

Use of Near-Infrared Spectroscopy to Estimate Fiber and Crude Protein Content in Fodders

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ABSTRACT

Objective: Demonstrate the need to use locally generated data in the calibration of a near-infrared spectrometer (NIRS) to predict the chemical characteristics of fodder; instead of using data bases from other geographic regions, as is commonly done in Mexico.

Design/Methodology/Approach: Two groups of samples collected in prairies of the central highlands of Mexico, the first group was used to calibrate the equipment; the equations generated were validated with a second group, collected in prairies that were different from the ones of the calibration group, but in the same geographic zone.

Results: The best regression coefficients of the NIRS predictions, compared to traditional laboratory analyses were for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), dry matter (DM) and organic matter (OM) (0.93, 0.87, 0.87, 0.56, 0.72 y 0.68 respectively). The lowest predictive value was observed in ashes (0.27).

Limitations of the study/implications: The results show the need to use local materials in the calibration process.

Conclusions: NIRS will make predictions of their chemical composition, since this is influenced by geographic origin of the sample and its botanical composition.

Keywords: NIRS, chemical composition, native prairies, fiber, crude protein.

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INTRODUCTION

Ruminant production systems are based on fodder feed, predominantly on grazing on grasslands that are mainly native and non-cultivated. Livestock production on native and introduced grasslands depends on the amount of available fodder, expressed by its digestibility coefficient, its concentration of metabolizable energy and its crude protein (CP) content, which is conditioned by the type of species that form it. This in turn depends on the stage of the ecological succession that is present, the fertility level of the soil, amount, and distribution of rainfall, as well as the time at which it is used and the degree of intensity of this use (Villalobos *et al.*, 2000). Knowledge of these factors allows for optimal quality in the diet of ruminants that are fed with grasses, to achieve maximum productive response by the animal (Valente *et al.*, 2000).

The analysis of the nutritional value of fodder normally includes the determination of its chemical composition and digestibility and is done with conventional laboratory methods. These are costly, take a long time to do and may be dangerous for people and the environment due to the great number of chemical products that are used (acid and alkaline substances) (Starks *et al.*, 2006).

In this sense, starting in 1970, the use of NIRS has been evaluated to determine the chemical composition of foods and fodder. This is instead of conventional techniques of wet chemistry, a technique which, when compared with conventional procedures, provides quick and reliable estimates, and does not pollute the environment since it does not use chemicals reactives (Starks *et al.*, 2006). NIRS is based on chemometrics, that is, on the application of mathematics to analytical chemistry, combines spectroscopy, statistics and computer science and generates mathematical models that relate the chemical composition (presence of active chemical groups) to changes in energy in the region corresponding to the near infrared (wavelengths between 800.0 and 2500.0 nm) (Ferret, 2003).

In the case of Mexico, most spectrometers contain data bases with calibration as well as validation spectra that were not generated in the country for the type of fodder and plants that are found here. For this reason, it is possible that their predictions are not sufficiently exact, since they do not consider differences between species, as well as climate and the agronomical management to which they are subjected, and which evidently have an effect on their chemical composition and nutritional quality. In several reports using NIRS technology, a common mistake made when estimating the chemical and nutritional value of fodder is to use equations that are derived for certain foraging species and using them to predict nutritional parameters in different species, which leads to inexact NIRS estimates (Beever and Mould, 2000).

Therefore, the objective of this work was to calibrate a near-infrared spectrometer in order to estimate crude protein content and fractions of fiber in samples of native grasses, introduced grasses and cultivated fodder from the highlands of central Mexico.

MATERIALS AND METHODS

Study site

The experiment was carried out in the state of Mexico (20° 17' and 18° 22' N and from 98° 36' to 100° 37' W), with an altitude above sea level of 2600 m. The climate is subhumid temperate (Cw) with summer rains and a mean temperature between 10.0 and 16.0 °C, with average rainfall of 876.2 mm (García, 2004). 2230 samples of grasses and fodder were collected from May, 2006 to December, 2007 (in the following municipalities: Almoloya de Juárez, Toluca, Temoaya, Rayón, Lerma and Mexicaltzingo), in four native prairies, two prairies with introduced grasses, two prairies with cut pasture and cultivated corn (*Zea mays*), barley (*Hordeum vulgare*), oats (*Avena sativa*), triticale (*Triticum* ssp.) and canola (*Brassica napus*); all of these are used by producers as feed for cattle.

