



# Monitoring of Calcium and Phosphorus in Ruminant Feed by Smartphone-Assisted Colorimetry

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## ABSTRACT

Routine laboratory analysis for ruminant feed minerals is often unavailable in a field setting; therefore, rapid mineral monitoring is necessary. The present study aimed to evaluate a smartphone-assisted colorimetry method for estimating calcium and phosphorus in forage extracts and compare its performance with UV-Visible spectrophotometry. Standard solutions ranging from 1 to 5 ppm were prepared for smartphone-assisted colorimetry and UV-Visible spectrophotometry, and reagent-treated extracts from four forage species, including *Pennisetum purpureum* cv. Mott, *Pennisetum purpupoides*, *Leucaena leucocephala*, and *Sesbania grandiflora* were measured by a Genesys 10S spectrophotometer and by smartphone imaging under controlled lighting. Linear regression was used to develop calibration models, and method agreement was assessed using Pearson's *r* and mean absolute percentage error. Both methods indicated strong linearity for calcium and phosphorus. For the smartphone method, the coefficient of determination ranged from 0.9448 to 0.9974, compared to 0.9514 to 0.9610 for the UV-Vis method, with absolute differences between the two methods remaining below 0.5%. Smartphone-assisted colorimetry and UV-Visible spectrophotometry produced closely comparable estimates for each tested forage sample for the tested forages. Smartphone-assisted colorimetry provided accurate estimates of calcium and phosphorus in forage extracts, comparable to those produced by spectrophotometry, and demonstrated potential as a low-cost tool for routine screening of ruminant feed minerals.

**Keywords:** Calcium, Colorimetry, Forage, Nutrition, Phosphorus, Ruminant feed, Smartphone

## INTRODUCTION

Ruminant production systems, which depend on nutritionally balanced diets, contribute substantially to food security and rural livelihoods. Among dietary components, minerals are essential for skeletal development, enzyme-mediated reactions, osmotic balance, and reproductive performance. The mineral composition of forages and other feed ingredients can differ with botanical source, soil conditions, fertilizer input, processing, and season, which complicates accurate ration formulation (Hawu et al., 2022; Fanelli et al., 2023; Pérez-Reverón et al., 2024). Without appropriate monitoring of mineral supply, deficiencies or excesses can occur, potentially reducing animal performance and increasing nutrient losses (Wasson et al., 2022; Šaljić et al., 2024).

While conventional analytical techniques such as atomic absorption spectrometry and inductively coupled plasma methods remain reliable, their high cost, dependence on laboratory facilities, and limited accessibility make these techniques impractical for routine farm-level screening (Jamarun et al., 2020). Additionally, wet-chemical methods are beneficial, although they require careful handling and can be time-consuming when processing a high volume of samples. To address these limitations, digital image analysis has been explored as an alternative analytical approach in agricultural samples. Smartphone-assisted colorimetry relies on a mineral-specific reaction to generate a visible color response that can be quantified from red-green-blue image analysis under controlled illumination. Within an appropriate linear range, this optical response can be related to analyte concentration, following a principle comparable to the Beer-Lambert law used in spectrophotometric analysis (Yanti et al., 2021; Ünlü et al., 2022). Smartphone-based assays are well-suited for field use because a portable device can handle image capture, data storage, and numerical processing at relatively low cost. Recent studies indicated that calibrated digital imaging can support quantitative evaluation of agricultural and feed samples when acquisition conditions are carefully managed (Ünlü et al., 2022; Batista and Campos, 2024). The portable nature of these assays may shorten the time required for mineral screening in ruminant feeding systems and facilitate faster supplementation decisions in regions with limited access to centralized laboratories (Sapkota et al., 2019; Poulsen et al., 2021; Ponnampalam et al., 2024).

Many available applications focus on single analytes, simplified matrices, or tightly controlled laboratory

conditions, whereas practical feed samples are chemically diverse and can react differently during extraction and color development (Khan *et al.*, 2020; Lucio *et al.*, 2020; Uushona *et al.*, 2021). Differences in device response, calibration strategy, and ambient light can influence comparability among studies. Therefore, direct validation against a reference method remains essential before smartphone imaging can be recommended for routine mineral screening. The present study aimed to compare smartphone-assisted colorimetry with UV-Visible spectrophotometry for determining calcium and phosphorus in forage extracts and to evaluate the reliability of smartphone image-based regression models for routine feed screening.

## MATERIALS AND METHODS

### Ethical approval

The present study was conducted in accordance with the guidelines of Mataram and Mandalika University of Education, Mataram, Indonesia.

