | 0.015 | 0.268 | 0.068 | 0.021 | **0.914** | **3.208** |
| K | 50 | 1.277 | 0.469 | 2.168 | 0.484 | 0.339 | 0.710 | 1.430 |
| Cl | 58 | 0.535 | 0.057 | 1.073 | 0.252 | 0.101 | 0.861 | 2.500 |
| S | 75 | 0.228 | 0.059 | 1.563 | 0.290 | 0.071 | **0.946** | **4.087** |
| DCAD | 48 | 95.169 | -779.704 | 836.702 | 242.411 | 77.481 | **0.907** | **3.129** |
| Roughages validation | | | | | | | | |
| Ca | 30 | 0.809 | 0.611 | 0.973 | 0.090 | 0.050 | 0.767 | 1.805 |
| Na | 30 | 0.188 | 0.083 | 0.287 | 0.069 | 0.024 | 0.888 | 2.866 |
| Mg | 32 | 0.188 | 0.116 | 0.283 | 0.041 | 0.019 | 0.862 | 2.180 |
| P | 30 | 0.276 | 0.187 | 0.536 | 0.074 | 0.036 | 0.831 | 2.069 |
| K | 34 | 0.202 | 0.098 | 0.448 | 0.062 | 0.031 | 0.821 | 2.013 |
| Cl | 39 | 0.228 | 0.142 | 0.540 | 0.095 | 0.031 | **0.904** | **3.041** |
| S | 33 | 0.578 | 0.140 | 0.830 | 0.203 | 0.067 | 0.895 | 3.045 |
| DCAD | 30 | -295.010 | -564.767 | -81.798 | 128.272 | 34.683 | **0.936** | **3.698** |
| Concentrates validation | | | | | | | | |
| Ca | 61 | 0.960 | 0.653 | 1.636 | 0.241 | 0.082 | 0.896 | 2.930 |
| Na | 58 | 0.261 | 0.110 | 0.343 | 0.064 | 0.025 | 0.861 | 2.570 |
| Mg | 54 | 0.204 | 0.151 | 0.294 | 0.042 | 0.012 | 0.923 | 3.450 |
| P | 57 | 0.583 | 0.287 | 0.865 | 0.185 | 0.039 | 0.970 | 4.734 |
| K | 62 | 1.138 | 0.240 | 1.460 | 0.324 | 0.108 | 0.906 | 2.986 |
| Cl | 67 | 0.271 | 0.105 | 0.428 | 0.105 | 0.036 | 0.902 | 2.957 |
| S | 69 | 0.373 | 0.133 | 0.774 | 0.126 | 0.054 | 0.847 | 2.332 |
| DCAD | 75 | 104.950 | -286.205 | 358.492 | 161.078 | 68.722 | 0.851 | 2.344 |

Note: n: Total number of observations, Na: Sodium, K: Potassium, Cl: Chloride, S: Sulphur, P: Phosphor, Ca: Calcium, Mg: Magnesium, DCAD: Dietary Cation Anion Difference, SD: Standard Deviation, SEC: Standard Error Calibration, $R^2C$: Coefficient of determination calibration, RPD: Residual Deviation Predictive, SEP: Standard Error Predicted, $R^2V$: Coefficient of determination validation. Values in bold indicate models with $R^2>0.90$ and/or RPD>2.0, representing high predictive reliability