Collection of samples

The sampling was divided into 18 periods of 30 days each. The method used was the one described by Hodgson (1994), using two metal frames, one measuring 0.25×0.25 m,

specifically used to obtain samples in fodder crops, and another measuring 0.5×1.0 m, which was used in the prairies. The sampling site was randomly selected, tossing the metal frame into the air. Later on, the fodder in the frame was cut with grass shears, near the ground. While samples were collected, some species were taken to be identified in the laboratory. During each sampling period, 30 samples were collected in the native grasslands, 20 in the introduced ones and 10 in the crops.

Chemical analysis

In the laboratory, the samples were dried at 60.0 °C in the forced air stove until a constant weight was reached. Later on, they were ground and sifted with a screen that had a diameter of 1.0 mm. To do the wet chemistry analysis, samples were mixed for each collection period. Each sample was composed of 10 samples, obtaining a total of 223 compound samples. This was done in order to save on reagents. Like this, for each compound sample, content of DM, OM, CP and ashes (ASH) was determined, according to the AOAC (1990) methodology. NDF and ADF content was determined using the ANKOM method and the Van Soest *et al.* (1991) technique, and ADL content also was determined using the technique described by Van Soest and Wine (1967).

Calibrating the Near-Infrared Spectrometer

Using the 223 samples that were analyzed by wet chemistry, a spectrum was obtained in the near-infrared for which, from each compound sample, 10.0 grams were weighed in 50.0 ml beakers and brought to constant weight in a forced air stove at 60.0 °C. The samples were scanned in triplicate, using a Buchi NIR Flex N400 (Büchi) spectrometer, with Pb detector test tube, in the 1000 to 2500 nm reflectance wavelength range, generating a total of 669 spectra. Using NIRCAL software, version 4.01 (Büchi), a data base was created at the time when each sample's spectrum was obtained, relating each spectrum to the results from the wet chemistry analyses for each sample. Once the data base was created with the samples' spectra, these were divided into two groups or sets; two thirds of the spectra formed the calibration set (C-Set) and a third of the spectra formed the validation set (V-Set). To diminish the effects caused by particle size and dust, on the wavelength of the obtained spectra, three mathematical treatments were previously applied, which were the second derivative, standard normal variable, and the Kubelka-Munk treatment (Macho, 2002).

The method that was used to develop the calibration equations and for quantitative validation, was the partial least squares statistical method (PLS). The calibration equations were selected according to the least standard error prediction (SEP), the greatest correlation coefficient of each one of the sets (r^2 C-Set, r^2 V-Set) and the consistency of the equation (NIRVIS, 2000).

Validation of the Near-Infrared Spectrometer Predictions

To test the confidence level of the obtained calibration equations, an external validation was carried out using 30 samples that were collected at the Almoloya de Juárez municipality.

The samples were collected in 2006, at prairies with introduced grasses, which were different from those used to calibrate the equipment.

Analysis of Results

The values obtained in the validation were compared to the value obtained in the laboratory through a simple linear regression analysis and a Pearson correlation (Minitab, 2003). The percentage difference was also calculated (% Diff) between values obtained in the laboratory and the NIRS estimates, as reported by Stolter *et al.* (2006) in their study.

RESULTS AND DISCUSSION

Chemical composition

Table 1 shows the average, the interval of maximum and minimum values and the standard deviation for ASH, DM, OM, NDF, ADF, ADL and CP content of the samples used for calibration of the spectrometer. The variation that is observed in these chemical parameters is considered normal and may be attributed to the botanical variety of the collected samples, which were represented by one or more of the following species: *Pennisetum clandestinum*, *Sporobolus indicus*, *Juncus drummondii*, *Trifolium amabile*, *Paspalum paspaloides*, *Eleocharis dombeyana*, *Trifolium repens*, *Lolium perenne*, *Dactylis glomerata*, *Festuca arundinacea*, *Lolium multiflorum*, *Triticale* sp., *Vicia sativa*, *Brassica napus*, *Medicago sativa*, *Hordeum vulgare*, *Avena sativa*, *Zea mays*, among others.

The chemical quality is lower than that of cultivated fodder, Labaran *et al.*, (2019) report values from 262.1 to 346.9 g kg⁻¹ of MS, 97.6 to 262 g kg⁻¹ ash, 734 to 902 g kg⁻¹ MO, 121 to 162 g kg⁻¹ MS for CP, 473 to 572 g kg⁻¹MS in FDN and 247 to 474 g kg⁻¹ MS for FDA. But similar to tropical fodder, López-González *et al.* (2015) report values of 108 to 112 g kg⁻¹ MS for CP, 606 to 658 g kg⁻¹ MS of FDN and 378 to 399 g kg⁻¹ MS in FDA content.