### Study design

The present study was a comparative analytical validation study. The target analytes were calcium and phosphorus in forage extracts, and the performance of smartphone-assisted colorimetry was compared directly with UV-Visible spectrophotometry as the reference method under the same experimental conditions (Yanti *et al.*, 2021; Batista and Campos, 2024). The evaluation of calcium and phosphorus in forage extracts focused on calibration linearity, correlation, and agreement between methods for standard solutions and forage samples.

### Materials

The laboratory equipment included a UV-Visible spectrophotometer (Genesys 10S, Thermo Scientific, USA), a smartphone equipped with a digital camera, beakers, graduated cylinders, funnels, filter papers, sieves, micropipettes, volumetric flasks, and an analytical balance. During image acquisition, the smartphone was fixed on a stand, and measurements were conducted under controlled illumination to minimize variation among images (Uushona *et al.*, 2021; Yanti *et al.*, 2021).

### Reagents and standards

Analytical-grade reagents included distilled water, nitric acid, ammonium molybdate, tin (II) chloride, Eriochrome black T, and ammonium chloride–ammonium hydroxide buffer (pH 10.0). Stock standards of calcium and phosphorus (each at 100 ppm) were prepared and diluted to 1-5 ppm to establish calibration curves within the tested calibration range (Pérez-Reverón *et al.*, 2024; Lambert, 2025).

### Sample preparation

The experiment was conducted at the Animal Science Laboratory of the University of Mataram and the Chemistry Laboratory of Mandalika University of Education, Indonesia. Four forage materials used in the comparison were *Pennisetum purpureum cv. Mott*, *Pennisetum purpureoides*, *Leucaena leucocephala*, and *Sesbania grandiflora* that were obtained from a local forage shop. The samples were homogenized, finely ground, and sieved before analysis. Representative subsamples were weighed, digested with 5% nitric acid to release soluble minerals, and filtered through Whatman filter paper to obtain purified extracts for colorimetric measurement.

### Solution preparation

A 5% nitric acid solution was prepared by dilution with distilled water. Calcium and phosphorus stock solutions (100 ppm) were serially diluted to 1-5 ppm, and fresh color-developing reagents were prepared on the day of analysis. Calibration curves were constructed using standard solutions, whereas method performance under sample conditions was assessed using the forage extracts.

### Measurement procedures

Absorbance of the reacted solutions was measured at the appropriate wavelength for each mineral using the Genesys 10S. In parallel, images of the same reacted solutions were captured under fixed distance, focus, ISO, and lighting conditions. Phosphorus was determined using the ammonium molybdate-tin (II) chloride reaction, which produced a blue complex, whereas calcium was determined using Eriochrome Black T. After color stabilization, Red Green Blue (RGB) values were extracted from the images and used as the digital response variable (Grünberg *et al.*, 2019; Uushona *et al.*, 2021).

### Quality control and validation

All measurements were performed in triplicate, and analytical blanks were included to assess contamination and baseline shift. Calibration was repeated across analytical sessions, and color reference charts were used during smartphone imaging to reduce the effect of lighting and sensor variation. Validation of the smartphone-assisted procedure was based on direct comparison with UV-Visible spectrophotometry under the same experimental conditions (Yanti et al., 2021; Batista and Campos, 2024). Accordingly, the smartphone-assisted colorimetric method was evaluated as a practical screening approach for calcium and phosphorus in forage extracts under established laboratory conditions.

### Calibration and data analysis

Calibration curves were constructed by plotting concentration (ppm) against absorbance (UV-Visible) and against the RGB-derived response (smartphone). Linear regression equations were generated for calcium and phosphorus, where X represents the instrumental response and Y the estimated concentration (ppm). The regression equations followed the general formula  $Y = mX + c$ , where Y was mineral concentration (ppm), X was the measured optical response, m was the slope, and c was the intercept. Model performance was evaluated using the coefficient of determination ( $R^2$ ), Pearson correlation coefficient (r), and mean absolute percentage error (MAPE), using the following Formula. Data processing and graph preparation were performed in Microsoft Excel (Ciaccheri et al., 2023).

$$\text{MAPE} = (1/n) \times \sum |(\text{observed}_i - \text{predicted}_i)/\text{observed}_i| \times 100$$

where n is the number of paired observations included in the calculation,  $\text{observed}_i$  is the reference concentration measured by UV-Visible spectrophotometry, and  $\text{predicted}_i$  is the concentration estimated using the smartphone-assisted colorimetric method.