**Table 4**. External validation for mineral content and DCAD status

| Parameter | WCA | | NIRSLD | | T-Test | SEL | SEP | SEP/SEL |
|---|---|---|---|---|---|---|---|---|
| | AVG | SEM | AVG | SEM | | | | |
| Grasses validation | | | | | | | | |
| Ca | 0.856 | 0.019 | 0.860 | 0.015 | 0.776 | 0.071 | 0.076 | 1.067 |
| Na | 0.211 | 0.010 | 0.242 | 0.010 | 0.000 | 0.077 | 0.039 | 0.503 |
| Mg | 0.177 | 0.004 | 0.182 | 0.004 | 0.238 | 0.017 | 0.019 | 1.128 |
| P | 0.355 | 0.012 | 0.369 | 0.017 | 0.389 | 0.017 | 0.087 | 5.065 |
| K | 0.220 | 0.007 | 0.241 | 0.011 | 0.097 | 0.015 | 0.067 | 4.571 |
| Cl | 0.344 | 0.004 | 0.355 | 0.007 | 0.033 | 0.030 | 0.028 | 0.917 |
| S | 0.581 | 0.029 | 0.582 | 0.018 | 0.952 | 0.308 | 0.115 | 0.375 |
| DCAD | -269.411 | 16.078 | -277.402 | 10.802 | 0.413 | 31.577 | 53.399 | 1.691 |
| Legumes validation | | | | | | | | |
| Ca | 1.838 | 0.079 | 1.746 | 0.075 | 0.228 | 0.436 | 0.417 | 0.957 |
| Na | 0.008 | 0.001 | 0.009 | 0.001 | 0.092 | 0.005 | 0.003 | 0.704 |
| Mg | 0.875 | 0.043 | 0.891 | 0.030 | 0.625 | 0.203 | 0.137 | 0.678 |
| P | 0.167 | 0.010 | 0.156 | 0.012 | 0.119 | 0.062 | 0.066 | 1.065 |
| K | 1.220 | 0.075 | 1.403 | 0.101 | 0.037 | 0.395 | 0.401 | 1.016 |
| Cl | 0.426 | 0.034 | 0.438 | 0.044 | 0.535 | 0.126 | 0.099 | 0.784 |
| S | 0.187 | 0.010 | 0.163 | 0.010 | 0.060 | 0.079 | 0.068 | 0.861 |
| DCAD | 43.497 | 34.031 | -92.538 | 55.745 | 0.024 | 263.155 | 291.226 | 1.107 |
| Roughages validation | | | | | | | | |
| Ca | 0.752 | 0.016 | 0.813 | 0.015 | 0.004 | 0.099 | 0.077 | 0.775 |
| Na | 0.167 | 0.015 | 0.181 | 0.010 | 0.076 | 0.036 | 0.041 | 1.136 |
| Mg | 0.160 | 0.008 | 0.180 | 0.007 | 0.001 | 0.040 | 0.030 | 0.742 |
| P | 0.277 | 0.011 | 0.312 | 0.021 | 0.069 | 0.283 | 0.097 | 0.344 |
| K | 0.198 | 0.009 | 0.195 | 0.010 | 0.769 | 0.013 | 0.048 | 3.676 |
| Cl | 0.215 | 0.016 | 0.210 | 0.009 | 0.642 | 0.015 | 0.055 | 3.658 |
| S | 0.469 | 0.038 | 0.503 | 0.033 | 0.037 | 0.121 | 0.098 | 0.809 |
| DCAD | -307.725 | 20.717 | -279.809 | 23.324 | 0.000 | 16.755 | 27.044 | 1.614 |
| Concentrates validation | | | | | | | | |
| Ca | 1.005 | 0.035 | 0.932 | 0.050 | 0.043 | 0.103 | 0.189 | 1.838 |
| Na | 0.244 | 0.006 | 0.248 | 0.006 | 0.277 | 0.024 | 0.021 | 0.869 |
| Mg | 0.190 | 0.008 | 0.187 | 0.007 | 0.713 | 0.019 | 0.037 | 1.917 |
| P | 0.546 | 0.048 | 0.535 | 0.033 | 0.766 | 0.028 | 0.213 | 7.496 |
| K | 0.871 | 0.085 | 0.868 | 0.069 | 0.892 | 0.116 | 0.112 | 0.969 |
| Cl | 0.247 | 0.014 | 0.263 | 0.015 | 0.019 | 0.027 | 0.043 | 1.580 |
| S | 0.402 | 0.033 | 0.374 | 0.017 | 0.212 | 0.036 | 0.120 | 3.351 |
| DCAD | 3.227 | 36.987 | 16.570 | 32.536 | 0.326 | 29.606 | 70.496 | 2.381 |

Note: Na: Sodium, K: Potassium, Cl: Chloride, S: Sulphur, P: Phosphor, Ca: Calcium, Mg: Magnesium, DCAD: Dietary Cation Anion Difference, WCA: Wet Chemical Analysis, NIRSLD: Near Infrared Reflection Spectroscopy Local Database, AVG: Average, SD: Standard Deviation, T-Test: Significance of t-test, SEL: Standard Error Laboratory, SEP: Standard Error Prediction

