



REVIEW ARTICLE

## Use of near infrared spectroscopy for the evaluation of forage for ruminants

### *Uso da espectroscopia do infravermelho próximo na avaliação de forragens para ruminantes*

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#### PALAVRAS-CHAVE

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**ABSTRACT:** Near infrared spectroscopy (NIRS) is a technology that has been applied to evaluate the quality of forage for ruminants. This paper describes how the NIRS technique has been applied to the evaluation of fresh, dried and ground forage with, for example, laboratory bench equipment, portable equipment, and the use in drones and tractors. The technology has been widely implemented in the evaluation of chemical composition (dry matter, crude protein, neutral and acid detergent fiber, lignin, and ether extract), digestibility, gas production, intake and other parameters of forage quality with the benefits of not destroying samples, not using reagents and providing rapid analyses, among others.

**RESUMO:** *Espectroscopia do infravermelho próximo (NIRS) é uma tecnologia que tem sido aplicada na avaliação da qualidade da forragem de ruminantes. Neste artigo é descrito de que forma a técnica NIRS tem sido aplicada na avaliação de forragens frescas em pasto ou secas e moídas, tanto com o equipamento de bancada no laboratório quanto com o equipamento portátil e sua utilização em drones e tratores, por exemplo. A tecnologia tem sido amplamente implementada na avaliação da composição química (matéria seca, proteína bruta, fibra em detergente neutro e ácido, lignina, extrato etéreo), da digestibilidade, da produção de gás, do consumo e de outros parâmetros de qualidade da forragem, com os benefícios de não destruir amostras, não utilizar reagentes, fornecer rápido resultado das análises, entre outros.*

# 1 Introduction

Forages are the main and most important source of nutrients for ruminant livestock (Molano et al., 2016). Thus, it is important to understand and know forage nutritional values for improved business gains, because they directly influence the productive and reproductive performance of animals (Molano et al., 2016). This knowledge also allows animal requirements to be met to avoid unnecessary losses in the environment (De Boever et al., 1997), as is a prediction of animal performance and the subsequent development of the livestock industry (Herrero et al., 1996).

Nutritional value estimation is generally done using classical wet chemical methods, such as those of Tilley & Terry (1963) and Goering & Van Soest (1970), to obtain nutritional information about forage (Herrero et al., 1996); however, some of these techniques are time-consuming, require skilled labor, are often expensive, use chemical reagents that in some cases may be hazardous contaminants and can be inaccurate (Herrero et al., 1996; Molano et al., 2016).

The most appropriate methods to determine the nutritional value of ruminant feed are *in vivo* assays assessing animal production and digestibility. However, these require a high number of animals, labor, feed, time and elevated financial investments, which limits their applicability (Maurício et al., 2003). According to Andrés et al. (2005), *in vivo* analyses also cannot describe the dynamics of nutrient supply and are not readily applicable to large sample numbers or when small quantities of feedstuff are available.

The near infrared spectroscopy (NIRS) technique has been applied since the 1960s in neurology and in the feed and raw material (pharma) industries; it has also been used in the evaluation of forages. This technique is rapid and has the possibility of not requiring sample processing, allowing for large-scale sampling. It requires no reagents (Stuth et al., 2003), is cheaper and more precise, and predicts crude protein (CP), fiber fraction and *in vivo* or *in vitro* digestibility more accurately than other laboratorial analyses (Murray, 1993). It has been used in the estimation of the nutritional constituents of forages since the work of Norris et al. (1976); therefore, it is a suitable and efficient tool that provides valuable information (Andreu-Rodríguez et al., 2017).

The aim of this literature review was to explore the utilization of NIRS to measure the chemical composition, digestibility, gas production and intake of forages by ruminants.

## 2 Development

### 2.1 Principles of NIRS

Near infrared spectroscopy is a rapid, non-destructive and valid alternative technique, which represents a radical shift from conventional chemical methods, in which the whole matrix is characterized in terms of its absorption properties (Tassone et al., 2014). All the organic bonds, such as C-H, N-H, and O-H, have absorption bands in the near infrared (NIR) region (Osborne, 2000); this shows that NIRS can detect the bonds of fractions of fats, proteins, and carbohydrates in forage (Ibáñez & Alomar, 2008).