## RESULTS

### Calibration models for calcium and phosphorus

Calibration models demonstrated a linear relationship between instrumental response and concentration for both analytes. For calcium, the UV-Visible equation was  $Y = 228.41X - 0.0174$  with  $R^2 = 0.961$  and  $r = 0.980$ , whereas the smartphone equation was  $Y = 212.28X - 0.0615$  with  $R^2 = 0.9448$  and  $r = 0.972$ . For phosphorus, the UV-Visible equation was  $Y = 524.34X - 0.0442$  with  $R^2 = 0.9514$  and  $r = 0.975$ , while the smartphone equation was  $Y = 145.94x - 0.0986$  with  $R^2 = 0.9974$  and  $r = 0.999$ . In these calibration equations, Y represents the estimated concentration of calcium or phosphorus expressed in ppm, whereas X represents the instrumental response, namely absorbance for UV-Visible spectrophotometry and the RGB-derived response for smartphone-assisted colorimetry. The  $R^2$  denotes the coefficient of determination, which indicates the proportion of concentration variation explained by the linear regression model, while r denotes the Pearson correlation coefficient, which reflects the strength of the linear relationship between instrumental response and concentration. The present findings indicated strong linearity for both methods within the tested concentration range.

### Goodness-of-fit summary

Across both analytes, the UV-Visible calibration models yielded mean  $R^2$  and r values of 0.9562 and 0.9775, respectively, indicating that the fitted models accounted for most of the variation in the calibration data.

### Method agreement

Agreement between the two methods was close for both analytes. Mean absolute differences were 0.012% for calcium and 0.007% for phosphorus, indicating minimal deviation between smartphone-based estimates and UV-Visible measurements.

### Sample-level results

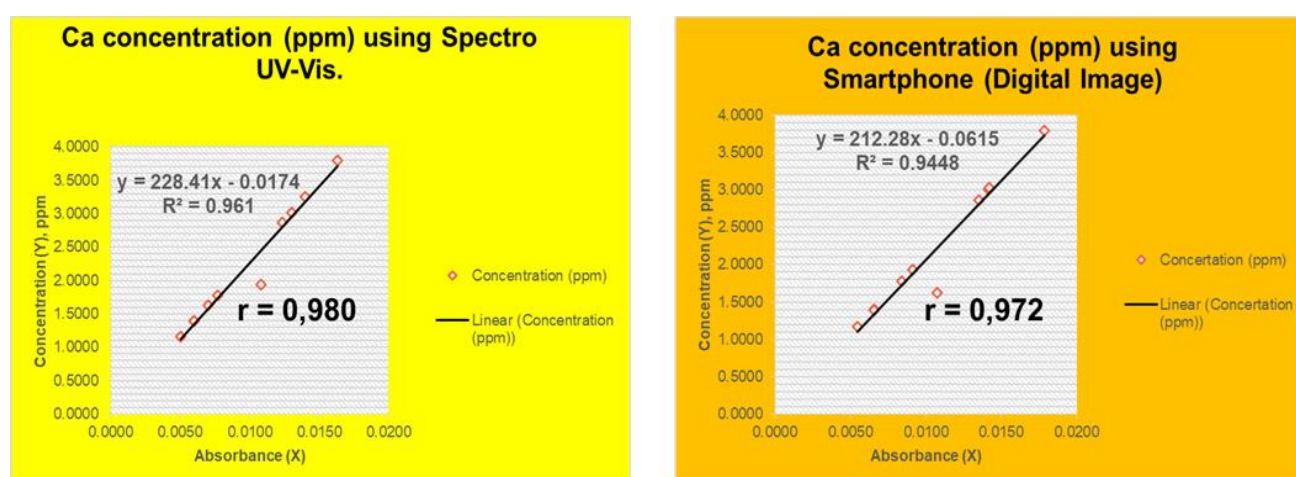
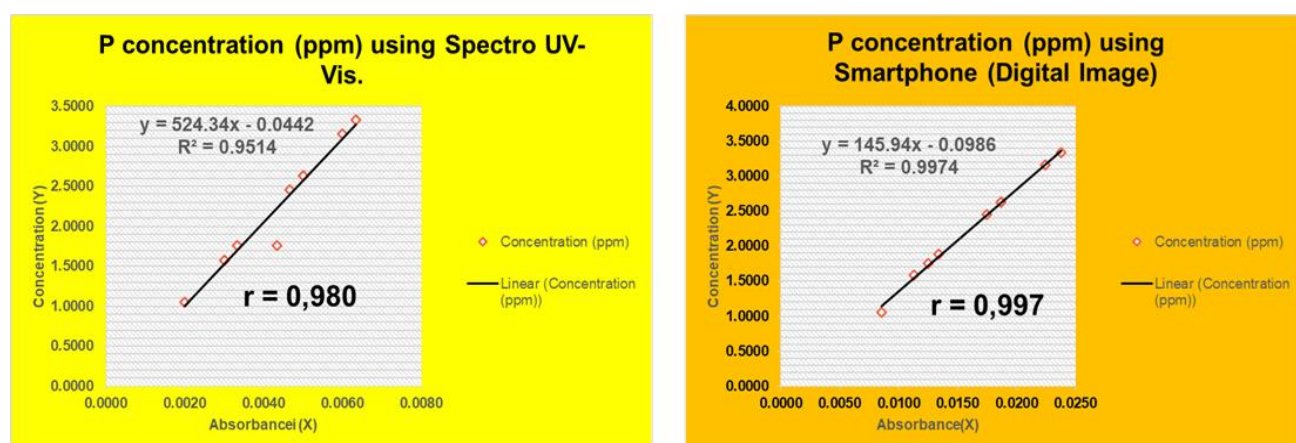
At the sample level, smartphone-derived concentrations were close to UV-Visible values for all tested forages. For calcium in *Pennisetum purpuroides*, the smartphone and UV-Visible methods yielded concentrations of 3.7936 and 3.7984 ppm, respectively. In *Leucaena leucocephala*, the corresponding values were 3.0238 and 3.0233 ppm for smartphone and UV-Visible methods, respectively. For phosphorus, the smartphone and UV-Visible methods yielded values of 3.1585 and 3.1579 ppm in *Pennisetum purpureum* cv. Mott and 3.3450 and 3.3333 ppm for *Sesbania grandiflora*, respectively. Table 1 presents the regression equations and goodness-of-fit statistics for calcium and phosphorus. Figures 1 and 2 illustrate the linear calibration relationships obtained for the two analytes by the present methods.

