DANIEL MARTINS DE OLIVEIRA

PREDICTABILITY OF DRY MATTER INTAKE, DIET COMPOSITION AND DIGESTIBILITY IN BEEF CATTLE USING FECAL NEAR-INFRARED REFLECTANCE SPECTROSCOPY

LAVRAS – MG

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Dissertation presented to the University of Lavras, as part of the requirements of the Graduate Program in Animal Science, area of concentration in Ruminant Production and Nutrition, to obtain the title of Master.

Prof. Marina de Arruda Camargo Danés, PhD.
Advisor
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PREDICTABILITY OF DRY MATTER INTAKE, DIET COMPOSITION AND DIGESTIBILITY IN BEEF CATTLE USING FECAL NEAR-INFRARED REFLECTANCE SPECTROSCOPY

PREDIÇÃO DO CONSUMO DE MATÉRIA SECA, COMPOSIÇÃO DA DIETA E DIGESTIBILIDADE EM BOVINO DE CORTE USANDO ESPECTROSCOPIA DO INFRAVERMELHO PRÓXIMO EM FEZES

Dissertation presented to the University of Lavras, as part of the requirements of the Graduate Program in Animal Science, area of concentration in Ruminant Production and Nutrition, to obtain the title of Master.

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2019

To my parents Márcio and Enery and my uncles Vanildo (in memoriam) and Nilva for being my base, responsible for what I became today, for all the love and affection, for all the support and for teaching me the true sense of charity.

I DEDICATE

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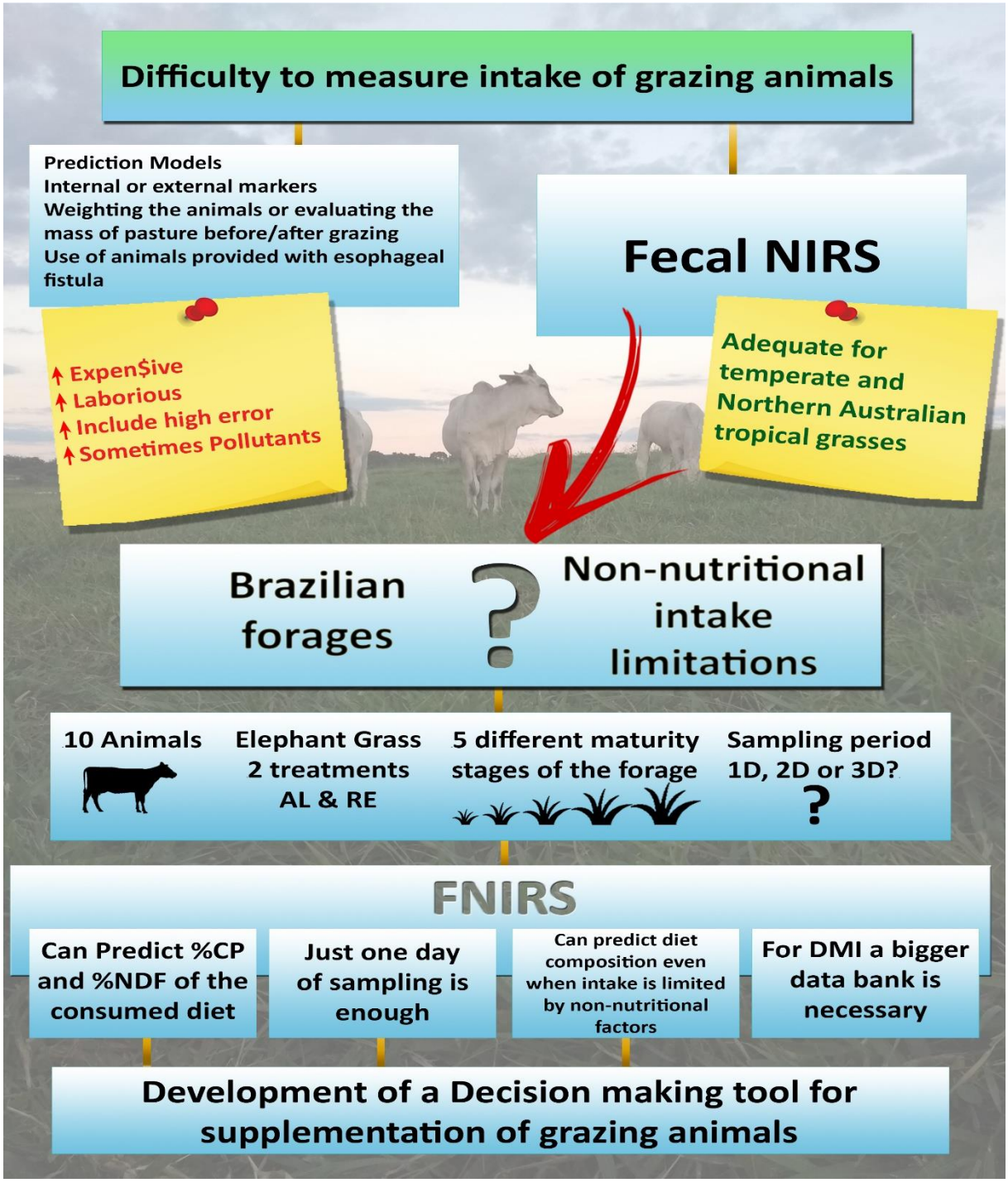
“Continuous effort — not strength or intelligence — is the key to unlocking our potential.”

Winston Churchill.

INTERPRETATIVE SUMMARY

Measuring intake of grazing animals is hard, laborious and expensive. Fecal NIRS (F.NIRS) has been proven adequate to predict forage intake and diet composition of animals grazing temperate and Australian tropical grasses. We decided to verify if it works for Brazilian forages and if the technique is able to predict intake when it is limited by non-nutritional factors. We used ten Nelore heifers, two treatments: ad libitum and restricted at five different maturity stages of forage and evaluated three sampling days. Our results show that F.NIRS can predict % CP and % NDF of the consumed diet, requiring just one day of sampling and can predict diet composition even when intake is limited by non-nutritional factors. For DMI a bigger data set might be necessary, however, such results open ways to the development of a decision making tool for supplementation of grazing animals.



RESUMO

O objetivo deste projeto foi usar o Fecal NIRS (F.NIRS) para desenvolver equações de calibração para predizer o consumo de forragem, composição da forragem consumida e digestibilidade de nutrientes de gramíneas tropicais quando o consumo é limitado por fatores nutricionais ou não nutricionais. Dez novilhas Nelore foram alojadas em baias individuais, com piso de concreto e acesso livre a água fresca durante 5 períodos experimentais divididos em 10 subperíodos de 7 dias cada. Os animais foram blocados em pares de acordo com a idade e peso corporal e, dentro de cada bloco, foram divididos aleatoriamente em uma sequência de dois tratamentos: alimentação ad libitum ou restrita. A dieta era oferecida duas vezes ao dia (às 07:00 e 14:00), composta exclusivamente por Pennisetum purpureum (capim-elefante), colhido e picado diariamente. O consumo individual de forragem foi medido diariamente, pesando-se a forragem oferecida e as sobras. Durante o período de amostragem (3 dos 7 dias de cada subperíodo), amostras de forragem oferecida e das sobras foram coletadas diariamente e a produção fecal total diária foi medida coletando todas as fezes no chão de cada baia a cada 6 horas, imediatamente após os animais evacuarem, as amostras foram secas e moídas em peneira de 1 mm para análise posterior. Amostras fecais individuais de cada animal foram submetidas à varredura no NIRS. Os espectros foram registrados no modo de refletância difusa usando um espectrômetro FT-NIR e armazenados usando o Software OPUS. Os espectros fecais foram então utilizados para desenvolver equações para predizer a composição da dieta, digestibilidade e consumo de matéria seca. Os coeficientes de determinação para calibração (R^2c) e validação cruzada (R^2cv) para predição da composição da dieta foram excelentes para % Proteína Bruta (PB) ($R^2c = 0,99$; $R^2cv = 0,97$), % Fibra em Detergente Neutro (FDN) ($R^2c = 0,97$; $R^2cv = 0,95$) e % Matéria Orgânica (MO) ($R^2c = 0,98$; $R^2cv = 0,97$) com um RPD maior que 4,5 para todos os parâmetros. Para digestibilidade de PB (dPB), dFDN e dMO, R^2c e R^2cv foram maiores que 0,90 e RPD maior que 3. Para o Consumo de Matéria Seca (CMS) ($R^2c = 0,96$; $R^2cv = 0,77$) e dMS ($R^2c = 0,93$; $R^2cv = 0,88$) e RPD menor que 3 para ambos os parâmetros. F.NIRS mostrou seu potencial para predição de % PB e % FDN da dieta consumida, exigindo apenas um dia de amostragem, mesmo quando a ingestão é limitada por fatores não nutricionais. Mais pesquisas são necessárias para desenvolver equações de calibração mais robustas e com maior número de amostras para a predição de CMS, no entanto, nossos resultados fornecem boas evidências de que calibrações aprimoradas de F.NIRS podem ser usadas como um método alternativo para monitorar a composição da dieta de animais em pastejo.

