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# ESTIMATION OF THE *IN SITU* DRY MATTER DIGESTIBILITY OF TEMPERATE CLIMATE FORAGE DIGESTIBILITY BY SPECTROSCOPY (FT-NIRS)

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**Abstract:** The objective of the trial was to evaluate the use of near-infrared reflectance spectroscopy based on Fourier transform technology (FT-NIRS) to estimate *in situ* dry matter digestibility (DISMS) of samples (n=182) of different temperate forage varieties. DISMS was determined using Ankom bags in two adult bovines with cannula in the rumen for 24 h of incubation. The spectrum of the samples was obtained with the help of an FTIR spectrophotometer in the spectral range between 4,000 to 10,000 waves /cm-$^{1}$. With the DISMS values, the information contained in the spectra, a multivariate partial least squares (PLS) model and the TQ Analyst program, the calibration equation was developed. The selection of the calibration equation was based on the maximization of the coefficient of determination (R2), and the minimization of the calibration standard error (EEC) and prediction standard error (EEP) was considered. In addition, to evaluate the predictive ability of the calibration equation based on the ratio (RDP) of EEP to the standard deviation of the validation The range of DISMS of all samples in the study varied from 48.4 to 93.7 %. The statistics for calibration equation were R2=0.96 and EEC=1.96 and for the validation model R2=0.91, EPP=2.98 and RDP=3.30. Based on these results, it can be concluded that FT-NIRS technology accurately and reliably predicts DISMS of temperate forages.
**Keywords:** Forages, Spectroscopy, *in situ* Digestibility.

## INTRODUCTION.

Forage development programs usually generate many samples for evaluating multiple agronomic characteristics. However, the assessment becomes more complicated and expensive when nutritional quality parameters (for example, crude protein content, detergent fiber, and digestibility are considered in the evaluation. Although analytical techniques to measure digestibility *in vitro* or *in situ* allow the evaluation of a large number of samples in a relatively short time, they still require a large amount of labor and material resources. For example, the *in situ* technique, one of the most widely used, requires the use of dacron bags with standard pore sizes and animals with a cannula in the rumen; however, because several bags can be inserted simultaneously in the rumen, it is possible to evaluate a significant number of samples of forages (KEMPTON, 1980; HUNTINGTON; GIVENS, 1995). However, the time required and the costs of the analysis continue to stimulate the evaluation of new technologies that allow rapid, precise, reliable, and low-cost evaluations. One of these techniques is near-infrared spectroscopy (NIRS) has been especially fast, cheap, and accurate in determining the protein and fiber content of forages (YANG *et al*., 2017). TODOROV *et al.* (1994) conclude that NIRS spectroscopy has a high potential to determine the nutritional characteristics of forage, such as the degradation of fiber and crude protein, which could vary widely.

In contrast, there are fewer studies with NIRS to evaluate the prediction of the nutritional content of ingredients when the component or animal response is involved (rumen degradation of protein, starch, or carbohydrates). In addition, Fourier Transform Infrared Spectroscopy (FT-NIRS) has recently appeared, which allows collecting spectra that contain more information and, together with better chemometric programs, more robust analyses can be achieved. Thus, despite the variability of a set of samples, it has been possible to accurately and precisely estimate the chemical composition of natural and introduced pastures with the FT-NIRS technique (PARRINI *et al.*, 2018). However, whether the predictive power is maintained

when an animal component is involved is unknown.

## OBJECTIVE

Develop a calibration equation using near-infrared reflectance spectroscopy, based on the Fourier transform (FT-NIRS), to estimate the *in situ* digestibility of temperate climate forages.

## MATERIAL AND METHODS

For this study, 181 samples of different varieties of sorghum (*Sorghum spp*; n=41), oats (*Avena spp*; n=19), bromine (*Bromus spp*; n=11), fescue (*Festuca spp*; n=10),, Guinea (*Panicum spp*; n=5), ryegrass (*Lolium spp*; n=4) and rhodes (*Chloris spp*; n=3) were collected on the 2019 to 2022 cycles. The forages were cultivated in the bank of Forager Genetic Resources of the Temperate Zone, belonging to the National Center for Disciplinary Investigations in Physiology and Animal Improvement, located in Ajuchitlán, Colón, Querétaro, México. The samples were dried at 55°C in a forced-air oven for 72 h and then ground with a Wiley-type mill (Thomas Scientific, Swedesboro, NJ) with a 2-mm sieve in the laboratory. Of each sample, 5 ± 0.1 g was placed in triplicate in a dacron bag (10 x 20 cm; Ankom Technologies Corp, Macedon, NY), with a ratio of 13.5 mg of sample/cm2 of bag surface (HUNTINGTON; GIVENS, 1995). The bags were heat-sealed using a pulse sealer (American International Electric). Next, the bags were grouped into sets of 50, and each set was placed in a 45 x 40 cm nylon bag for insertion into the rumen. Before insertion, each nylon bag was immersed in water at 37°C for 10 to 15 min to moisten the forage in the dacron bags. The bags were incubated for 24 h in the rumen. Observing the guidelines of the Official Mexican Standard (NOM-062-ZOO-1999). and CIOMS; (1985), two adult bovines with a cannula in the rumen were used; the animals grazed natural rangeland with free access to water and a mineral block, At the end of the incubation period, the bags were removed from the rumen and mechanically washed utilizing a commercial detergent. Next, the bags were dried for 72 h in a forced air oven and then weighed; With these data, the residue weight was determined by difference. Thus, the *in situ* digestibility of dry matter (DISMS) was calculated as the percentage of the weight of the original sample that disappeared from the bag after incubation, as DISMS (%)= 100 - (100* ((weight of the sample - the weight of the residue) / initial weight of the sample If DISMS of a sample had a coefficient of variation greater than 5%, the sample was incubated again..