The NIR region is located just outside the red band, with a wavelength range between 700 and 2500 nm in the electromagnetic spectrum; infrared (IR) light is emitted and absorbed by all biological compounds (Stuth et al., 2003). When a sample is scanned, the NIR spectrometer projects NIR light in the sample,

and the radiant energy is absorbed by the sample molecules according to the frequency of a specific vibration, which results in a unique spectrum for that sample (Ibáñez & Alomar, 2008), which is then stored in a computer (Stuth et al., 2003).

Near infrared spectroscopy is a very rapid technique with a low maintenance cost. It provides results at the time of analysis, which makes it a very interesting tool for animal feed control (Pujol et al., 2007) and a reliable tool in the determination of forage quality parameters (Castro, 2002; Ibáñez & Alomar, 2008; Molano et al., 2016).

### 2.2 Calibration and validation process

Before using NIRS, calibrations are required. The calibration process establishes a relationship between a spectrum and a reference property (e.g., composition parameter) through the creation of a spectrochemical prediction model (Shenk & Westerhaus, 1993). This model aims at making the most accurate and precise prediction for a parameter/variable of interest (Decruyenaere et al., 2015).

Calibration equations can be calculated from the relationship between the spectral properties of samples and the results obtained by a reference laboratory method (Marten et al., 1989); the development of robust and accurate NIRS predictions depends on a database of samples that represents the predicted forage characteristics (Parrini et al., 2018).

As for the quantity of forage samples that are necessary to build a robust calibration, the initial idea is to have a calibration for each species of forage or, in practical terms, more than one species, if they are closely related. Samples need to cover the variability (heterogeneously) of the sample being predicted, especially with respect to different species, from different years and distinct agronomical conditions, among other characteristics, to build a robust calibration.

As previously explained, the number of samples required can vary widely. Durmic et al. (2017) used 1231 samples for a calibration equation predicting nitrogen, 427 samples for a neutral detergent fiber (NDF) calibration, 402 samples for an acid detergent fiber (ADF) calibration, and 405 samples for an *in vitro* dry matter loss calibration. Parrini et al. (2018) used 105 samples of natural and naturalized pastures from Tuscany (Italy) and affirmed that NIRS was able to precisely and accurately estimate the chemical composition of the pastures, even with a reduced number of samples.

Therefore, it is necessary that the laboratory values (from wet chemistry procedures) and techniques be precise and accurate to develop suitable calibration equations; the lower the error in reference values, the better the precision of the model (Decruyenaere et al., 2015; Osborne, 2000). This is a key point in the quality of the NIRS technique used, as NIRS requires a large number of reference samples for instrument calibration (Pujol et al., 2007).

In terms of sample preparation, it is ideal if the sample is measured fresh, but frequently, there is a lack of fresh material available, so the spectra are collected from dried and ground samples, such that calibrations are more often performed with processed samples. In this case, attention should be given to the drying and grinding procedures; due to the fact that water is a strong absorber of NIR light and that particle size also affects the spectrum, it is essential that the conditions under which NIR spectra are obtained be as uniform as possible (Stuth et al., 2003).

Some disadvantages about the technique are related to the need of the reference method, the complexity about the calibration and validation process, the high financial investment in the inclusion of the technology, and qualification of the specialist.

According to Landau et al. (2006), the quality of the calibrations on NIRS can be evaluated in terms of the coefficient of determination ( $R^2$ ), which represents the proportion of variability in the reference data that is accounted for by the regression equation. Other important variables are SEC (standard error of calibration) or SECV (standard error of cross validation), which are the variability in the difference between the predicted values and the values obtained by the reference methods when the equation was developed from the calibration data set (Landau et al., 2006) and RMSEC (root-mean square error of calibration).

When the spectra and the reference data are included and the calibration is finalized with high  $R^2$  and low SEC values, it is necessary to validate them, which will evaluate the prediction and accuracy of the calibration process (Landau et al., 2006). After this first process, it is necessary to include new sample spectra and new reference data to evaluate the quality of the calibration equation, which will generate validation equation and parameters, such as  $R^2$ , SEP (standard error of prediction), and RMSEP (root-mean square error of prediction). With good validation equation parameters and the inclusion of new spectra, it is then possible to make a prediction of the selected parameters of new samples. To select the NIRS calibration and validation equations, they must have the highest  $R^2$  and the lowest errors associated with the measurements in each phase (Molano et al., 2016).