Palavras-chave: Animais em pastejo. Monitoramento de dieta. Zootecnia de precisão

ABSTRACT

The objective of this project was to use F.NIRS to develop calibration equations to predict forage intake, composition of the consumed forage and nutrient digestibility of tropical grasses when the intake is limited by either nutrition or non-nutritional factors. Ten Nelore heifers were housed in individual pens with concrete floors and free access to fresh water during 5 experimental periods divided into 10 sub periods of 7 days each. Animals were blocked in pairs according to age and body weight and, within each block, randomly assigned to a sequence of two treatments: *ad libitum* or restricted fed. Diet was offered twice daily (at 07:00 and 14:00), composed exclusively of *Pennisetum purpureum* (Elephant Grass), daily harvested and chopped. Individual feed intake was measured daily by weighing feed offered and orts. During the sampling period (3 out of 7 days per sub period), samples of the feed offered and orts were collected daily and total daily fecal production was measured by collecting all feces in each pen's floor as soon as the animal excreted them every 6 hours. Dried and ground individual fecal samples from each animal were subjected to NIRS scanning. Spectra were recorded in diffuse reflectance mode using a Fourier transformation NIR spectrometer and stored using OPUS Spectroscopy Software. Fecal spectra were then used to develop equations to predict diet composition, digestibility and dry matter intake. Coefficients of determination for calibration (R^2c) and cross-validation (R^2cv) for prediction of diet composition were greater for % CP (R^2c = 0.99; R^2cv = 0.97), % NDF (R^2c = 0.97; R^2cv = 0.95) and % OM (R^2c = 0.98; R^2cv = 0.97) with an RPD higher than 4.5 for all parameters. For CPd, NDFd and OMd, R^2c and R^2cv were greater than 0.90 and RPD higher than 3. For DMI (R^2c = 0.96; R^2cv = 0.77) and DMd (R^2c = 0.0.93; R^2cv = 0.88) and an RPD lower than 3 for both parameters. F.NIRS showed its potential for prediction of % CP and % NDF of the diet consumed, requiring just one day of sampling, even when intake is limited by non-nutritional factors. Further research is needed to develop more robust and larger calibration equations for the prediction of DMI, however, our results provides good evidence that improved F.NIRS calibrations can be used as an alternative method to monitor diet composition of grazing animals.

Keywords: Diet Monitor. Grazing animals. Precision farming.

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LIST OF ABBREVIATIONS

AL *Ad Libitum* fed treatment

BW Body Weight

CP Crude Protein

CPd Crude Protein digestibility

DM Dry Matter

DMd Dry Matter digestibility

DMI Dry Matter Intake

F. NIRS Fecal Near Infrared Spectroscopy

LV Latent Variables

MSC Multiplicative Scatter Correction

NDF Neutral Detergent Fiber

NDFd Neutral Detergent Fiber digestibility

NIR Near Infrared

NIRS Near Infrared Reflectance Spectroscopy

OM Organic Matter

OMd Organic Matter digestibility

PLS-R Partial Least Squares Regression

RE Restricted fed treatment

RESEP Root Mean Standard Error of Prediction

RMSEC Root Mean Standard Error of Calibration

RMSECV Root Mean Standard Error of Cross-Validation

RPD Ratio of Performance to Deviation

SD Standard Deviation

SNV Standard Normal Variate

SUMMARY

1. INTRODUCTION

Grazing ruminant performance is closely related to intake and the digestibility of forages, with 60 to 90% of animal performance being explained by variations in intake, leaving only 10 to 40% for digestibility (Mertens, 1994). Determining voluntary intake of animals in confinement is facilitated due to man-controlled conditions, such as the amount of feed supplied, processing, individual assessment capacity, etc. On the other hand, when animals are under grazing conditions, measuring intake can be difficult. In terms of quality and quantity, estimations of intake by grazing animals have usually been based on weighting the animals or evaluating the mass of pasture before and after grazing, use of animals provided with esophageal fistulas or use of internal and external markers from plant constituents (e.g. alkanes and long chain fatty acids). However, such measurements are laborious, time consuming, costly and are often associated with large errors (Poppi et al., 2000).

Holloway et al. (1981) proposed the concept of using fecal components to predict intake of beef steers and determined that 70% of the between-animal variation in intake and digestibility could be explained through feces. The collection of feces is easy and does not harm or interfere with animals. According to the same authors, the chemical information contained in feces is inherently representative of the consumed diet and is related to intake and digestibility. Based on this principle, another approach developed to estimate diet characteristics and intake was the application of near infrared reflectance spectroscopy of feces (F.NIRS). Near infrared reflectance spectroscopy has been shown to be an accurate tool in predicting the digestibility and chemical composition of forages (Coates & Dixon, 2007; Tolleson & Schafer, 2014), but the application of F.NIRS to predict voluntary intake is only at the beginning. With the appropriate calibration equations, F.NIRS is a rapid and non-destructive method that could predict the digestibility and intake of a large set of similar samples.

Although previous research have demonstrated the ability of this technique to predict voluntary intake, diet quality and digestibility (Agnew et al., 2004, Decruyenaere et al., 2009, Johnson et al., 2017), the technology has not yet been used to provide information about intake when the limitation relates to non-nutritional characteristics of the canopy, such as sward structure. In addition, further research is necessary to generate robust and accurate prediction equations to estimate forage intake across a variety of forages, maturity stages and production systems with unfavorable sward conditions.

2. OBJECTIVE

The objective of this project was to use F.NIRS to develop calibration equations to estimate forage intake, composition of the consumed forage and nutrient digestibility of tropical grasses when the intake is limited by either nutrition or non-nutritional factors.

3. LITERATURE REVIEW

3.1. Forage Intake in Grazing Cattle

The majority of livestock in Brazil and in the world is produced in grassland areas. These areas cover about 3 billion hectares on the planet (Hedley, 1993).

The efficiency of available forage intake related to grazing management directly affects animal performance (Reis & Silva 2006). Voluntary intake is essential to nutrition and is the major responsible for animal responses as it determines the nutrients ingested in a diet and the efficiency in which they are used in metabolic processes (Van Soest, 1994). The voluntary intake is affected by grazing management, forage plant, environment and the animals. Understanding these factors correctly is important to apply measures aimed at optimizing the productive process, improving animal performance. The pattern of animal intake is directly influenced by the structure of sward, which determines the degree of ease that animals will have when grazing (Carvalho & Moraes, 2005).

Intake is influenced by canopy characteristics, and may be related to the ingestive behavior of grazing animals (non-nutritional factors) and aspects related to forage chemical composition, digestibility and metabolic factors (nutritional factors) (Hodgson, 1990). The rate of passage in the digestive tract of the animal, digestibility and chemical composition including the amount of antinutritional substances in forage plant, are nutritional characteristics that influence intake. Non-nutritional characteristics are related to the canopy structure, such as sward height, density of plant biomass, presence of barriers to defoliation, and the spatial arrangement of preferred plant components (Minson, 1990). The rate of selection and efficacy of forage harvesting by grazing animals are factors determined by these characteristics (Stobbs, 1995), and these factors have the greatest control in the limitation of forage intake in grazing animals (Poppi et al., 1987).

To evaluate intake, the management of characteristics of the canopy is important, as it directly influences the behavior of ingestion of animals, mainly in the amount of forage obtained per bite. The forage mass apprehended per bite is the most important variable in determination of pasture intake and the most influenced by canopy structure (Hodgson, 1990). The mass per bite summarizes between the product of the bulk density of forage and the volume of bite in the grazed layer. The quantity of forage ingested per bite is very sensitive to variations in canopy structure, especially sward height (Coleman, 1992). With a reduced mass per bite, a drop in intake rate occurs, even if there are more bites taken. Daily forage intake will also be

impaired if there is a reduction in intake rate without being compensated by an increase in grazing time.

In canopies kept with low forage supply (lower height) the bite mass decreases, but the bite rate and / or grazing time increase (Penning & Johnson, 1983). When managed with higher height, the pasture is evaluated as unfavorable to the animal's forage intake, with a high production of forage mass that will hinder the apprehension and ingestion of the mass (Palhano et al., 2007). An even bigger problem in canopies too high is the competition for light among the plants, which promotes stem development. The greater proportion of stems acts as an obstacle for the animals to graze the green leaves and decreases forage intake (Hodgson, 1990). Both situations (pastures too low and too high) are common in Brazil.

In very extensive systems, with no fertilization, pastures are usually overgrazed and forage availability is limited, which compromises intake. On the other hand, on intensive systems, with high doses of fertilization, tropical grasses have very rapid growth rates and if the grazing management is not very good, canopies can easily become too high. Therefore, it is logical to assume that non-nutritional factors are more frequently limiting intake than the forage composition.

3.2. Methods to estimate forage intake by grazing animals

The estimation of animal intake in pasture is challenging, since it is affected by several variables, which are classified as nutritional and non-nutritional, as well as animal-related factors (Hodgson, 1990). When the dry matter intake (DMI) is estimated, it is possible to determine the intake of a specific nutrient by multiplying the DMI by the concentration of nutrient in the dry matter consumed. All methods used present limitations, which can induce significant errors for the livestock production or research system.

Weigh animals during grazing (or before and after) is one of the most direct methods to determine DMI. Even though it has several limitations, it can be used when there is interest in the ingestive behavior, it can be applied to relatively short grazing periods and it is applicable to large and small ruminants. The short time to record data and the need to correct possible losses through respiration, feces and urine and gains from ingestion of minerals, water, and soil that are not related to forage are its greatest limitation. Considering intake of fresh material and not dry matter, due to the lack of information on the chemical composition of grazed material is another negative point of this technique (Berchielli et al., 2011).

Another possible way to measure intake is to evaluate the mass of forage before and after grazing. However, this technique can overestimate the DMI for not taking into account

some factors that contribute to the disappearance of the mass such as trampling, assuming that all the disappeared mass was grazed (Minson, 1990; Burns et al., 1994). Another negative point of this technique is the necessity of a large number of samples collected in the paddock, in order to have a reliable estimate of the difference before and after grazing.

The use of collecting bags for total fecal collection is another alternative to estimate DMI by means of dry matter digestibility (Lippke, 2002). It has the advantage of being a fast method to obtain results, since it only requires the analysis of dry matter and mineral matter. The disadvantage is that it can reduce animal performance, since the bags may interfere with the animal's ingestive behavior, cause distortion in the legs of the animals, incomplete collection due to losses and by the bags being of expensive material (Burns et al., 1994). It is a more recommended technique for small ruminants, because their feces have a higher content of dry matter and the animals are smaller.

The use of animals provided with an esophageal fistula allows for the collection of samples to be analyzed in order to obtain the chemical composition or the digestibility of the forage actually consumed by the animal. The analysis may be by in vitro digestibility assays (Pond et al., 1989), in situ (Nocek, 1988) or by the in vitro gas production method (Malafaia et al., 1996). However, using fistulated animals is a very invasive method, which is currently hardly approved by ethics committees in the use of experimental animals, in addition to the animals being of different breeds and physiological state of experimental animals. Variations in the results of the method is also an inconvenience in its use.