On the other hand, the spectra of the absorbances of the samples were obtained with a Nicolet 6700 FTIR unit (Thermo Scientific Inc.) in the spectral range of 4,000cm-1 to 10,000 waves cm-1. The absorbance values of the spectra were stored as log(1/ Reflectance). Calibration development for DISMS used the TQ Analyst program (v8.0; Thermo Scientific Inc.) and a partial least squares (PLS) multivariate model. Selection of calibrations was based on the minimization of standard errors of calibration (EEC), prediction (EEP), cross-calibration (EECC), and the maximization of the coefficient of determination of the calibration (R2). The ratio (RDP) of the EEP to the standard deviation of the validation group is a dimensionless statistic proposed for the evaluation of the reliability or the predictive power of the NIRS calibration models (WILLIANS, 2019). Thus, based on the RDP, if a calibration equation has an RDP less than 2.0, it is considered to have poor performance, and its use is not recommended; RDP greater than 2.0 and less than 2.5 indicates that the calibration has low performance, but it could only be

used for a lesser rigorous selection, that is, to discriminate between high and low values of the variable; RDP greater than 2.5 and less than 3.0, the calibration is helpful for a stricter selection and an RDP greater than 3.0 and less than 3.5, indicates that the calibration can be used to make quantitative estimates or quality control; finally, RDP greater than 3.5, the calibration model is valuable in control processes (WILLIAMS, 2019).

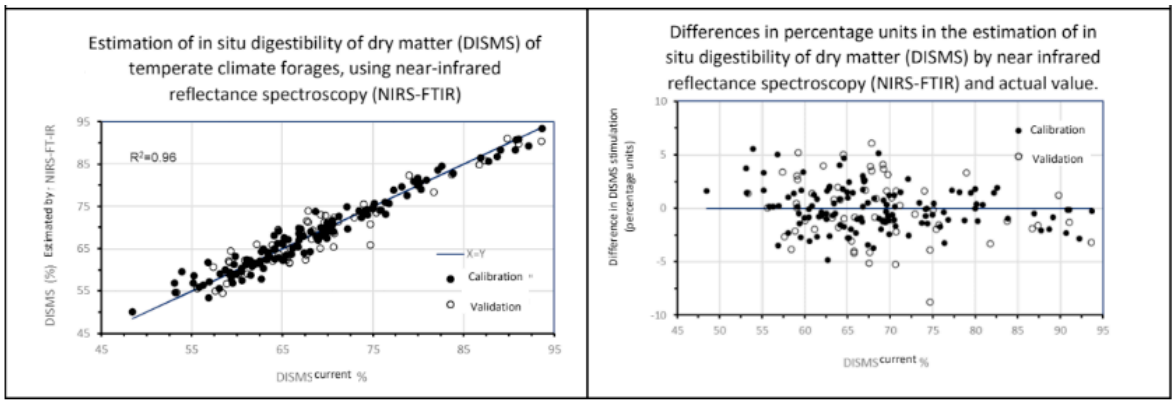
## RESULTS AND DISCUSSION.

The table shows the descriptive statistics of the *in situ* digestibility for each set of samples: general, calibration, and validation; also, the statistics for the FTIR-NIRS calibration equation are shown. According to these statistics, the calibration equation explains more than 96% of the variation (R2) with a low EEC of DISMS. Regarding the validation set, the calibration equation explains 91% variation with an EEP=2.98 and EEC=2.75 percentual units. Following the established by WILLIAMS (2019), the RDP statistic indicates that the calibration has an excellent predictive capacity and can be used for quantitative analysis. In a preliminary study (RAMIREZ et al., 2022), the database consisted almost entirely of sorghum varieties, and the DISMS range was only 26.7 percentage units. In these conditions, the prediction power of the calibration equation had just an RDP=2.05; this means that the equation could discriminate only high or low values of DISMS. These authors (RAMIREZ *et al*., 2005) suggested that the prediction capacity could improve if the inclusion of more samples or species increased the variability of DISMS in the database, In the present trial, the range of the DISMS was 45 percentage units and with extreme values, from 48 to 93% of the DISMS. Graph (a) shows the relationship between the predicted by NIRS-FTIR and actual values of the samples, where the calibration equation explains more than 90% of the variation in the validation set. Graph (b) shows the dispersion of the prediction errors for the calibration and validation sets. It can appreciate that there is no systematic trend in the prediction of the DISMS by the calibration.