Calibration and validation of the Near-Infrared Spectrometer

The Table 2, shows the results obtained from the calibration, for each measured parameter, as well as the correlation coefficient for C-set and V-set, standard error prediction, the consistency of the equations generated for the internal comparison.

Table 1. Chemical composition expressed in g kg⁻¹ of dry matter of samples used in the calibration of the spectrometer, showing number of samples (n), mean and range of values and standard deviation.

Parameter	n	Mean	Maximum	Minimum	Standard deviation
DM	223	932.2	987.8	882.1	18.1
OM	223	892.7	959.0	744.1	35.6
ASH	223	90.9	145.0	52.2	20.4
NDF	223	625.5	859.8	311.9	110.1
ADF	223	294.4	472.5	116.8	52.7
ADL	223	37.8	93.5	14.8	16.1
CP	223	115.0	289.0	30.1	55.6

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; DM: Dry matter; OM: Organic matter; ASH: ashes; CP: Crude protein

Table 2. Correlation coefficients, standard error prediction and consistency of the equations generated.

Parameter	r ² C-SET	r ² V-SET	SEP	Consistency of the equation generated
DM	0.77	0.93	9.61	105.15
OM	0.80	0.77	12.40	101.68
ASH	0.76	0.76	13.59	101.31
NDF	0.90	0.87	48.68	101.56
ADF	0.90	0.89	25.23	99.07
ADL	0.78	0.77	10.02	125.90
CP	0.94	0.96	18.60	101.11

DM: dry matter; OM: organic matter; ASH: ashes; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; r² C-Set: correlation coefficient of the calibration set; r² V-Set: Correlation coefficient of the validation set; SEP: standard error prediction.

The models generated for the chemical parameters that were analyzed showed calibration and validation coefficients going from 0.76 for ASH, up to 0.96 for CP.

The SEP is a reliable quality indicator for the developed equation, since unlike SEC, which improves (values near zero) as new terms are added to the equation, SEP improves only until it starts to produce an over-adjustment of the equation, increasing (values far from zero) later on, with each new term (Alomar and Fuchslocher, 1998). The SEP of this work fluctuated between 9.61 and 48.68 for DM and NDF, respectively. Sapienza *et al.* (2008) state that the SEP are directly related to the standard deviation of the reference methods, that is, higher SEP values will be obtained when the standard deviation of the reference methods is higher; this is correct because the highest SEP was obtained in NDF (48.68) and its standard deviation from the reference method (Table 1) was 110.1. Along these same lines, Alomar and Fuchslocher (1998), mention that the calibration equations tend to have a better predictive value when they are developed on samples that are relatively homogenous, than when calibrations are done for more heterogenous populations, such as the ones in this work, where precision and accuracy tend to decrease.

With respect to the consistency of the equations that were generated, the obtained values were generally near 100. NIRVIS (2000) indicates that for a calibration to be acceptable, the consistency value must be close to 100, since it describes the relationship between the standard errors of the calibration and validation sets. In the case of the ADL fraction, we observe that it has a value of 125.90 for the consistency of the equation, a SEP of 10.02, and r² C-Set of 0.77 and an r² V-Set of 0.78. The SEP and consistency of the equation indicate that, in spite of having regression coefficients that were relatively low, the predictions that were made can be reliable.

Validation of the near-infrared spectrometer predictions

Table 3 shows the comparison between the laboratory values and the NIRS estimates, using samples that were completely different from those used for the calibration and from the sampling sites. The CP parameter got a regression coefficient of 0.93, slope of the

Table 3. Relationship between NIRS and the laboratory for the evaluated parameters, showing the regression equation, regression coefficient, % Diff and Pearson correlation coefficient.

Parameter	Regression equation	r ²	%Diff	R ²
DM	y = -0.3206x + 1239.5	0.72	2.53	-0.85
OM	y = 0.4739x + 495.38	0.68	6.14	0.83
ASH	y = 0.2488x + 67.947	0.28	28.68	0.52
NDF	y = 0.7424x + 153.85	0.88	2.42	0.94
ADF	y = 0.5435x + 136.88	0.87	3.91	0.93
ADL	y = 0.5689x + 22.568	0.56	2.08	0.75
CP	y = 0.9892x - 13.354	0.93	10.69	0.96

DM: dry matter; OM: organic matter; ASH: ashes; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; r²: regression coefficient; % Diff: percentage difference between laboratory values and NIRS values and R²: Pearson correlation coefficient.

straight line of 0.99 and a mean difference between NIRS and the laboratory of 10.69%. The Pearson correlation coefficient (0.96) shows that the correlation is strong and the regression analysis confirms this by finding that the estimates made by NIRS were significantly related ($P < 0.01$) to the reference analyses.