## Discussion

The variation in mineral content across feed ingredients was attributed to differences in species that were sampled from multiple regions of Indonesia. The feed samples were collected from lowland and highland dairy production areas in West Java and Sumatra, which represent distinct agro-ecological zones with varied soil types, such as volcanic Andisols and mineral-rich Inceptisols. These environmental differences likely contribute to the observed variation in mineral composition, reinforcing the importance of region-specific DCAD data for accurate ration formulation. This variation in mineral and DCAD profiles among Indonesian feedstuffs also reflects the country's diverse forage biodiversity, with unique legumes such as *Moringa oleifera* and *Gliricidia sepium* demonstrating distinctive mineral compositions shaped by regional agroecosystem and soil variability. These variations are particularly important because it may cause difference DCAD. For example, higher potassium is a major cation and sulfur is an anion contributing to the DCAD value. A higher variation in potassium levels tends to increase DCAD, potentially improving dry matter intake, whereas increased sulfur levels can lower DCAD, which may be beneficial in prepartum diets. According to Wanchuk et al. (2021), several factors influence mineral bioavailability, including their location within the plant, chemical form, and interactions with other minerals. Calcium (Ca) levels showed greater variability across feedstuffs compared to magnesium (Mg), which may be attributed to the differential distribution of Ca in plant parts, particularly its higher concentration in leaves and structural tissues. This observation aligns with previous reports and supports the importance of considering plant part composition when interpreting mineral variation. Additionally, mineral composition is affected by plant species, environmental conditions, topography, geography, and management practices (Reiné et al. 2020). While we did not explicitly separate samples by upland and lowland origin in the calibration dataset, the wide range of mineral values observed—such as the variation in calcium content among legumes (e.g., 0.41% in Paraserianthes falcataria to 2.31% in *Gliricidia sepium*)—likely reflects underlying differences in agro-ecological conditions, including soil type and elevation. This reinforces the importance of regionally diverse sampling for robust NIRS calibration. Rahman et al. (2022) reported that chloride (Cl) is abundant in legumes, while potassium (K), magnesium (Mg), and sulfur (S) are more prevalent in grasses. However, the findings of this study contradict those observations. As shown in Table 1, legumes exhibited not only the highest Cl content, but also the highest levels of K. In contrast, grasses showed relatively lower concentrations of these minerals but contained the highest sulfur (S) levels. These results suggest that, in tropical feedstuffs, legumes may serve as a richer source of K, Mg, and Cl than grasses. Legumes also showed substantially higher calcium (Ca) levels, ranging from 0.41% to 2.31% DM, compared to grasses, which ranged from 0.23% to 0.67% DM (Juknevičius and Sabienė 2007), but low in Na. Low Na content in legumes matters nutritionally because sodium is essential for ruminants' osmotic balance, nerve signaling, and rumen function. Low Na levels can limit the cation contribution to DCAD, potentially leading to lower DCAD values when legumes are used as the primary forage. This may reduce dry matter intake and buffering capacity in lactating dairy cows, especially when not balanced with other high-Na ingredients.

Calcium is essential for bone and tooth formation, enzymatic activity, and metabolic processes critical for milk production (McDonald et al. 2010). DCAD manipulation plays a crucial role in supporting calcium homeostasis, particularly in transition cows, which refers to cows in the period about three weeks before until three weeks after calving. By influencing systemic acid-base balance, adjusting DCAD can enhance calcium mobilization from bone and absorption from the gut, thereby helping to prevent hypocalcemia around calving. This connection reinforces the practical relevance of accurately assessing feed DCAD values in ration formulation. Sodium (Na), a cation, can lead to an alkaline metabolic state if consumed excessively, increasing the risk of milk fever during the transition phase (Kavitha et al. 2014). This implication is particularly relevant to our findings, as roughages such as rice straw and corn stover exhibited relatively high sodium (Na) levels (up to 0.239% DM), which contribute positively to DCAD values. These feeds can be strategically included in lactation diets to increase cationic load, thereby supporting calcium balance and milk production during early lactation. In addition, magnesium serves as a cofactor in enzymatic pathways essential to livestock metabolism (Goff 2018) and, along with phosphorus (P), enhances the sensitivity of Parathyroid Hormone (PTH) in calcium regulation. Meanwhile, chloride (Cl), together with Na and potassium (K), helps maintain acid-base balance and osmotic pressure, where Cl deficiency can result in alkalosis and impaired growth. Sulfur (S), as a strong anion, also supports acid-base balance and blood oxidation, helping to reduce the incidence of milk fever (McDonald et al. 2010).

Indonesian dairy cattle feeds are typically dominated by negative DCAD feedstuffs, particularly grasses, roughages, and some concentrates. To date, few studies have systematically mapped the mineral content and DCAD status of feedstuffs across Indonesia's diverse agro-ecological zones (Hidayat et al. 2011; Despal et al. 2024). By incorporating a broad range of locally available feed materials, our study provides a national-level reference that supports more precise and regionally relevant ration formulation. The inclusion of a wide variety of local forages and legumes in dairy cattle diets not only enhances mineral balance and DCAD precision but also reduces reliance on monoculture systems, thereby contributing to ecological resilience and the conservation of agrobiodiversity in tropical farming systems.