**Table 1.** Regression models for calcium and phosphorus determined by UV-Visible spectrophotometry and smartphone imaging using estimated calcium concentration (Y) from the instrumental response (X)

No	Mineral Elements	UV-Visible spectrophotometry	Digital image (Smartphone)
1	Calcium	$Y = 228.41X - 0.0174$ ; $R^2 = 0.961$ ; $r = 0.980$	$Y = 212.28X - 0.0615$ ; $R^2 = 0.9448$ ; $r = 0.972$
2	Phosphorus	$Y = 524.34X - 0.0442$ ; $R^2 = 0.9514$ ; $r = 0.975$	$Y = 145.94 - 0.0986$ ; $R^2 = 0.9974$ ; $r = 0.999$

Y: The estimated concentration of calcium or phosphorus expressed in ppm, X: The instrumental response,  $R^2$ : The coefficient of determination, r: The Pearson correlation coefficient

$R^2$  measures the proportion of variance in the dependent variable (Y) that was explained by the independent variable(s) (X). The  $R^2$  Value ranges from 0 to 1 (or 0% to 100%). r measures the strength and direction of the linear relationship between two variables. In the regression equations, X represents the instrumental response, namely absorbance for UV-Visible spectrophotometry or the RGB-derived response for smartphone imaging, while Y represents the estimated mineral concentration expressed in ppm.  $R^2$  represents the coefficient of determination, with values closer to 1 indicating better model fit, whereas r represents the Pearson correlation coefficient, with values closer to +1 indicating a stronger positive linear relationship between X and Y.

**Figure 1.** Regression equation used to estimate calcium concentration (Y, ppm) from the instrumental response (X) in ruminant feed extracts**Figure 2.** Regression equation used to estimate phosphorus concentration (Y, ppm) from the instrumental response (X) in ruminant feed extracts

## DISCUSSION

### Model performance and predictive strength

The calibration results indicated that smartphone-assisted colorimetry can generate quantitative responses that closely track those obtained by UV-Visible spectrophotometry. The high  $R^2$  and r values observed for both analytes were consistent with the findings of Yanti et al. (2021) and Batista and Campos (2024), who reported that controlled digital imaging can support analytical measurements in agricultural matrices. The present findings suggested that under

established conditions, smartphone imaging was sufficiently sensitive for screening calcium and phosphorus in forage extracts.

### **Interpretation of correlations and calibration**

The present study found that smartphone-derived RGB responses were strongly associated with calcium and phosphorus concentrations in forage extracts, indicating that smartphone-assisted colorimetry could provide calibration performance comparable to UV-Visible spectrophotometry under controlled laboratory conditions. This proportional relationship was consistent with the Beer-Lambert principle, which established a linear relationship between optical response and analyte concentration over an appropriate concentration range (Madzingira et al., 2021).