Another very usual, but expensive and laborious way to determine the fecal production and flow of the duodenal digest, which from these values, allows the estimation of parameters such as digestibility and intake, is the use of external or internal markers. Markers are indigestible substances, generally of easy determination, that can be administered with the feed or in some segment of the digestive tract, being later identified and quantified in feces. The ideal marker must have fundamental properties to generate reliable data. The main ones are: (I) being inert; (II) not be toxic to the animal or to the person administering the marker; (III) have no physiological function; (IV) not be metabolized; (V) mix with the food and remain evenly distributed in the digest; (VI) have no influence on motility or intestinal secretions; (VII) not cause disturbance or enhance the microflora of the digestive tract and its hosts; (VIII) have a specific and sensitive method of determination; (IX) have physical-chemical characteristics that do not interfere in the digestive processes (Berchielli et al., 2011). The frequency of administration of the marker is a major difficulty of the method, which is difficult to ensure continuous administration, necessitating the use of slow release capsules which do not always

meet the release rate specified by the supplier company. Sampling frequency is also an inconvenience because it depends on the frequency of administration of the marker and it is recommended to collect the feces directly from the rectum of the animals, requiring containment and management of the animals to collect feces free of soil contamination. Another aspect that makes the method laborious is the recovery of the marker, which often can not be recovered efficiently (Lippke, 2002), because it has been transformed into other compounds or absorbed in the digestive tract and depends on factors such as diet, type of marker used, animal species and variability among animals.

3.3. Near Infrared Spectroscopy

Near Infrared Spectroscopy (NIRS) is a type of vibrational spectroscopy based on electromagnetic radiation at wavelengths 700 to 2500 nm. NIRS basic principles involve creation, recording and interpretation of spectra resulting from interaction of electromagnetic radiation with organic matter (Manley et al., 2008). This radiation relies on the sample state (liquid or solid, opaque or transparent) that can be absorbed, transmitted or reflected when interacting with the analyzed sample. Thus, there are several modes of measurement in NIRS, which process different applications, being diffuse transmittance and diffuse reflectance the most used (Huang et al., 2008).

According to Woodcock et al. (2008), the reaction of molecular bonds (e.g. CH, NH, OH) to electromagnetic radiation in NIRS creates a characteristic spectrum of a sample acting as a "fingerprint". Since it contains chemical and physical information of sample, this spectrum can generate rich information about composition of a product of interest (Katsumoto et al., 2001). It is the shape of the spectral line, or the rate of change in slope with wavelength, which transmits chemical information (Murray et al., 1987). Spectrum generated in forage NIRS include water, in two bands at 1450 nm (first harmonic band) and 1940 nm (combined band); lipids (CH) in bands at 1210, 1400, 1725 and 2310 nm; and carbohydrates (OH) in bands at about 1600 and 2100 nm (Murray, 1983). Absorbance of NH due to starch structures present in protein occur largely at 2180 and 2055 nm (Wetzel, 1983), but these are often masked by the wide carbohydrate band at 2100 nm. Demanding subsequent pre-treatments to minimize particle size effects.

The main components of a NIRS apparatus are light source, beam splitter system (to select wavelength), optical detector, sample holder and data analyzer system (Manley et al., 2008). There are several devices available for NIRS analysis. The most commonly used to select

wavelengths are monochromators that scan the full wavelength range using a grid or prism and provide maximum versatility.

Different chemical bonds have absorption at specific wavelengths and can be used to define chemical composition of different substances. The problem is that different molecules have absorption peaks overlapping at various points in the spectral region. Due to this, mathematically processing the spectral data is necessary in order to obtain valuable information on the chemical properties of the samples. Chemometrics is the chemical discipline that uses mathematical and statistical methods to plan or select optimal conditions of measurements and experiments, and extract as much information as possible from chemical data.

Calibration is the construction of a prediction model, being the most important part in the development of an equation in NIRS. Calibration is a multivariate analysis model that will allow the prediction of chemical composition of sample based on spectral data. Several steps are involved in calibration process: Acquisition of spectral data; use of appropriate reference methods to determine concentration of what has been analyzed in a number of samples; pre-treatment of spectrum data to reduce the effects of particle size dispersion and improve the spectrum obtained; use of chemometrics in order to relate the spectra to the analyzed samples; and finally validate the models using another set of samples not used in the calibration set (Cen and He, 2007).

NIRS can also be used to estimate variation among animals in diet composition, digestibility and intake based on the spectra generated from chemical components of the animals' feces. About 70% of variation in digestibility and intake among animals is explained through a diverse range of chemical components found in feces (Holloway et al., 1981). This application has been evaluated as a means of predicting digestibility (Garnersworthy and Unal, 2004; Tran et al., 2010), diet quality (Jancewicz et al., 2017; Johnson et al., 2017) and voluntary intake (Tran et al., 2010; Johnson et al., 2017) in beef and dairy cattle.

4. MATERIAL AND METHODS

4.1. Experimental Animals and Design

The experiment was conducted in the Beef Cattle Research Center of the Department of Animal Sciences of the University of Lavras. All animal-use procedures were approved by the Ethics Committee for Animal Research of the University of Lavras (protocol #115/18).

Ten Nelore heifers were housed in individual pens with concrete floors and free access to fresh water during the experimental periods. Animals were blocked in pairs according to age and body weight (BW) and, within each block, randomly assigned to a sequence of two treatments: *ad libitum* (AL) or restricted (RE) fed. Diet was offered twice daily (at 07:00 and 14:00), composed exclusively of *Pennisetum purpureum* (Elephant Grass), daily harvested to ground level and chopped. The AL treatment was offered an amount of grass which allowed at least 5% orts (fresh matter basis), while the RE treatment was fed to 1.1% of their live weight.

The experiment was carried out in 5 periods of 14 days, divided into 2 sub periods of 7 days, when inversion of the treatments within blocks occurred. Within each sub period, 4 days were devoted to adaptation of the animals to treatment and 3 days were for data and samples collections. The periods were designed to provide forages with different stages of maturity (and composition), in order to increase the variability of the response variables (intake, diet composition and digestibility). This is a desirable feature when developing calibration equations. Another important feature is the restricted treatment, simulating a situation with unfavorable sward conditions, in order to evaluate if the F.NIRS can predict intake, diet composition and digestibility under such situations, another point is that in well-managed grazing systems, the chance of good prediction is better. Therefore, the 5 periods took place throughout a period of 8 months being two periods during the dry season (from May to July) and 3 during the rainy season (from October to December). In between periods, the animals were kept in a pasture area of the Department of Animal Sciences.

4.2. Data and Sample Collection and Laboratory Analysis

Individual feed intake was measured daily by weighing feed offered and orts. During the sampling period, samples of the feed offered and orts were collected daily. Dry matter intake was calculated after drying the samples at 65º C for 72 hours in a forced-air oven to measure their DM content. Dried samples were ground through a 1-mm screen in a Wiley mill for laboratory analysis. Equal amounts of daily samples were combined to make one composite sample per period (feed) or per animal per period (orts).

During the sampling period, total daily fecal production was measured by collecting all feces in each pen's floor every 6 hours. At each collection time, feces produced by the animal were weighed and a spot sample were taken as soon as the animals excreted them. The spot sample was always taken from the top of the fecal pile to assure it had no contact with the pen floor. Spot fecal samples were dried and ground in the same way as the feed samples. Equal amounts of ground fecal samples from each animal in each period were combined to form a composite sample.

Composite samples were analyzed according to the Association of Official Analytical Chemists (AOAC, 1990) for dry matter (DM) by oven-drying at 105ºC for 24 h (method 934.01), for ashes by burning in the furnace at 500ºC for 6 h (method 924.05), organic matter (OM) were calculated based on ash, for CP by Kjeldahl method (method 920.87). Neutral detergent fiber (NDF) by using a 5x5 cm non-woven textile (NWT – 100 g/m^2) with 5 g per bag, after being heat-sealed, the bags were autoclaved for 1 hour at 100ºC. The neutral detergent solution was produced according to recommendations by Mertens (2002). The neutral detergent: sample ratio was maintained at 100 mL/ g DM, adapted from Van Soest et al., (1991).

Total tract digestibility of DM (DMd), OM (OMd), NDF (NDFd) and CP (CPd) were calculated by dividing the amounts of nutrients excreted in feces by the amount ingested and subtracting the result from 100%.

4.3.F.NIRS Analysis

Individual fecal samples (i.e. from each time point of fecal collection, 4 per animal per sampling day, not the composites) from each animal were subjected to NIRS scanning. Before scanning, each sample was packed into quartz-lens sample cups and kept in a climate-controlled room (20º C and relative humidity of approximately 65%) during 24 h for samples to reach an equilibrium moisture of approximately 6%. Spectra were recorded in diffuse reflectance mode using a Fourier transformation NIR spectrometer (MPA, Bruker Optik GmbH, Ettlingen, Germany) and stored using OPUS Spectroscopy Software version 7.5. Spectral analysis were performed within the 12.000–4000 cm^{-1} range at 2-cm^{-1} resolution.

The spectra of each sample was obtained by the mean of 16 scans performed twice in the quartz-lens sample cups, one reading obtained after packing the samples and another after mixing the sample inside the quartz-lens sample cups, that is, 2×16 scans per sample.

4.4. Spectral Pretreatment and NIRS Calibration Development

Before calibration, spectral data was transformed using either a standard normal variate (SNV) or a multiplicative scatter correction (MSC) method. Mathematical treatments used to enhance spectral differences were 1.4.4.1 or 2.4.4.2, in which the numbers represent: the derivative; the gap over which the derivative is calculated; the number of points in a moving average, that is, first smoothing procedure; and the number of nm over which the second smoothing is applied, respectively.

Calibration equations were developed from the treated spectral data using the partial least-squares routine of The Unscrambler® software (CAMO AS, Oslo, Norway, v. 9.7). Partial least squares regression (PLS-R) were adjusted based on NIR spectra (matrix X, independent variables) and DMI, OM, CP, NDF contents of the diet, DMd, OMd, CPd and NDFd values, creating the Y matrix as dependent variables.