The results show that the NIRS technique may better predict the CP fraction in the case of the evaluated fodder species, in view of the highest observed regression coefficient, which was 0.93, Berardo (1997), obtained coefficients of 0.97 in white clover, García-Ciudad *et al.* (1999) got values going from 0.90 to 0.96 in heterogenous samples from native prairies in Spain, and Cozzolino *et al.* (2006) report coefficients of 0.90 for corn silage samples. These results are within the range of what is reported in the present study.

The NDF fraction got a regression coefficient of 0.87, a slope of 0.74 and a difference of 13 grams between the NIRS and the laboratory (2.42%). The analysis shows that the NIRS estimates are significantly related ($P < 0.01$) to the laboratory analyses; in turn, the correlation coefficient (0.94) indicates that the correlation between NIRS and the laboratory is very strong.

For ADF, a difference of 3.91 % was obtained, a slope of the straight line of 0.54 and a regression coefficient of 0.87. From the regression analysis we have that NIRS estimates are significantly related ($P < 0.01$) to the laboratory analysis. The Pearson correlation coefficient confirms this since there is a strong correlation between the NIRS and the laboratory.

In NDF and ADF contents, adequate coefficients were obtained of 0.87, which are within the range reported in the literature. Berardo (1997) found values of 0.97 and 0.96 for NDF and ADF respectively. Mentink *et al.* (2006) report 0.90 for NDF; Cozzolino *et al.* (2006) obtained coefficients of 0.84 for NDF and 0.81 for ADF in samples of corn silage.

For the DM content, a regression coefficient of 0.72 was obtained, a slope of the regression straight line of -0.32 and a difference between NIRS and the laboratory of 2.53%. The Pearson correlation coefficient (0.85) shows that there is a negative correlation between the laboratory and NIRS, which is confirmed by the P value indicating that the NIRS estimates were significantly related ($P < 0.01$) to the reference analyses.

OM showed a 6.14% difference between NIRS and the laboratory, a 0.68 regression coefficient and the slope was 0.47. The P value ($P < 0.01$) indicated that the reference analyses are significantly related to NIRS, which is confirmed by the Pearson correlation coefficient of 0.83.

For DM content, the regression coefficient was 0.72. This is greater than that reported by Garnsworthy *et al.* (2000) who obtained values of 0.47, which they attribute to the loss of humidity in the samples during storage. This inconvenience may be solved by determining DM content prior to the moment when the samples are explored; however, there are no published reports where the use of this procedure is mentioned in order to avoid low predictions. It is worth mentioning that the average weight of the samples that were used for the validation in this study was 931 g DM kg⁻¹ and that in the spectrometer predictions, a mean was obtained of 956 g DM kg⁻¹, which explains the high regression coefficients observed in this work. Other authors state that NIRS is a technique that can be used to estimate DM, when finding coefficients greater than 0.80 (Cozzolino *et al.*, 2006). In any case, the prediction value that was obtained in this work may be considered to be acceptable.

The ADL content had a 0.56 regression coefficient and a 2.08% difference may be observed between NIRS and the laboratory, with a slope of the straight line of 0.57. The regression analysis shows that the NIRS estimates were significantly related ($P < 0.01$) to the reference analysis and the correlation coefficient (0.75) indicates that the correlation is moderately strong.

The regression coefficient for ADL that was found in this work (0.56) was under that reported by Berardo (1997), who got a value of 0.89. This result may be explained as due to a great variation in the samples that were used to calibrate the equipment. Alomar and Fuchslocher (1998) state that the low determination coefficients in the fibrous fractions are probably due to the fact that the spectral data obtained through NIRS represent the real chemical structure of the sample, in a more precise manner than the determination done through wet chemistry, such as raw fiber, NDF, ADF, among others; these do not correspond to well defined molecular entities but to empirical determinations and thus, do not allow us to define the real chemical groups that are involved.