## 2.3 Forage analysis

Since the work of Norris et al. (1976), the NIRS technique has been used to evaluate the quality of forages; researchers have found that NIRS has been successfully used in the prediction of nutritional value through direct scanning of forage samples (Boschma et al., 2017; Stuth et al., 2003). In the following sections and in the Tables 1 and 2, we will demonstrate how NIRS has been used to predict the composition of forages, from which the nutritional value can be extrapolated, and to predict the intake, digestibility, and gas production of forages.

### 2.3.1 Chemical composition

#### 2.3.1.1 Dry matter (DM) and organic matter (OM)

According to Cozzolino (2014), the DM and OM yields are two of the most important parameters in forages and crops; they are directly related to production costs and also are also well analyzed by NIRS. Castro et al. (2005) evaluated the OM content of 366 dried and ground samples, finding a  $R^2$  of 0.92, a SEC of 0.73, and a SECV of 0.89. Fernandes (2015) compared the results of 145 fresh and 140 processed (dried and ground) samples, obtaining DM values with a  $R^2$  of 0.87 and SEC of 0.99, for fresh samples, and an  $R^2$  of 0.89 and a SEC of 0.78, for processed samples, demonstrating that the NIRS methodology can be applied in fresh samples of forage.

**Table 1.** NIRS statistics parameters for chemical composition of forages

**Tabela 1.** Parâmetros estatísticos NIRS para composição química de forragens

Property	N	$R^2$	SEC	SECV	SEP	RMSEC	RMSEP	Author
DM/OM	145	0.87	0.99	-	-	-	-	Fernandes (2015)
	158	0.73	-	-	23.50	-	-	Cozzolino & Labandera (2002)
CP/N	50	0.98	-	-	-	1.02	-	Bezada et al. (2017)
	1231	0.98	0.84	0.88	-	-	-	Durmic et al. (2017)
	182	0.99	0.81	1.04	-	-	-	Ullmann et al. (2017)
	1025	0.98	-	-	1.00	-	-	Andueza et al. (2016)
	310	0.99	0.80	0.90	-	-	-	Molano et al. (2016)
	141	0.60	0.71	-	-	-	-	Fernandes (2015)
	147	0.97	-	-	-	-	-	Simeone et al. (2015)
	158	0.83	-	-	19.90	-	-	Cozzolino & Labandera (2002)
NDF	50	0.90	-	-	-	1.01	-	Bezada et al. (2017)
	427	0.96	26.10	27.70	-	-	-	Durmic et al. (2017)
	262	0.91	20.76	-	23.80	-	-	Ullmann et al. (2017)
	228	0.99	1.50	3.50	-	-	-	Molano et al. (2016)
	139	0.62	2.51	-	-	-	-	Fernandes (2015)
	147	0.95	-	-	-	-	1.74	Simeone et al. (2015)
ADF	402	0.97	17.4	18.5	-	-	-	Durmic et al. (2017)
	155	0.95	1.70	2.10	-	-	-	Molano et al. (2016)
	140	0.78	1.34	-	-	-	-	Fernandes (2015)
	147	0.93	-	-	-	-	1.46	Simeone et al. (2015)
LIG	182	0.86	4.44	7.19	-	-	-	Ullmann et al. (2017)
	147	0.94	-	-	-	-	-	Simeone et al. (2015)
EE	50	0.94	-	-	-	0.29	-	Bezada et al. (2017)
	245	0.94	2.17	2.80	-	-	-	Ullmann et al. (2017)
	115	0.51	0.63	-	-	-	-	Fernandes (2015)

N= number of samples,  $R^2$ = coefficient of determination, SEP= standard error of prediction, SEC= standard error of calibration, SECV= standard error of cross-validation, DM= dry matter, OM= organic matter, CP= crude protein, N= nitrogen, NDF= neutral detergent fiber, ADF= acid detergent fiber, LIG= lignin, EE= ether extract

### 2.3.1.2 Crude protein (CP) and nitrogen (N)

According to Stuth et al. (2003), the total nitrogen (N) or CP (usually  $N \times 6.25$ ) contents are two of the most commonly measured components of forages using the NIRS technique. Usually, researchers find very high  $R^2$  values for these components, mainly due to the strong  $-N-H$  absorptions in the NIR region (Stuth et al., 2003). This is confirmed by the results obtained in the literature, which can vary from 0.60 to 0.99, representing good  $R^2$  values from different species of forage sample, as tropical forage and *Brachiaria* spp. (Fernandes, 2015; Molano et al., 2016; Simeone et al., 2015; Ullmann et al., 2017).