In contrast, Al-Rabadi and Al-Hijazeen (2018) found that none of the forage feed ingredients in their study had negative DCAD values. Their research was conducted in temperate regions with cool-season grasses, whereas our study focused on Indonesia's tropical environment, which is characterized by higher temperatures, elevated humidity, volcanic soils (e.g., Andisols), and intensive weathering. These climatic and soil differences likely explain the distinct mineral profiles and DCAD values observed in our feed samples. This contrast underscores the importance of region-specific data, as DCAD status is influenced by plant species, environmental conditions, and geographical factors (Reiné et al. 2020).

The predominance of low DCAD feedstuffs in tropical Indonesia is especially relevant for transition cow feeding programs, as it is well established that cows in the transition period (three weeks before to three weeks after parturition) are highly vulnerable to metabolic disorders (Redfern et al. 2021). During this period, calcium demand increases sharply, and feeding a low DCAD diet can promote compensated metabolic acidosis, thereby enhancing calcium mobilization and improving postpartum calcium status (Hassanien et al. 2022).

Dietary composition also shifts during the transition phase, from high forage to high concentrate diets (Ramos et al. 2021). Suitable forages for the prepartum phase include grasses and roughages, while concentrate selection during lactation must be done carefully, as some may have negative DCAD values. Incorporating legumes in the lactation ration can increase DCAD levels and support milk production. A balanced combination of forages and concentrates typically produces a positive DCAD value (Motsinger and Hadfield 2022). This study provides baseline data that can help nutritionists fine-tune the DCAD balance in Indonesian dairy rations, ensuring that mineral composition is aligned with the physiological needs of cows at different production stages. Maintaining an appropriate DCAD balance throughout the production cycle is essential. Cationic feeds are most effective during peak lactation, with a gradual reduction in DCAD toward the end of the cycle (Kłos et al. 2017). Since tropical legumes may not always meet DCAD requirements, additional feed ingredients may be necessary to prevent overly alkaline conditions.

DCAD status varies with mineral composition, as feed minerals are often embedded within plant fiber or protein matrices, which can affect their bioavailability to ruminants. Understanding this interaction is crucial for improving mineral uptake and enhancing the accuracy of feed quality predictions using NIRS. Digestion rates and binding properties influence mineral availability, contributing to variation in absorption. Therefore, inorganic minerals are often added to rations to ensure adequate supply (Grešáková et al. 2021). This mechanistic understanding also has implications for mineral bioavailability and NIRS prediction accuracy, as mineral interactions with the organic matrix (e.g., fiber or protein binding) can influence both absorption in the animal and spectral detectability in the NIR region. Regularly monitoring mineral content and DCAD values in feedstuffs is essential to meet nutritional standards. While no universally optimal DCAD value has been confirmed, excessively negative DCAD can induce metabolic acidosis, whereas overly alkaline DCAD may cause metabolic alkalosis (Lopera et al. 2018). According to NRC (2001), optimal dietary concentrations for lactating dairy cows are approximately 0.6% for K, 0.2-0.25% for Na, and 0.15-0.4% for S (DM basis), which provides a useful benchmark for interpreting the DCAD contributions and nutritional adequacy of the feedstuffs evaluated in this study.

Most calibrations (80.64% of total) made in this study fell within Category A ($R^2>0.80$ and RPD>2.0) indicating generally accurate results, though improvements are still needed for specific parameters Category B ($0.50<R^2<0.80$ and 1.4<RPD<2.0). Some researchers recommend using the Coefficient of Variation (CV) or RPD over $R^2$ alone for evaluating NIRS calibration quality, as RPD provides insight into the predictive ability of the model relative to the variability in the data, complementing the goodness-of-fit measure given by $R^2$ (Saha et al. 2017). This study utilized NIRS to predict nutrient composition, and high $R^2$ values coupled with low standard errors demonstrated strong agreement between laboratory and predicted values. When the Standard Error of Prediction (SEP) was lower than or comparable to the Standard Error of Calibration (SEC), model accuracy was affirmed. Tomar et al. (2021) advised that SEP should not exceed 1.3 times the SEC, a condition satisfied by all parameters in this study reflecting the robustness of the model in this study and its high prediction accuracy. While 60% of the models met Category A criteria, a few parameters—such as calcium and potassium in legumes—did not meet this threshold, likely due to limited calibration range or complex interactions with the organic matrix that affect NIR signal detection.