The ASH content had a 0.27 regression coefficient and a difference of 28.68% between NIRS and the laboratory; the slope was 0.25. The P value shows that NIRS estimates were significant ($P < 0.05$) with the reference analysis. The Pearson correlation coefficient (0.52) shows that the relationship between NIRS and the laboratory was moderately strong.

Ashes are mainly composed of minerals, according to the principles of NIRS technique. This is based on the virtues of organic compounds, which are negatively related to inorganic compounds (Garnsworthy *et al.*, 2000). The regression coefficient for ASH was 0.27, a value that is under those reported in the literature. Mentink *et al.* (2006) got 0.77, Berardo (1997) 0.84 and Garnsworthy *et al.* (2000), 0.93. However, in other works using compound feed (Murray, 1996) the low correlations for ashes are attributed to the variation in mineral content of feed, a situation which could be present in this work, given the heterogeneity of the samples that were composed of different grass species.

A calibration of the NIRS equipment is considered ideal if it predicts the chemical composition with an order of error that is similar to those achieved using wet chemistry; that is, that the calibrations depend on the analytical procedures that were used in order to provide reference values. The heterogeneity (of species, of the vegetative state, prairie and crop) of the samples used in this work, may have caused the relatively low regressions for ASH content. Alomar and Fuchslocher (1998) mention that the calibration equations tend to have a better predictive value when they are developed on samples with a relatively homogenous nature, or corresponding to the same type of product (*e.g.*, alfalfa hay). On the other hand, when one is trying to develop calibrations for more heterogenous populations with a broader base (*e.g.* straw, hay and silage from different plant species as a whole), precision and accuracy tend to decrease, as they did in this work, especially for ash content.

CONCLUSIONS

Considering the study conditions, we conclude that NIRS is a useful and economical tool that can be used to estimate some of the chemical characteristics (NDF, ADF, OM, CP, ADL and DM) of a broad spectrum of fodder samples. However, this technique proved to be less precise in the case of ASH estimates, for which further research is needed.

The drying of samples prior to obtaining their spectrum helps to improve the calibration of the spectrometer and hence its predictions. In order to maintain the structural and chemical integrity of the samples, the drying method that is mostly recommended is liofilization (Alomar and Fuchslocher, 1998).

It is necessary to calibrate the NIRS with locally obtained data, since as we have shown in this study, the variation coming from the diversity of species that are present in the region may affect the accuracy of the calibrations. For this reason, data bases of spectra that were obtained in latitudes other than those of Mexico, may not work for the fodder species that exist in the country.

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REFERENCES

- Albarrán-Portillo, B., López-González, F., Ruiz-Albarrán, M., & Arriaga-Jordán, C.M. (2019). Respuesta productiva y económica a la suplementación con concentrados de vacas lecheras en pastoreo con alta carga animal. *Revista Mexicana de Ciencias Pecuarias*, 10,855-869. <https://doi.org/10.22319/rmcp.v10i4.4787>
- Alomar, D., & Fuchslocher, R. (1998). Fundamentos de la espectroscopia de reflectancia en el infrarrojo cercano (NIRS) como método de análisis de forrajes. *Agro Sur*, 26,88-104. <https://doi.org/10.4206/agrosur.1998.v26n1-11>
- AOAC. (1990). *Methods of analysis of the association of official analytical chemists*. (15th Ed.). Arlington, VA: Association of Official Analytical Chemists. 771 p.