### 2.3.1.3 Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG)/acid-detergent lignin (ADL)

After CP, NDF and ADF contents are the next most frequently reported components in studies using NIRS technology for forage. Usually, researchers find good calibration equations, with  $R^2$  ranging from 0.62 to 0.99 for NDF, 0.75 to 0.97 for ADF, and 0.86 to 0.94 for LIG (Durmic et al., 2017; Ullmann et al., 2017; Molano et al., 2016; Fernandes, 2015; Simeone et al., 2015), which came from many different studies from different places and forage species, as various cultivars of *Brachiaria* spp. (Simeone et al., 2015). It is also noticed that the number of samples can vary from study to study, in this case from 139 to 427, and variability can bring robustness to the calibration equation.

### 2.3.1.4 Ether extract (EE)

According to Stuth et al. (2003), the prediction of ether extract (EE) or lipids (LIP) with NIRS is not common, which can be caused by the low quantification of this property on forage; thus, the results can vary from good calibrations, as found by Ullmann et al. (2017), who analyzed 245 samples of forage and obtained  $R^2$  of 0.94, SEC of 2.17, and SECV of 2.80, but different from those found in another study that used 115 samples of forage, but did not obtain so good calibration parameters ( $R^2$  of 0.51 and SEC of 0.63) (Fernandes, 2015).

### 2.3.2 Digestibility and gas production

Digestibility is another parameter that can be measured by the NIRS technique, but because digestibility is a property rather than a chemical parameter of forage, prediction can be more difficult (Stuth et al., 2003). This happens because errors in predicting animal responses are usually greater than those in predicting chemical compositions (Norris et al., 1976). Therefore, by comparison with the usual chemical parameters, the calibration and validation equation values of *in vivo* characteristics are higher due the variation between animals (Decruyenaere et al., 2015).

In these *in vivo* studies, Decruyenaere et al. (2015) found  $R^2$  and SECV values of 0.92 and 0.02, respectively, for the organic matter digestibility (OMD) of 951 samples, and Kneebone et al. (2015) observed  $R^2$  values of 0.85 and 0.82, and SEC values of 0.03 and 0.01 for DMD and OMD, respectively, showing that the NIRS technique is appropriate for the prediction of OMD and DMD values of *in vivo* studies.

Even within the *in vitro* methodology to evaluate digestibility, the values of NIRS parameters can vary due to the different methods as two-stage pepsin cellulose or gas production technique, but in all of them, values that indicate accurate and robust calibrations were observed (Table 2).

Andrés et al. (2005) compared chemical composition data and NIRS data to predict gas production parameters and noted a more accurate prediction using NIRS, perhaps because the NIR spectra contained a chemical component and physical property information for the sample.

### 2.3.3 Intake

A reduction in forage intake is relevant due to its negative effect on animal production (Benvenuti et al., 2014), and the NIRS technique has been used to estimate this parameter with good values obtained in the calibration equations, as observed in the works of Decruyenaere et al. (2015) and Kneebone et al. (2015), and is reported in Table 2. The estimate of intake by NIRS provides several benefits, such as speed in the result, besides not being necessary to develop an experiment, which would make use of animals, feed and later analyzes in the laboratory.

### 2.3.4 Fecal NIRS (FNIRS)

The chemical analysis of feces is usually conducted in the laboratory, which is associated with high costs and high labor. Based on how the sample is used (i.e., destroyed), it may be impossible to conduct different analyses when samples are small, as is often the case with fecal pellet samples (Ramanzin et al., 2017).

The use of NIRS to analyze feces to predict chemical composition, digestibility, or intake is based on the fact that the spectral information found in feces is enough to describe the composition of the ingested diet (Dixon & Coates, 2009). In the application of this technique, instead of a forage spectra, feces is collected, and from this, the calibration and validation equations are built.