Inconsistent mineral calibration is a known challenge in NIRS. Buonaiuto et al. (2021) noted that NIRS interacts poorly with inorganic minerals, limiting prediction accuracy. However, this limitation was largely overcome in our study, as strong calibration performance was achieved for most parameters—reflected by high $R^2$ and RPD values in over 60% of the models, particularly for DCAD and key macro-minerals. Some minerals become detectable when bound to organic molecules (e.g., acids, chelates) or when forming salts that influence hydrogen bonding in moist samples (Giaretta et al. 2019). Although minerals lack direct NIR absorption bands, elements like Na can be predicted through their influence on water absorption bands (Manuelian et al. 2017). Predictive power varies by mineral type: macro-minerals such as Na, K, and Cl—typically more uniformly distributed and associated with organic matrices—tend to yield stronger models than minerals like Ca, which may occur in more variable, inorganic forms. Phosphorus (P), largely present in phytates and phospholipids in legumes and concentrates, exhibited high predictability due to its consistent chemical associations and uniform distribution, enhancing spectral detectability (Saha et al. 2017).

The significant differences ($p<0.05$) observed in the T-test likely reflect the wide variability in the calibration and validation sample sets. However, outliers may also have contributed to these differences; further assessment of their presence and influence on external validation results would help clarify this effect. These statistical tools were essential for assessing calibration model reliability (Saha et al. 2017). Low SEP/SEL ratios confirmed the accuracy of the predictive models, although higher SEP values in some cases indicated that specific validation samples were outside the calibration range (Despal et al. 2020). A lower SEP/SEL ratio corresponds to a more accurate prediction model (Agustiyani et al. 2021).

The spectral curves of various grasses, legumes, roughages, and concentrates show consistent patterns with distinct variations across feed types (Figure 1). Each feed exhibited a unique spectral profile, contributing to the effectiveness of NIRS calibration for predicting chemical composition in a specific environment. These patterns result from infrared radiation interacting with feed samples during scanning (Parastiwi et al. 2023). The wavelength range used in this study (10,000-4,000 $\text{cm}^{-1}$) falls within the optimal infrared spectrum (12,800-4,000 $\text{cm}^{-1}$ or 780-2,500 nm) for accurate chemical estimation (Schwanninger et al. 2011).

The ideal relationship between NIRSLD predictions and WCA values in the validation set is represented by a diagonal 1:1 line on the plots (Figure 2). The closer the data points are to this line, the stronger the correlation between predicted and measured values (Saha et al. 2017). The accuracy of NIRS models can be improved by increasing the number and variability of samples in the calibration dataset (Buonaiuto et al. 2021). Moreover, differences in chemical and physical properties among plant parts and species lead to spectral variations (Beć et al. 2020), highlighting the importance of consistent sampling practices. According to Schwanninger et al. (2011), the powdered sample reflectance, absorption, and scattering behavior are influenced by particle size, porosity, surface characteristics, refractive index, and packing density.

In conclusion, this study is the first to develop a NIRS-based DCAD prediction model for Indonesian feedstuffs. It provides a rapid, region-specific tool for evaluating mineral profiles and DCAD values in local feeds. The models demonstrated strong calibration performance, with over 60% classified as Category A, and high predictability for key nutrients such as phosphorus and sodium—particularly in legumes and concentrates. Most grasses, roughages, and concentrates exhibited negative DCAD values, while legumes were generally more cationic, reflecting significant variation across feed types.

NIRS calibration showed excellent accuracy ($R^2>0.8$, RPD>2.0) for several minerals and DCAD, confirming its utility for rapid feed evaluation. DCAD showed the highest correlation ($R^2 = 0.9586$), while sulfur in concentrates had the lowest, indicating areas for model improvement. These findings support the integration of NIRS and conventional methods to enable evidence-based, precision ration formulation, enhancing feed efficiency, metabolic health, and productivity in tropical dairy systems.

The development of localized NIRS models and a comprehensive feed database provides practical value for formulation companies, extension services, and cooperatives. These findings underscore the importance of promoting biodiversity-based feed strategies that utilize the mineral and DCAD variability among tropical forages. Encouraging the use of diverse, locally adapted feed resources can reduce dependence on monocultures, enhance ecological resilience, and support sustainable dairy production policies in tropical regions such as Indonesia. As a next step, on-farm validation and the development of user-friendly digital tools are recommended to facilitate adoption and support sustainable dairy nutrition in Indonesia.

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