We generated three data sets to compare the calibration equations:

1) "PERIOD MEAN": all response variables and spectral data from the three collection days were averaged, generating one replication per animal per subperiod. Therefore, for each response variable, the n would be 100 (10 animals x 10 subperiods). However, the forage samples for the last subperiod were burned due to a malfunction of the forced-air oven. Thus, n was 100 for DMI and DMd, but for CP, NDF, OM, CPd, NDFd and OMd, n was 90;
2) "DAILY MEAN": included data for each day of the collection periods (spectral data of the four daily fecal samples were averaged) was averaged for each day of collection periods. Likewise, this dataset included DMI of each day of collection periods. Therefore, for each response variable, the n would be 300 (3 days x 10 animals x 10 subperiods). However, for the reasons explained before, the last subperiod did not have forage composition data. Thus, n was 300 for DMI and DMd, but for CP, NDF, OM, CPd, NDFd and OMd, n was 270;
3) "ONE DAY": database containing data from every first day of collection for each period ("D1"); database containing data from every second day of collection for each period ("D2"); and database containing data from every third day of collection for each period ("D3"). For each day, n = 100 for DMI and DMd and n = 90 for CP, NDF, OM, CPd, NDFd and OMd.

4.5. F.**NIRS Equation Validation**

The calibrations were validated by full cross validation (leave one out) and a test-set (external) validation. Cross-validation is often employed when an independent validation set is unavailable or when removal of samples from a calibration set results in too few samples for effective equation development. Briefly, this process involves removing a certain number of samples during the calibration procedure: for example, 25%, and predicting these with the remaining 75% of the dataset. This step is then repeated until all have served as validation samples. Cross-validation was performed using 7 segments with 14 samples (for PERIOD MEAN and ONE DAY databases) or 7 segments with 42 samples (for the DAILY MEAN database) chosen at random by the software. The external validation was based on two data sets composed by half of the samples in the calibration lot (AL samples) and the other half of the samples in the validation lot (RE samples). The number of latent variables was determined based on the minimization of the standard error of the validation and maximization of the coefficient of determination of the validation.

To develop the models, only wavelengths from 9000 to 4000 cm−1 (1100 – 2500nm) were used for calibrations and the Westad & Martens (2000) uncertainty test was applied for selecting significant wavenumbers to improve the signal-to-noise ratio. Outliers were detected by means of the student x leverage residuals graph and removed from the models.

4.6. Equations Evaluation

The selection of models were based on the following statistics: coefficient of determination for calibration (R^2c), cross validation (R^2cv) and test set validation (R^2p); root mean standard error of calibration (RMSEC); root mean standard error of cross-validation (RMSECV); root mean standard error of prediction (RMSEP), which comes from the test-set validation; and number of latent variables used in the models (LV). The RMSEC represents the variability in the difference between predicted values and reference values when the equation was developed from the calibration data set. The RMSECV/RMSEP represents the variability in the difference between predicted and reference values when the equation is applied sequentially to subsets of data from the calibration data set (Landau et al., 2006). Another criterion of quality of NIRS calibrations is that the ratio of performance to deviation (RPD, calculated as the ratio of standard deviation (SD) to root mean standard error of prediction (RMSEP) or, in the case of cross-validation, to RMSECV) be >2.5 (Williams, 2004). We obtained our RPD using an alternative formula for computing the RPD that is RPD = $1 \div (1 - R^2cv)^{\wedge 0,5}$ (Williams, 2014).

5. RESULTS AND DISCUSSION

5.1. Dry Matter intake, Diet Composition and Digestibility

Each parameter (response variable) and the range of data used to develop the F.NIRS calibration and prediction equations are presented in Table 1. Dry matter intake ranged from 1.7 to 11.7 kg/d. The average diet consumed by the animals varied from 4.8 to 11.3% CP, from 69.5 to 80.7% NDF and from 88.1 to 95.5% OM. Digestibility of all nutrients showed great variation, ranging from approximately 30 to 85% for DM, OM and NDF, and from 7,7 to 86% for CP. Such differences between maximum and minimum values for all the reference methods confirm that the forage chemical composition database is representative of a broad range of growth cycle and maturity stage, which are important factors influencing digestibility of elephant grass (Silva et al., 2007).

Table 1. Range in chemical data used to develop F.NIRS calibration equations for diet composition, digestibility and dry matter intake based on composite fecal samples from beef cattle.

Parameters	N	Minimum	Maximum	Mean	SD
Dry matter intake[1], kg/d	300	1.73	11.66	5.45	1.96
Diet Composition[2]					
CP, % of DM	160	4.79	11.28	6.71	1.92
NDF, % of DM	160	69.54	80.07	73.64	2.56
OM, % of DM	160	88.14	95.48	92.07	2.45
Digestibility					
DMd, %	300	29.14	83.45	62.74	10.60
CPd, %	160	7.72	86.53	57.78	15.71
NDFd, %	160	31.92	85.78	63.88	11.24
OMd, %	160	33.19	85.21	64.05	10.79

[1]Measured in each collection day of all sub periods
[2]CP = Crude Protein; NDF = Neutral Detergent Fiber; OM = Organic Matter

The NIRS analysis rely on developing a calibration that relates the spectra of a large number of fecal samples to their known characteristics such as CP or NDF. This calibration is then used to predict the CP or NDF of independent samples based on their NIR spectra. It is implicit on the technique that the "training" sets on which the calibrations are based contains the whole range of variation in the samples to be analyzed. The bigger the variation among samples, covering a wide range of chemical composition of the forage, the greater is the gain in precision and reliability of the models generated (Williams et al., 2017).

5.2. Prediction of Diet Composition and Nutrients Digestibility

The best calibration equations for diet composition and nutrient digestibility, for datasets "PERIOD MEAN" and "DAILY MEAN", are listed in Table 2, along with the best pretreatment used for calibrations; the final sample size for each calibration after removing outliers and the statistical parameters for model evaluation. The efficiency in terms of accuracy and robustness of F.NIRS equations can be evaluated by various statistical parameters, such as R^2, RMSECV and the RPD ratio. To be acceptable, the NIRS equations must have an R^2 higher than 0.80, a RMSECV close to the RMSEC and an RPD ratio higher than 2.5 for quality screening purposes or 3 to be used for quantitative applications (Williams, 2004).

Based on $R^2c = 0.98$, more of the variability in % CP is explained by F. NIRS analysis than for % NDF ($R^2c = 0.97$). Better calibrations were developed using the "PERIOD MEAN" data (Figure 1A and 1B). Crude protein may be the most commonly measured variable in forages and feedstuffs, but also in feces because there is a strong correlation between fecal CP and diet quality (Fanchone et al, 2009). The calibration equations for diet composition of % CP and % NDF gave R^2cv values >0.95 and RPD greater than 4, being satisfactory and consistent with results compiled from 21 studies using forages composed by temperate forage or tropical forage native to Australia (Dixon and Coates 2009). Our results show great potential in using F.NIRS to monitor diets of ruminants aiming to improve their performance as RMSEC and RMSECV are close to each other for both % CP and % NDF (0.21 and 0.31; 0.44 and 0.55, respectively) and R^2c and R^2cv are greater than 0.90.

Equations developed for % OM gave excellents R^2c and R^2cv, both greater than 0.90 (Table 2 and Figure 1C). Once again, the data set "PERIOD MEAN" showed superior calibrations compared to the data set "DAILY MEAN" with lower RMSEC and RMSECV (0.31 and 0.42; 0.58 and 0.63, respectively) and greater RPD (5.97 and 3.88). Nonetheless, both sets of values appear to be sufficiently robust. Decruyenaere (2009), using NIRS directly on forages obtained unsatisfactory values to estimate the OM voluntary intake ($R^2 = 0.30$; SEC = 7.29g/kg $BW^{0,75}$; SECV = 7.47 g/kg $BW^{0,75}$). However, when working with fecal spectra, they improved the statistical parameters of their NIRS models (R^2 between 0.80 and 0.90, SECV lower than 5g/kg $BW^{0,75}$, leading to RPD values between 2.31 and 2.52). Andueza et al. (2009) reported similar results for OM voluntary intake. Although we predicted the percentage of OM in the diet consumed, our results were much better because it is more related to the fecal spectra than is for OM voluntary intake. When voluntary intake is measured, we have to consider many variables including animal behavior, temperature of the day, management of the animal, forage characteristics and the interaction among them (Pulina et al., 2013).

Table 2. Calibration performance of the fecal NIRS equations for composition of the consumed diet and digestibility

	Data Set	Pretreatment[1]	N	Outliers[2]	R^2c	RMSEC	R^2cv	RMSECV	LV	RPD
Diet Composition										
CP, % of DM	PERIOD MEAN	1 - MSC	89	1	0.98	0.21	0.97	0.31	5	6.08
	DAILY MEAN	1 - SNV	264	6	0.96	0.39	0.95	0.43	4	4.27
NDF, % of DM	PERIOD MEAN	1 - MSC	89	1	0.97	0.44	0.95	0.55	4	4.50
	DAILY MEAN	2 - MSC	267	3	0.92	0.68	0.92	0.76	2	3.64
OM, % of DM	PERIOD MEAN	1 - SNV	86	4	0.98	0.31	0.97	0.42	5	5.97
	DAILY MEAN	1 - SNV	266	4	0.94	0.58	0.93	0.63	4	3.88
Digestibility										
CP, %	PERIOD MEAN	1 -MSC	90	0	0.96	2.95	0.94	3.89	5	4.05
	DAILY MEAN	1 - MSC	269	1	0.90	4.78	0.87	5.52	3	2.79
NDF, %	PERIOD MEAN	2 - MSC	88	2	0.93	2.85	0.90	3.46	2	3.13
	DAILY MEAN	2 - SNV	269	1	0.89	3.66	0.86	4.16	2	2.66
OM, %	PERIOD MEAN	1 - SNV	82	8	0.97	1.62	0.91	2.99	6	3.42
	DAILY MEAN	2 - SNV	269	1	0.90	3.31	0.86	3.93	3	2.70

R^2c and R^2cv represent coefficient of determination for calibration and cross-validation, respectively; RMSEC and RMSECV are the root mean standard error of calibration and cross-validation; LV = Latent variables; RPD = Ratio of prediction to deviation