Brogna et al. (2018), using the NIRS technology to predict fecal indigestible neutral detergent fiber for dairy cows, observed  $R^2$  of 0.77 and SEC of 0.90, DM and  $R^2$  of 0.93 and SEC of 0.74 for CP and  $R^2$  of 0.66 and SEC of 0.43 for starch, that can be considered good calibration models, as the results founded by Jancewicz et al. (2016) that developed NIRS equations to predict fecal composition (OM, starch, N, NDF, ADF, ADL, and EE) and digestibility [DM, OM, starch, CP, NDF, ADF and gross energy (GE)] and found good results ( $R^2 \geq 0.70$  and SEP  $\leq 6.85$ ) for OM, starch, N, NDF, and ADL, and less promising results for ADF and EE, which can be observed by the lower result of  $R^2$  (0.25) and high SEP ( $\leq 11.0$ ).

In this case, FNIRS could be applied in the OM, starch, N, NDF, and ADL prediction, almost similar to what was found by Ramanzin et al. (2017), who presented more accurate FNIRS predictions for N and progressively less accurate predictions, in descending order, for NDF, ash, ADF, and ADL contents.

Decruyenaere et al. (2015) developed equations to analyze *in vivo* organic matter digestibility (OMD), dry matter voluntary intake (DMVI; g/kg BW 0.75) and organic matter voluntary intake (OMVI; g/kg BW 0.75); they used 951 samples for OMD, finding  $R^2$  of 0.92, SEC of 0.02, and SECV of 0.02; 1012 samples for DMVI, finding  $R^2$  of 0.80, SEC of 5.32, and SECV of 5.53; and 936 samples for OMVI, finding  $R^2$  of 0.83, SEC of 4.28, and SECV of 4.53, showing that it is a very promising technique corroborating with Coates & Dixon (2011), who used 1052 samples to predict the dietary dry matter digestibility of cattle consuming tropical forages and found  $R^2$ , SEC, and SECV values of 0.90, 1.87, and 1.91, respectively.

**Table 2.** NIRS statistics parameters for digestibility and intake of forages**Tabela 2.** Parâmetros estatísticos NIRS para digestibilidade e consumo de forragens

Analyse	N	R <sup>2</sup>	SEC	SECV	SEP	RMSEP	Author
IVDMD	405	0.95	26	28	-	-	Durmic et al. (2017)
	1025	0.93	-	-	0.04	-	Andueza et al. (2016)
	195	0.95	1.7	2.0	-	-	Molano et al. (2016)
	147	0.94	-	-	-	2.60	Simeone et al. (2015)
IVOMD	251	0.96	10.8	10.97	-	-	Ullmann et al. (2017)
DMD	100	0.85	0.03	-	-	-	Kneebone et al. (2015)
OMD	951	0.92	0.02	-	-	-	Decruyenaere et al. (2015)
	100	0.82	0.01	-	-	-	Kneebone et al. (2015)
DMI	1012	0.80	5.32	5.53	-	-	Decruyenaere et al. (2015)
	100	0.84	6.7	-	-	-	Kneebone et al. (2015)
OMI	936	0.80	4.28	4.53	-	-	Decruyenaere et al. (2015)
	100	0.80	6.8	-	-	-	Kneebone et al. (2015)
CPI	100	0.91	1.4	-	-	-	Kneebone et al. (2015)
DDMI	100	0.82	5.3	-	-	-	Kneebone et al. (2015)
DOMI	100	82.00	5.0	-	-	-	Kneebone et al. (2015)

N= number of samples, R<sup>2</sup>= coefficient of determination, SEP= standard error of prediction, SEC= standard error of calibration, SECV= standard error of cross-validation, IVDMD= *in vitro* dry matter digestibility, IVOMD= *in vitro* organic matter digestibility, DMD= dry matter digestibility, DMI= dry matter intake, OMI= organic matter intake, CPI= crude protein intake, DDMI= digestible dry matter intake, DOMI= digestible organic matter intake.

## 2.4 Implementation of NIRS

### 2.4.1 Forage preparation

Forage can be dried or oven-dried and afterwards ground to 1 mm (Bezada et al., 2017; Simeone et al., 2015; Andueza et al., 2016; Molano et al., 2016; Durmic et al., 2017; Ullmann et al., 2017; Parrini et al., 2018) or 2 mm (Zhang et al., 2017). This is done for the sake of standardization, especially because the quality of the calibration can be increased with sample preparation and measurement standardization (Reddersen et al., 2013). However, it is also important to standardize all preparations of the sample (including temperature and amount of time), because this also changes the water content, which introduces the possibility of a different and new calibration curve for each preparation method.