### **Agreement with feed-matrix evaluation**

The close agreement between methods across four forage matrices indicated that the smartphone approach was not limited to a single plant source. These findings were important because forage composition can differ widely among species and processing conditions, potentially affecting extraction behavior and color development (Hawu et al., 2022; Fanelli et al., 2023; Fuentes et al., 2025). Thus, the present results supported the use of matrix-specific calibration as a practical strategy for routine feed evaluation.

### **Analytical principles**

Both UV-Visible spectrophotometry and smartphone-assisted colorimetry depended on the conversion of chemical reactions into measurable optical signals. While UV-Visible spectrophotometry measured absorbance directly, the smartphone method captured color intensity that was transformed into a comparable response variable under standardized image conditions. Similar photometric approaches have been applied in recent digital image-based analyses for agricultural samples (Uushona et al., 2021; Batista and Campos, 2024).

### **Reaction schemes**

The color reactions used in the present study supported the observed linear response. Phosphorus was determined by forming a molybdenum blue complex after reaction with ammonium molybdate and tin (II) chloride, whereas calcium was detected after color development with Eriochrome Black T. The distinct, concentration-dependent color responses produced by both reactions made these reactions suitable for detection by spectrophotometry and smartphone imaging. This finding was consistent with previous studies demonstrating that the molybdenum blue reaction, whose signal intensity is proportional to orthophosphate concentration, served as a reliable basis for phosphate determination and can be successfully adapted to smartphone-based colorimetric platforms. Consistent with previous studies, Eriochrome Black T is an established metallochromic reagent for alkaline-earth ions, such as calcium, and its color response has been reliably quantified in both spectrophotometric and smartphone-based digital-image methods. Therefore, the present results aligned with previous evidence demonstrating that visually measurable chromogenic reactions can produce robust analytical signals in both conventional optical instruments and image-based detection systems (Fiske et al., 1925; Fan et al., 2021; Graña-Dosantos et al., 2026).

### **Limitations and future prospects**

From a practical perspective, the smartphone-assisted colorimetric method can serve as a rapid, portable alternative for preliminary mineral screening in settings where conventional instrumentation is unavailable. In the context of feed management, such accessibility is important because rapid access to calcium and phosphorus levels allows producers to adjust rations and optimize mineral supplementation in ruminant systems (Poulsen et al., 2021; Ponnampalam et al., 2024; Šaljić et al., 2024). Further validation across additional feed ingredients and wider field conditions is needed before routine large-scale application. From a practical perspective, the smartphone-assisted colorimetric method can serve as a rapid, portable alternative for preliminary mineral screening in settings where conventional instrumentation is unavailable. In the context of feed management, such accessibility is important because rapid access to calcium and phosphorus concentrations allows producers to adjust rations and optimize mineral supplementation in ruminant systems (Poulsen et al., 2021; Šaljić et al., 2024; Ponnampalam et al., 2024).

## **CONCLUSION**

Smartphone-assisted colorimetry provided calibration models for calcium and phosphorus that were closely comparable to those obtained by UV-Visible spectrophotometry. The forages selected in the present study, including *Pennisetum purpureum* cv. Mott, *Pennisetum purpupoides*, *Leucaena leucocephala*, and *Sesbania grandiflora* were selected for their

suitability to tropical and subtropical climates, their nutritional quality, and their resilience in resource-limited settings. With the established conditions of the present study, smartphone-assisted colorimetry indicated strong linearity and minimal deviation from reference measurements, confirming its suitability as a low-cost screening tool for forage mineral analysis. The present study had several limitations. Validation was restricted to two minerals and conducted under controlled imaging conditions; therefore, broader application could be affected by feed matrix complexity, extraction efficiency, and environmental lighting variation. Future studies should validate smartphone-assisted colorimetry across several feed ingredients and mineral fractions, evaluate performance under diverse field conditions, and develop more detailed device standardization protocols to ensure reliable on-farm use translation.

## DECLARATIONS

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### Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

Suhubdy designed the study, supervised the article, and drafted the manuscript. Syamsul Hidayat Dilaga and Azhary Noersidiq conducted laboratory analysis and data collection. Ahmadi contributed to analytical procedures, data interpretation, and manuscript revision. All authors read and approved the final edition of the manuscript.

### Competing interests

The authors declared that they have no competing interests.

### Ethical considerations

The authors confirmed that the manuscript is original and has been checked for plagiarism, duplication, and other ethical considerations in accordance with the journal's principles. The manuscript has not been published previously and is not under consideration elsewhere. No AI tools were used to conduct the present study.

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