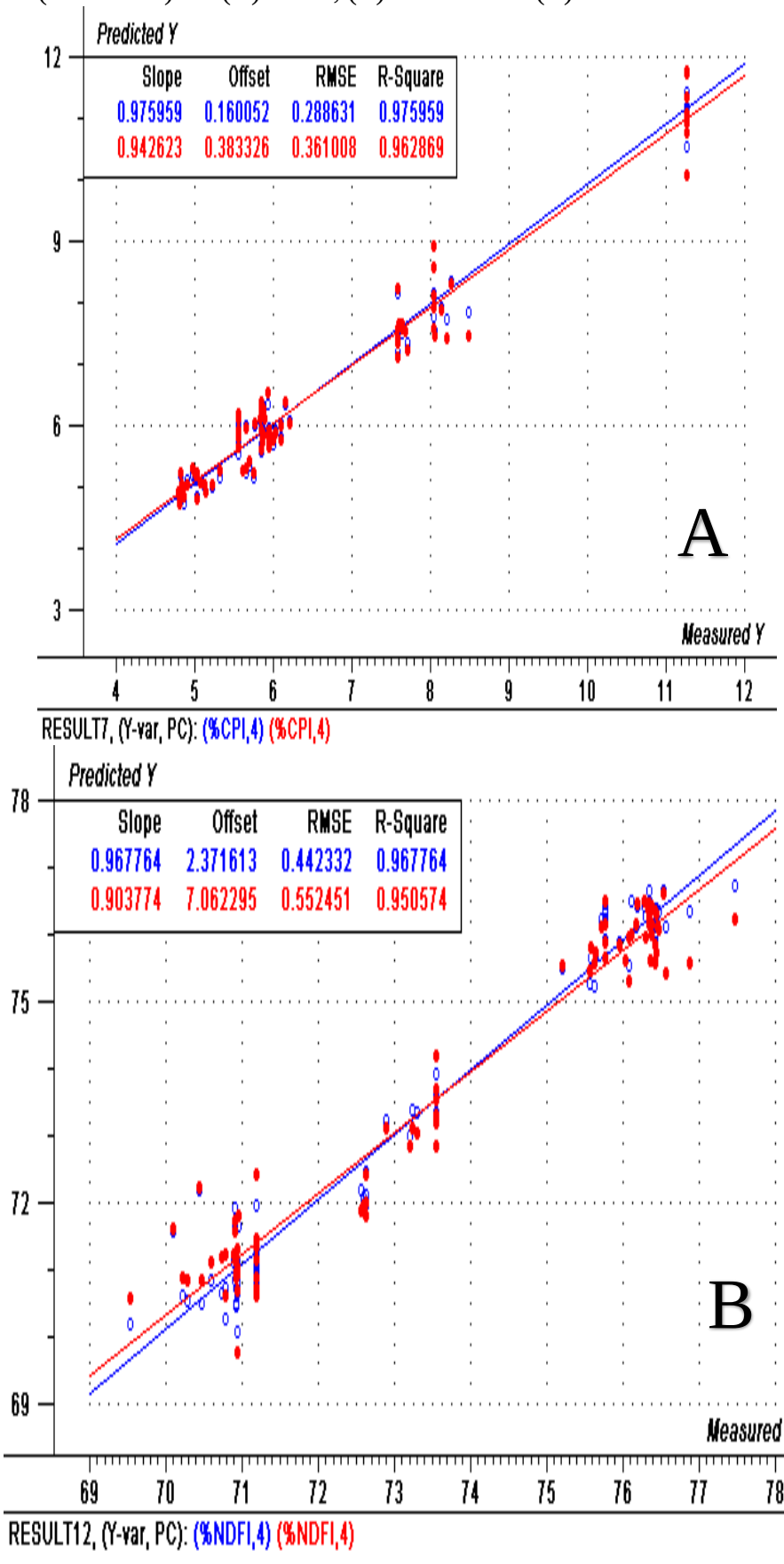
[1]Mathematical treatment and pretreatment applied on NIR spectra; 1 = first derivative and 2 = second derivative

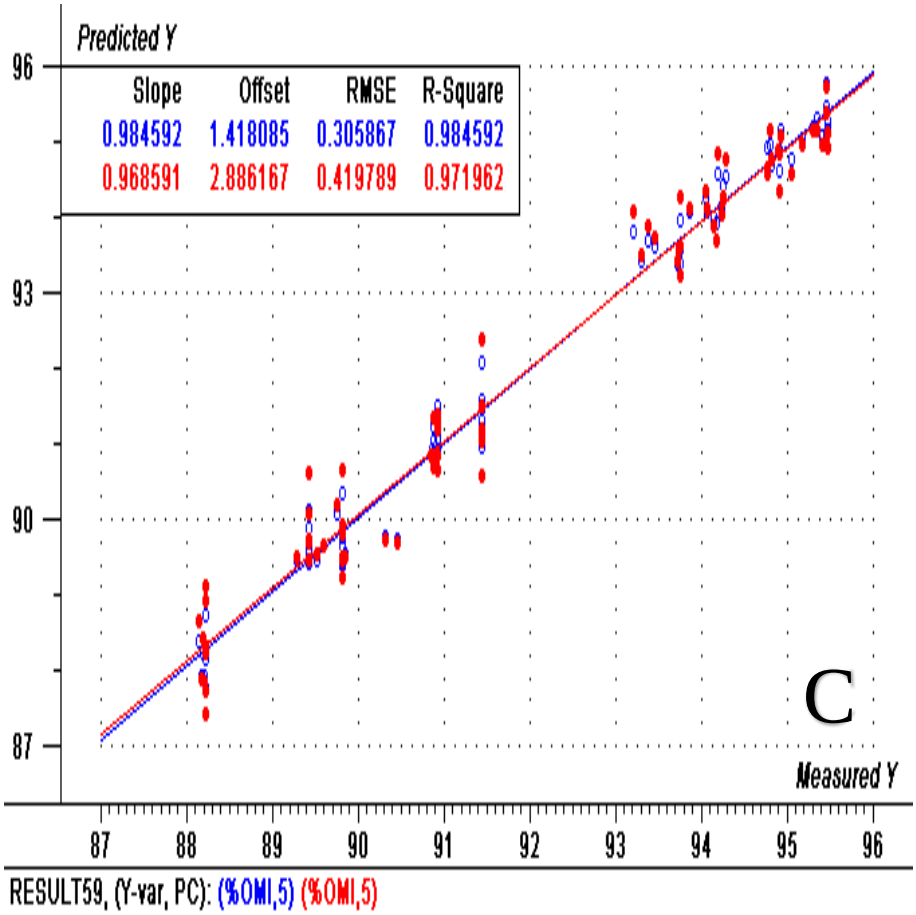
MSC = Multiplicative Scatter Correction

SNV = Standard Normal Variate

[2]Outliers were not included in the calibration equation

Figure 1. F.NIRS calibration for diet composition. The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (blue circles) and the cross-validation (red circles) for (A) % CP, (B) % NDF and (C) % OM.





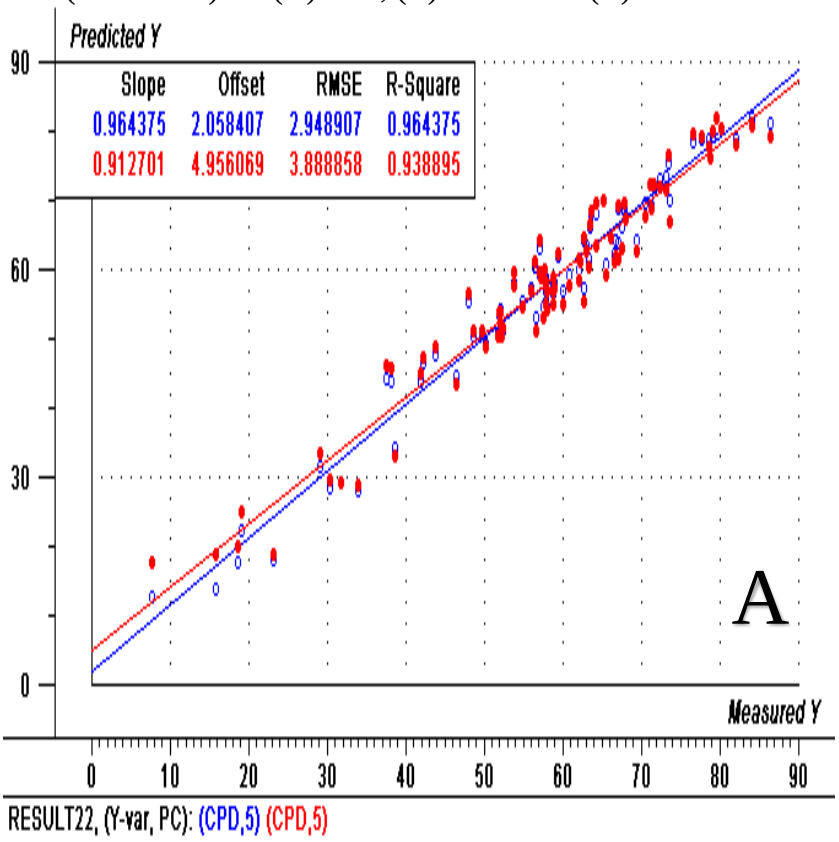
An important thing evaluated by our study is the inclusion of a restricted treatment simulating unfavorably conditions of sward, which allowed verifying if under such conditions, the NIRS would be able to predict dry matter intake, diet composition and digestibility. Our results showed that even when there are limitations related to non-nutritional characteristics the NIRS still a good technique to predict diet composition, since our dataset include a restricted treatment.