NIRS technology has successfully estimated various forage components, especially nitrogenous ones. However, other components, such as the fiber of forages, are estimated less precisely. The low prediction of fiber could be explained because the fiber is constituted by cellulose, hemicellulose, and lignin; in addition, their proportions in cell walls vary widely due to innumerable factors, such as species, phenological state, and climate (SHENK; WESTREHAUS, 1994; BELANCHE et al., 2014). However, SANDOVAL-MEJÍA et al. (2008) reported precise calibrations to estimate the fiber fractions of tropical grasses with high coefficients of determination (R2 > 0.91) and low prediction and validation errors (< 1.5). Even if the prediction and validation errors of the present study are greater than those reported by SANDOVAL-MEJÍA et al. (2008), it must consider that an animal factor was involved in the present study. This animal factor, indeed, affected the precision of NIRS. Similarly, BELANCHE et al. (2014) reported that their NIRS calibration poorly estimated i*n situ* degradation of forage dry matter (R2=0.53), but other parameters were accurately predicted. REEVES et (1991) evaluated the feasibility of NIRS to estimate the *in situ* digestibility of alfalfa and orchard grass samples and reported that the explained variation was R2=0.95, EEC=3.67, and EEP=3.56. This calibration is less precise than the one reported in the present test. On the other hand, FOSKOLOS et al. (2015) developed NIRS prediction equations of *in situ* degradation parameters for a wide variety of concentrated ingredients, forages, and by-products for animal feed (+800 samples) and

| | | Chemical analysis in the laboratory | | | | | FT-NIRS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Cluster | No. | Half | OF | MIN | MAX | R2cal | EEC | R2val | EEP | EECC | RDP |
| DISMS | you | 182 | 68.62 | 9.27 | 48.45 | 93.67 | 0.96 | 1.97 | 0.91 | 2.98 | 2.75 | 3.30 |
| | C. | 122 | 68.56 | 9.37 | 48.45 | 93.67 | | | | | | |
| | V | 60 | 68.83 | 9.82 | 53.29 | 93.62 | | | | | | |

Table. FTIR-NIRS calibration to estimate the in *situ* digestibility of dry matter (DISMS) of temperate forages. N=number of samples; SD=Standard deviation, MIN=Minimum value; MAX=Maximum value; R2cal= Prediction coefficient of the calibration equation; SEC=Standard error of calibration; SEE=Standard error of prediction; R2val= Prediction coefficient of the prediction equation; EECC=Standard error of cross calibration; RDP=DEval/EEP; Group: T=All samples analyzed, C=Calibration or V=Validation.




Graphics. Spread of values determined in the laboratory and values predicted by NIRS-FTIR (a) and spread band of prediction errors around the baseline centered at 0 (b).

reported that the CP and neutral detergent fiber content of the samples were accurately estimated using the NIRS; although in *situ* degradation parameters were estimated with less precision; for example, the calibration to determine the slowly degradable fraction of the forage or non-forage groups is not precise and can only be used to make a selection that is not very rigorous. In another study with mixtures of temperate climate grasses and legumes, CAJARVILLE et al. (2004) reported that the use of NIRS allowed to accurately estimate the *in situ* degradation of dry matter and crude protein of the mixtures; these authors report that the regression models had coefficients of determination 0.97 and 0.98, EEC 0.192 and 0.20, and EECC/DE rates of 0.13 and 0.18, respectively. TORODOV et al. (1994) evaluated the use of NIRS to estimate *in situ* degradation characteristics of forages of overall nutritional quality (grass, legume, prairie hay, haylage, wheat and oat straws, and alfalfa hay). NIRS calibration can predict 80% of the sum of the initial soluble fraction and the potentially digestible fraction (72 h), with a standard error of the calibration of 5.6 %. Considering these values and the RDP, the calibration reported by TORODOV et al. (1994) could have low predictive power.

## CONCLUSION

It concludes that the calibration equation developed with the use of the NIRS-FTIR technique accurately and confidently predicts the *in situ* digestibility of DM of temperate climate forages.

## IMPLICATIONS

In addition to the agronomic information offered by germplasm banks, using the NIRS-FTIR technique, varieties or accessions could be selected based on more complex traits without significantly increasing the evaluation cost.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