On the other hand, studies have been developed and applied to evaluate forage quality of fresh samples using NIRS technology (Fernandes, 2015). This facilitates the implementation of the technique, because it allows for the evaluation of forage quality at the production site, *in locus*. Cozzolino & Labandera (2002) considered the use of wet materials interesting, because it avoids the drying and grinding processes of sample preparation and is a technique that can be implemented in plant breeding programs with a large number of samples to be analyzed.

Cozzolino (2014), using a portable NIR spectrometer, affirmed a successful prediction of the DM, nitrogen, and protein components of fresh samples. The same result was found by Cozzolino & Labandera (2002), who obtained useful predictive models for the DM and CP contents of fresh forage, detecting similar results using dried samples. Some authors have also reported the use of a contact probe to collect the reflectance spectra of fresh samples (Lugassi et al., 2015). Mendarte et al. (2010) also obtained results that show the potential of portable NIRS technology in the determination of DM and CP contents of wet forage, but noticed that, in this case, a large number of samples was necessary to obtain a robust calibration equation.

### 2.4.2 Sample selection for different calibration equations

In this field, NIRS accuracy depends on a detailed database of samples and calibration equations for individual groups of materials and different methods of preparation prior to the spectra collection. To start the process, it is important to create a well-specified local calibration and, afterwards, work with a global calibration, which includes representative samples from several years, species, cuts, sample preparations, particle size, residual moistures, and other factors (Sinnavee et al., 1994).

When working specifically with forages, seasonal effects should be noted, because if there are differences between them, a new calibration for each season or year might be necessary to ensure good accuracy (Garcia & Cozzolino, 2006); these values can also be included in the universal calibration equation, increasing the spectral range and producing a more robust universal calibration. According to Andueza et al. (2016), the results obtained by global calibrations can be improved by the local approach, increasing the precision of the obtained results.

### 2.4.3 Equipment and new developments

The NIRS technique has also been used to analyze the quality of forages with equipment such as drones or cameras attached to tractors (Saari et al., 2017). In this system, NIR technology has been used to analyze hyperspectral images and describe the content of digestible organic matter in the dry matter of grass.

According to Hunt et al. (2010), it is also possible to post-process a raw digital camera image to produce a red, green, and NIR false-color image. For some commercial cameras, only the red channel is sensitive to NIR light; thus, these authors presented a new system in which a NIR, green, and blue digital image is obtained. They found good correlation between the leaf area index and the green normalized difference vegetation index (GNDVI).

To study the application of a complex visible and NIR camera system for parameters such as the leaf area index and

the growth stage of forage, Fan et al. (2018) mounted a camera system to a tripod and collected data in two growing seasons; they found good prediction values and identification with acceptable accuracy and reliability, highlighting the advantage of this easy operation.

According to the results obtained by different authors, this technology can be coupled with the various types of vehicles routinely used in farming (e.g., tractors, trucks), aiming, for example, at evaluating the chemical composition to make decisions about changes (e.g., entry and exit of animals in a picket, the balance of diets).

### 3 Final Considerations

As noted, NIRS has been extensively used, and technologies have been developed such that it can be applied both in analyses related to scientific research and in the farm environment.

There are limitations in the development of robust and accurate calibration and validation equations, and it is necessary that the reference data be of high quality and obtained using standardized laboratory methods. Another limitation is the application of the technique, which requires specialized labor for the creation and application of calibration and validation curves or the possible sharing of curves between equipment.

The prospects for NIRS technology are in the development of technologies that evaluate the quality of fresh on-farm forage, allowing daily variations in fresh fodder to be detected, which promotes instantaneous adjustments to animal nutrition. Research related to the use of NIRS to evaluate the chemical composition of forages is already well established and defined, but it is still necessary to search for other parameters (e.g., *in vitro* digestibility, gas production, intake) for the on-farm application of this technology. In addition, there is a need for more research on the use of NIRS technology with portable equipment, mainly attached to tractors and drones, for application in daily farm use.

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