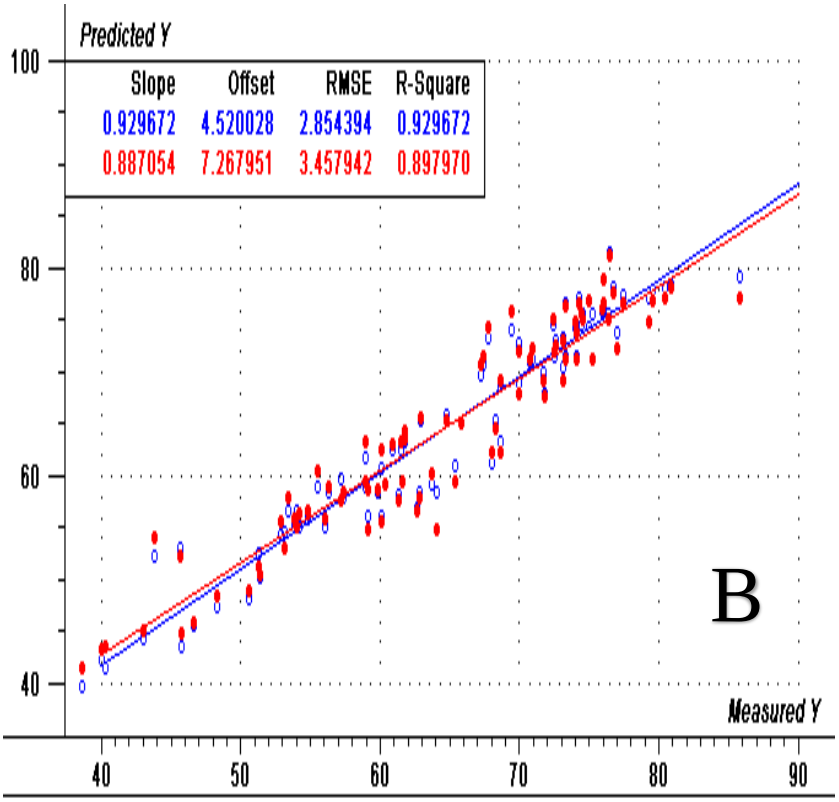
There is an increasing interest in F.NIRS calibrations of *in vivo* digestibility values as it allows substitution of laboratory techniques for predicting energy value (Stuth et al., 2003). Data for nutrient digestibility is shown in Table 2. Using the data set "PERIOD MEAN", calibration equations results were obtained for CPd (RMSEC = 2.95; RMSECV = 3.89) and NDFd (RMSEC = 2.85; RMSECV = 3.46), reinforcing the potential of F.NIRS as a tool to monitor diets of ruminants with R^2c and R^2cv greater than 0.90 (Figure 2A and 2B) and an RPD > 3. However, these results were not good when using the data set "DAILY MEAN" indicating that for digestibility such calibrations should be used carefully or maybe not used at all, considering the RPD less than 3 for both parameters. Another important aspect to consider when evaluating the difference between the datasets is that we used composite samples to the reference method causing a repetition for some values of the samples (Figure 3). It is important

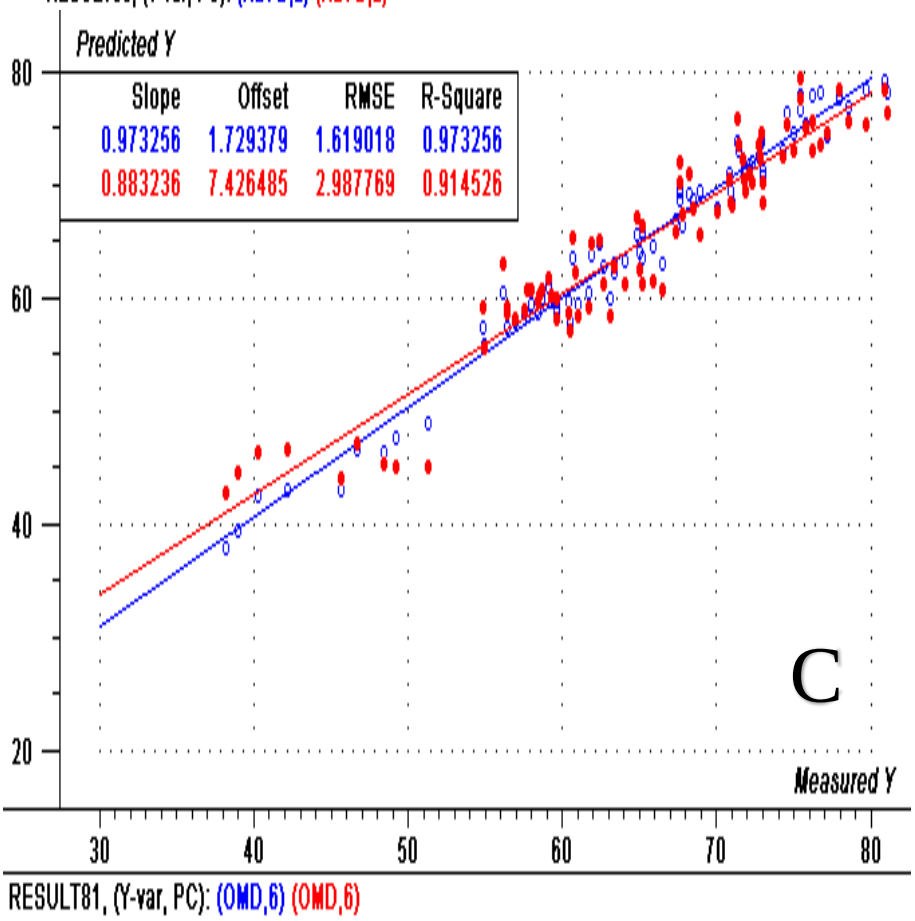
to mention that considering the complexity of forage composition and the variability of the sources of variation (like rainy or dry season and temperature), such calibrations would always demand an increase of variability and they should be frequently updated in order to obtain robust calibrations working accurately (Dardenne et al., 2000).

Calibration equations to estimate OMd showed great results using the dataset "PERIOD MEAN" (R^2c = 0.97 and R^2cv = 0.91; RPD = 3.42), with the RMSEC = 1.62 and RMSECV = 2.99 (Figure 2C). We relate such variation for RMSEC/RMSECV to the greater variation in digestibility (33 to 85%), caused by different growth stage and maturity of the forage used in this study. Viable relationships between NIRS data and *in vivo* nutritional values of fresh temperate forages were also reported by Decruyenaere et al. (2009) with an $R^2 > 0.90$ and RPD higher than 3 for all fecal databases used, with SECV varying from 0.021 to 0.018. According to Dixon and Coates (2009), feces reflects chemical and biological characteristics of the forage consumed by animals as well as their physiological status. This chemical composition can be detected by NIRS and successfully correlated to diet composition, explaining the relevance of F.NIRS for estimating nutrient intake and digestibility of the forage and feed.

Figure 2. F.NIRS calibration for digestibility of the consumed diet. The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (blue circles) and the cross-validation (red circles) for (A) CPd, (B) NDFd and (C) OMd.




Predicted Y
Slope Offset RMSE R-Square
0.929672 4.520028 2.854394 0.929672
0.887054 7.267951 3.457942 0.897970
100
80
60
40
B
Measured Y
40 50 60 70 80 90
RESULT60, (Y-var, PC): (NDFD,2) (NDFD,2)


Predicted Y
Slope Offset RMSE R-Square
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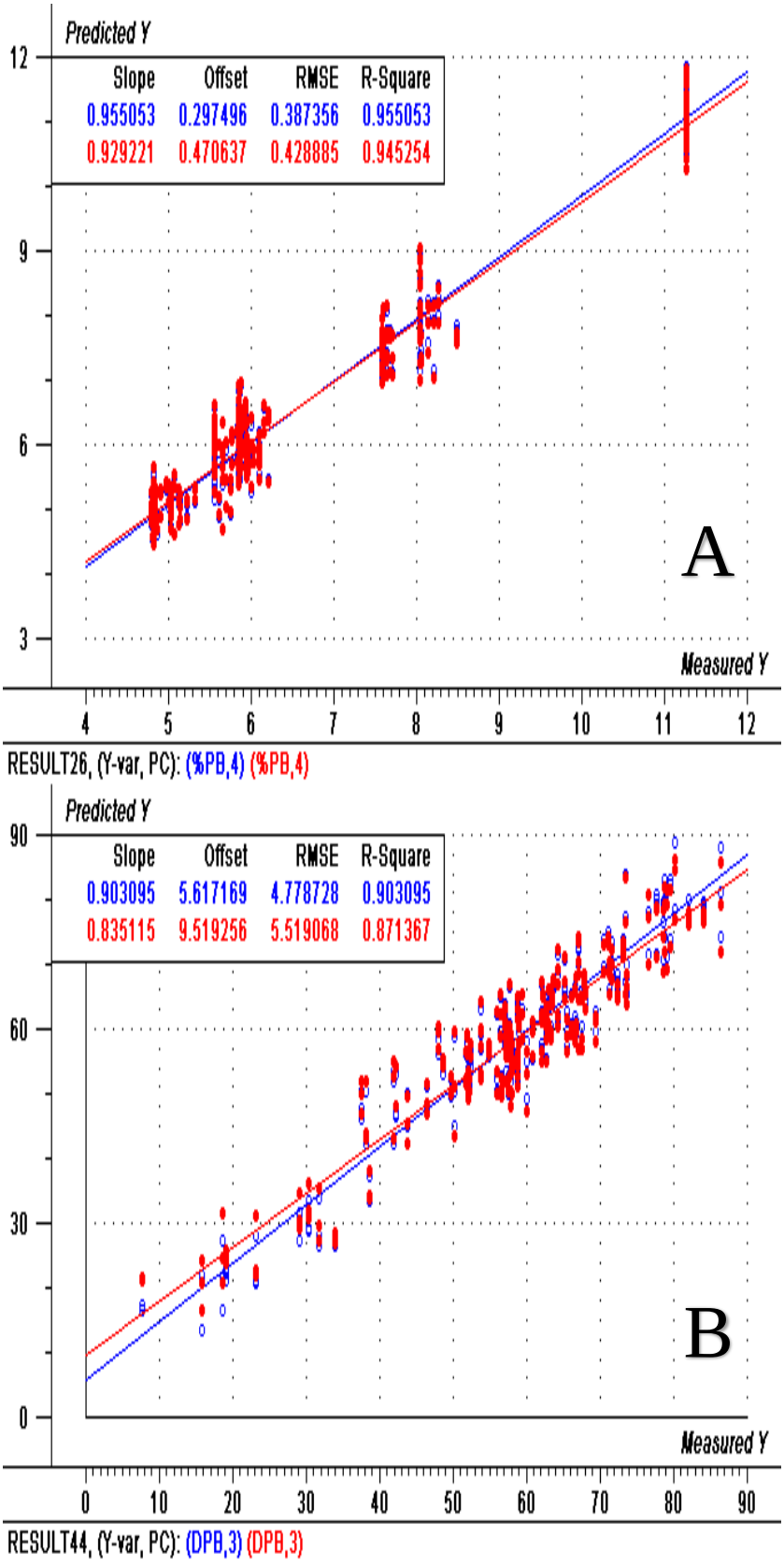
0.883236 7.426485 2.987769 0.914526
80
60
40
20
C
Measured Y
30 40 50 60 70 80
RESULT81, (Y-var, PC): (OMD,6) (OMD,6)

Figure 3. F.NIRS calibration with "DAILY MEAN" dataset for % CP and CPd of the consumed diet. The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (blue circles) and the cross-validation (red circles) for (A) % CP and (B) CPd of the consumed diet.