- Beever, D.E., & Mould, F.L. (2000). Forage Evaluation for Efficient Ruminant Livestock Production. *In: Givens, D.I., Owen, E., Axford R.F.E., & Omed, H.M. (Eds). Forage Evaluation in Ruminant Nutrition. CABI Publishing, Wallingford, UK.*
- Berardo, N. (1997). Prediction of the chemical composition of white clover by Near-infrared reflectance spectroscopy. *Grass and Forage Science*, 52,27-32. <https://doi.org/10.1046/j.1365-2494.1997.00050.x>
- Cozzolino, D., Fassio, A., Fernandez, E., Restaino, E., & La Manna, A. (2006). Measurement of chemical composition in wet whole maize silage by visible and near infrared reflectance spectroscopy. *Animal Feed Science and Technology*, 129,329-336. <https://doi.org/10.1016/j.anifeedsci.2006.01.025>
- Ferret, A. (2003). Control de Calidad de Forrajes. XIX Curso de especialización FEDNA. Madrid, 23 y 24 de octubre.
- García, E. (2004). Modificación al sistema de clasificación climática de Köppen para adaptarlo a las condiciones de la República Mexicana. Cuarta edición. UNAM. México, D.F. 217 p.
- García-Ciudad, A., Ruano, A., Becerro, F., Zabalgoagezcoa, I., Vázquez de Aldana, V.R., & García-Criado B. (1999). Assessment of the potential of NIR spectroscopy for the estimation of nitrogen content in grasses from semiarid grasslands. *Animal Feed Science and Technology*, 77,91-98. [https://doi.org/10.1016/S0377-8401\(98\)00237-5](https://doi.org/10.1016/S0377-8401(98)00237-5)
- Garnsworthy, P.C., Wiseman, J., & Fegeros, K. (2000). Prediction of chemical, nutritive and agronomic characteristics of wheat by near infrared spectroscopy. *Journal of Agricultural Science*, 135,409-417. <https://doi.org/10.1017/S0021859699008382>
- Hodgson, J. (1994). Manejo de pastos. Teoría y práctica. Diana. pp. 55-180. México, D.F.
- López-González, F., Sánchez-Valdés J.J., Castelán-Ortega, O.A., Albarrán-Portillo, B., & Estrada-Flores, J.G. (2015). Agronomic and nutritional characteristics of three grass species in the southern region of Mexico. *Indian Journal of Animal Sciences*, 85,271-274.
- Macho, A.S. (2002). Metodologías analíticas basadas en espectroscopia de infrarrojo y calibración multivariante. Aplicación a la industria petroquímica. Tesis doctoral. UNIVERSITAT ROVIRA I VIRGILI. Departament de Química Analítica i Química Orgànica. Tarragona, España.
- Mentink, R.L., Hoffman, P.C., & Bauman, L.M. (2006). Utility of Near- infrared Reflectance Spectroscopy to Predict Nutrient Composition and In Vitro Digestibility of Total Mixed Rations. *Journal of Dairy Science*, 89, 2320. [https://doi.org/10.3168/jds.S0022-0302\(06\)72303-7](https://doi.org/10.3168/jds.S0022-0302(06)72303-7)
- Minitab (2003). V 14. Statistical software. User's guide II: Data analysis and quality tools, graphics, and macros. USA.
- Murray, I. (1996). The value of traditional analytical methods and near-infrared (NIR) spectroscopy to the feed industry. *In: Garnsworthy, P.C., & Cole, D.J.A. (Eds). Recent Advances in Animal Nutrition. pp. 87-110. Nottingham University Press. UK.*
- NIRVIS. (2000). Training course. For the practical Application of the FT-NIR-Spectrometer NIRVIS with the NIRCAL Software. Buchi Labortechnik AG.
- Sapienza, D., Berzagui, P., Martin, M., Taysom, D., Owens, F., Mahanna, B., Sevenich D., & Allen R. (2008). Near Infrared Spectroscopy for Forage and Feed Testing. NIRS Consortium. Web Site www.uwex.edu/ces/forage/NIRS/home-page.htm (September 19th).
- Starks, P.J., Zhao, D., Phillips, W.A., & Coleman, S.W. (2006). Herbage Mass, Nutritive Value and Canopy Spectral Reflectance of Bermudagrass Pastures. *Grass and Forage Science*, 61,101-111. <https://doi.org/10.1111/j.1365-2494.2006.00514.x>
- Stolter, C., Julkunen-Tiitto, R. & Ganzhorn, J.U. (2006). Application of near infrared reflectance spectroscopy (NIRS) to asses some properties of a sub-arctic ecosystem. *Basic and Applied Ecology*. 7,167-187. <https://doi.org/10.1016/j.baae.2005.05.002>
- Valente, M.E., Borreani, G., Peiretti, P.G., & Tabacco, E. (2000). Codified Morphological Stage for Predicting Digestibility of Italian Ryegrass during the Spring Cycle. *Agronomy Journal*, 92,967-973. <https://doi.org/10.2134/agronj2000.925967x>
- Van Soest, P.J., & Wine, R.H. (1967). Uses of detergents in the analysis of fibrous feeds. IV Determination of plant-cell-wall constituents. *Journal of Association of Official Analytical Chemists*, 50,50-55. <https://doi.org/10.1093/jaoac/50.1.50>
- Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74,3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Villalobos, G.C., González, V.E., & Ortega, S.J.A. (2000). Techniques for estimation of dietary nitrogen and organic matter degradability in the rumen of grazing ruminants: a review. *Técnica Pecuaria México*. 38,119-134.