The best calibration equations for each day separately (dataset "ONE DAY") are shown in Table 3. For these datasets, reported R^2c were greater than 0.95 and R^2cv greater than 0.90 for all measured parameters in diet composition. Such results highlight an important potential application for NIRS in the field, especially for % CP with an RMSEC = 0.29 and RMSECV = 0.39 using the D1 dataset (Figure 4), considering that only one day of sampling would be enough to generate consistent results to monitor the diet composition of grazing animals. For instance, for grazing animals in a pasture of tropical forage (i.e. elephant grass) lacking CP in a dry season, one day of sampling feces would be enough to measure how much supplementation is necessary to supply the requirements of the animals, improving their performance and consequently reducing the costs with supplementation.

Figure 4. F.NIRS calibration for % CP of the consumed diet. The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (blue circles) and the cross-validation (red circles) for % CP of the consumed diet.

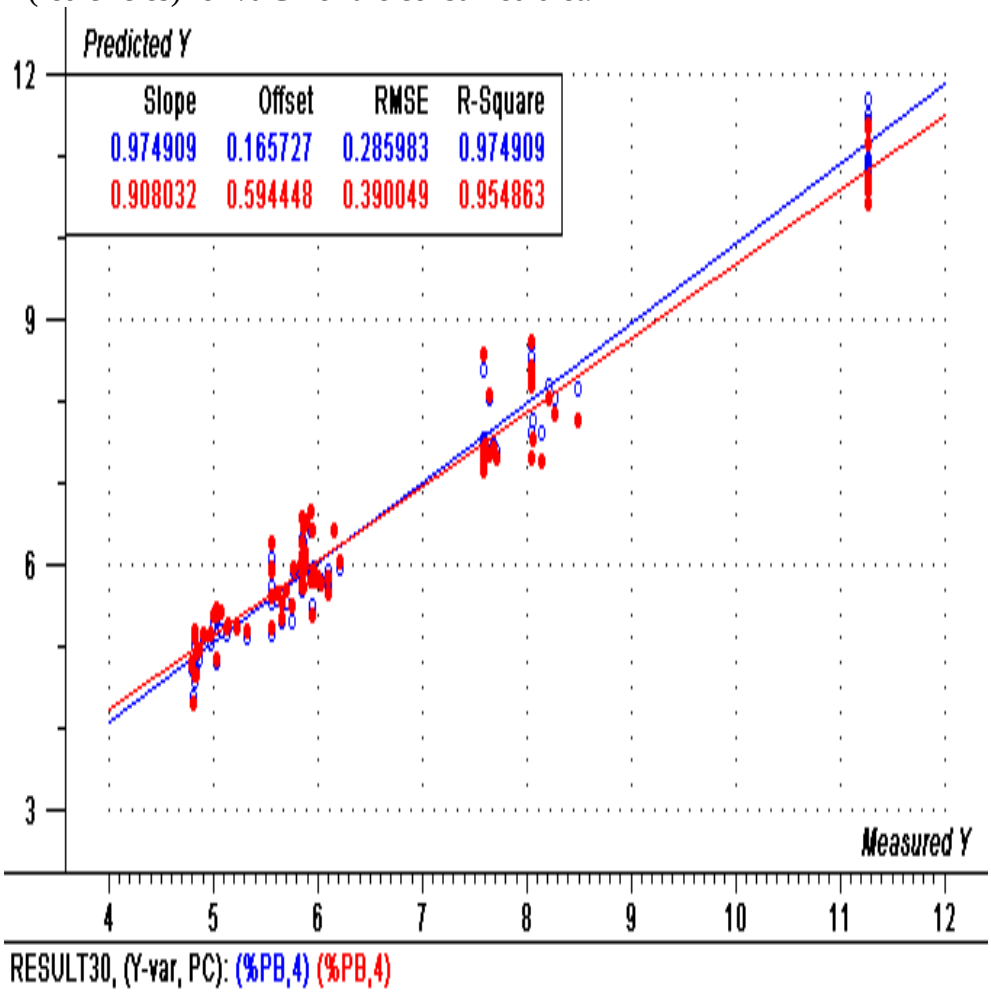


Table 3. Calibration performance of the fecal NIRS equations for composition of the consumed diet and digestibility for each day of data and fecal collection.

	Data Set	Pretreatment[1]	N	Outliers[2]	R^2c	RMSEC	R^2cv	RMSECV	LV	RPD
Diet composition										
CP, % of DM	D1	1 - SNV	88	2	0.97	0.29	0.95	0.39	4	4.71
	D2	1 - SNV	87	3	0.98	0.25	0.96	0.39	4	4.76
	D3	1 - SNV	86	4	0.99	0.23	0.96	0.41	5	4.84
NDF, % of DM	D1	1 - MSC	89	1	0.98	0.33	0.93	0.65	4	3.82
	D2	1 - MSC	89	1	0.96	0.48	0.91	0.77	3	3.25
	D3	1 - SNV	86	4	0.96	0.47	0.93	0.66	4	3.73
OM, % of DM	D1	1 - SNV	89	1	0.98	0.35	0.96	0.52	4	4.73
	D2	1 - SNV	89	1	0.95	0.53	0.93	0.63	3	3.90
	D3	1 - SNV	89	1	0.95	0.57	0.93	0.68	4	3.66
Digestibility										
CPd, %	D1	1 - MSC	89	1	0.98	2.29	0.93	4.05	4	3.68
	D2	1 - SNV	89	1	0.94	3.71	0.87	5.33	4	2.80
	D3	2 - MSC	86	4	0.95	3.43	0.88	5.52	2	2.84
NDFd, %	D1	1 - SNV	89	1	0.93	3.03	0.87	4.01	4	2.80
	D2	1 - MSC	89	1	0.96	2.03	0.92	3.03	3	3.57
	D3	1 - SNV	90	0	0.90	3.49	0.86	4.30	4	2.64
OMd, %	D1	2 - MSC	89	1	0.94	2.63	0.92	3.18	2	3.43
	D2	2 - MSC	89	1	0.95	2.32	0.90	3.24	2	3.21
	D3	1 - SNV	89	1	0.93	2.86	0.86	4.16	4	2.63

R^2c and R^2cv represent coefficient of determination for calibration and cross-validation, respectively; RMSEC and RMSECV are the root mean standard error of calibration and cross-validation; LV = Latent variables; RPD = Ratio of prediction to deviation

[1]Mathematical treatment and pretreatment applied on NIR spectra; 1 = first derivative and 2 = second derivative

MSC = Multiplicative Scatter Correction; SNV = Standard Normal Variate

[2]Outliers were not included in the calibration equation

We then tried to use calibration curves developed only with the AL samples to predict the response variables for the RE dataset (test-set validation). Results are presented in table 4. We observed that for % CP (R^2c = 0.96; R^2p = 0,93; RMSEC = 0.36; RMSEP = 0.51 and RPD = 3.73) and % NDF (R^2c = 0.99; R^2p = 0,89; RMSEC = 0.31; RMSEP = 0.77 and RPD = 3.01), our results were satisfactory, reinforcing the potential of this technique, even with a low number of samples. However, for the other variables the results were not adequate. This could be due to the low number of samples used to calibrate the model when we split the samples by half between calibration and validation. In fact, because of the lower number of samples used for test-set, it may not be recommended to any application, even with good RPD. Such results suggest that more trials should be taken to amplify the dataset and verify if the results are consistent.

5.3. Prediction of Dry Matter Intake and Digestibility

Individual DMI, as reported in Table 1, ranged from 1.7 to 11.7 kg/ day during the sampling periods. The best models for DMI and DMd are listed in table 5, including the sample size after removing outliers and the statistical parameters. The R^2c and R^2cv were substantially better for the prediction of DMI using the dataset "PERIOD MEAN" (R^2c = 0.96; R^2cv = 0.77) than for the dataset "DAILY MEAN" (R^2c = 0.66 e R^2cv = 0.55), as shown in Figure 5A and 5B. Unexpectedly, using the "ONE DAY" (D1, D2 or D3) resulted were better than using all three days in the model (Figure 5C). However, using the average of the three days of each sub period (dataset "PERIOD MEAN") was still superior to the others due to its lower RMSECV value (0.88) and greater RPD (2.09).

In developing F.NIRS calibrations, the most important thing to evaluate is the accuracy of the reference values. Such accuracy from diet do not rely only on accurate laboratory analysis but also on measures taken to avoid or minimize miss-match error which can lead to poorer calibrations statistics and equation performance. In general, fecal spectra associated with large miss-match errors will be identified as outliers and eliminated during calibration. Even when the animals are pen-feds, there is likely to occur some between-day variation in the composition of forage offered and forage ingested leading to some degree of miss-match error (Dixon and Coates, 2011). It could explain why the dataset "PERIOD MEAN" were better than "DAILY MEAN", by "reducing" the miss-match error by averaging the intake for the three days.

Table 4. Calibration and prediction performance using test-set validation of the fecal NIRS equations for dry matter intake of diet composition and digestibility.

	Data Set	Pretreatment[1]	N	Outliers[2]	R^2c	RMSEC	R^2p	RMSEP	LV	RPD
Intake										
DMI, kg/ day	PERIOD MEAN	2 - SNV	100	0	0.56	1.12	-0.10	2.77	1	0.95
CP, % of DM	PERIOD MEAN	2 - SNV	90	0	0.96	0.36	0.93	0.51	2	3.73
NDF, % of DM	PERIOD MEAN	2 - SNV	89	1	0.99	0.31	0.89	0.77	4	3.01
OM, % of DM	PERIOD MEAN	2 - MSC	89	1	0.99	0.25	0.87	0.88	4	2.82
Digestibility										
DMd, %	PERIOD MEAN	1 - SNV	100	0	0.87	3.41	0.76	5.67	4	2.05
CPd, %	PERIOD MEAN	1 - SNV	90	0	0.90	4.46	0.76	8.34	3	2.06
NDFd, %	PERIOD MEAN	1 - SNV	90	0	0.91	3.06	0.80	5.40	4	2.22
OMd, %	PERIOD MEAN	1 - SNV	90	0	0.90	3.12	0.78	5.49	3	2.11

R^2c and R^2p represent coefficient of determination for calibration and prediction, respectively; RMSEC and RMSEP are the root mean standard error of calibration and prediction; LV = Latent variables; RPD = Ratio of prediction to deviation

[1]Mathematical treatment and pretreatment applied on NIR spectra; 1 = first derivative and 2 = second derivative

MSC = Multiplicative Scatter Correction

SNV = Standard Normal Variate

[2]Outliers were not included in the validation equation

Table 5. Calibration performance of the fecal NIRS equations for dry matter intake and digestibility

	Data Set	Pretreatment[1]	N	Outliers[2]	R^2c	RMSEC	R^2cv	RMSECV	LV	RPD
Dry matter intake										
	PERIOD MEAN	1 - SNV	100	0	0.96	0.36	0.77	0.88	8	2.09
	DAILY MEAN	1 - SNV	300	0	0.66	1.14	0.55	1.32	5	1.49
	D1	1 - SNV	99	1	0.91	0.63	0.79	1.00	6	2.16
	D2	1 - SNV	100	0	0.83	0.77	0.68	1.08	5	1.76
	D3	1 - SNV	100	0	0.92	0.49	0.66	1.03	6	1.72
Dry matter digestibility										
	PERIOD MEAN	2 - SNV	96	4	0.93	2.67	0.88	3.44	3	2.90
	DAILY MEAN	2 - SNV	298	2	0.88	3.59	0.84	4.19	3	2.48
	D1	1 - MSC	97	3	0.93	2.62	0.88	3.56	3	2.89
	D2	2 - MSC	99	1	0.95	2.28	0.90	3.22	2	3.14
	D3	2 - MSC	95	5	0.92	2.83	0.84	4.23	2	2.47

R^2c and R^2cv represent coefficient of determination for calibration and cross-validation, respectively; RMSEC and RMSECV are the root mean standard error of calibration and cross-validation; LV = Latent variables; RPD = Ratio of prediction to deviation

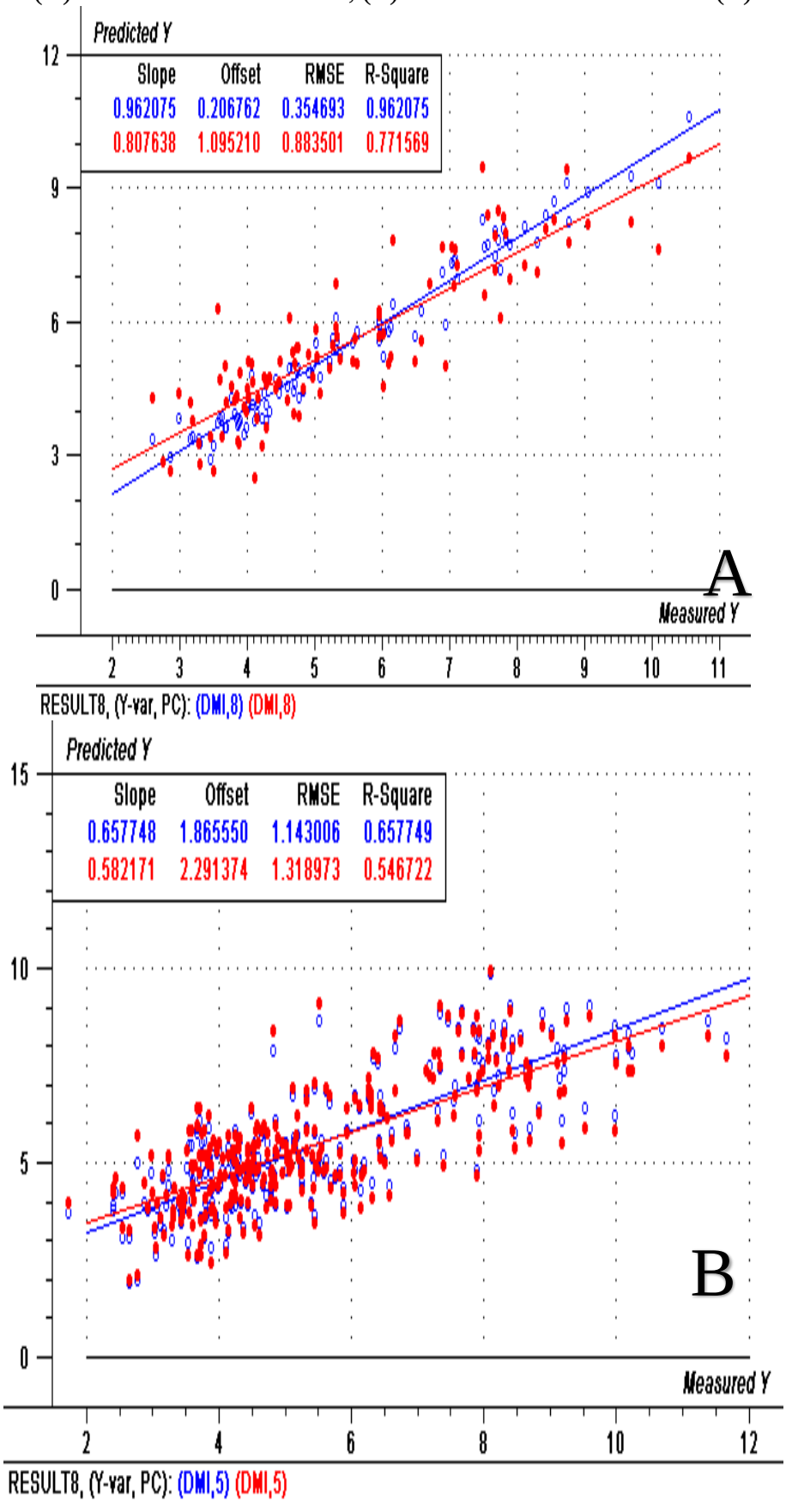
[1]Mathematical treatment and pretreatment applied on NIR spectra; 1 = first derivative and 2 = second derivative

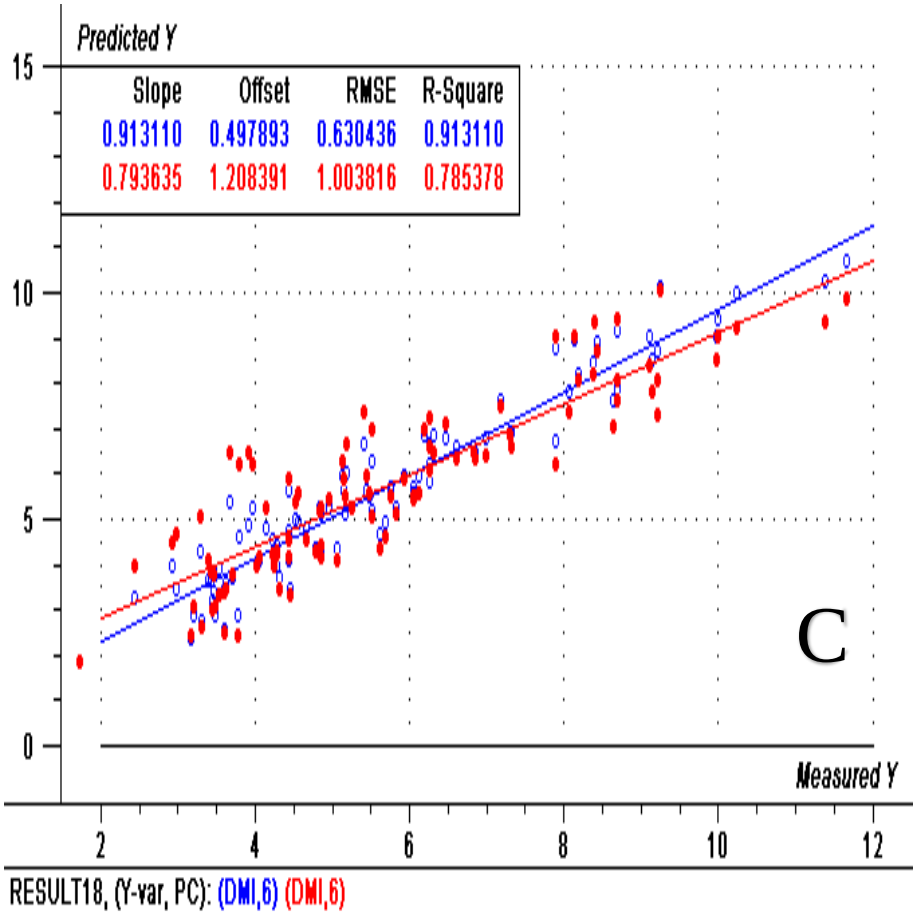
MSC = Multiplicative Scatter Correction

SNV = Standard Normal Variate

[2]Outliers were not included in the calibration equation

Figure 5. F.NIRS calibration for dry matter intake. The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (blue circles) and the cross-validation (red circles) for (A) PERIOD MEAN dataset, (B) DAILY MEAN dataset and (C) D1 dataset.



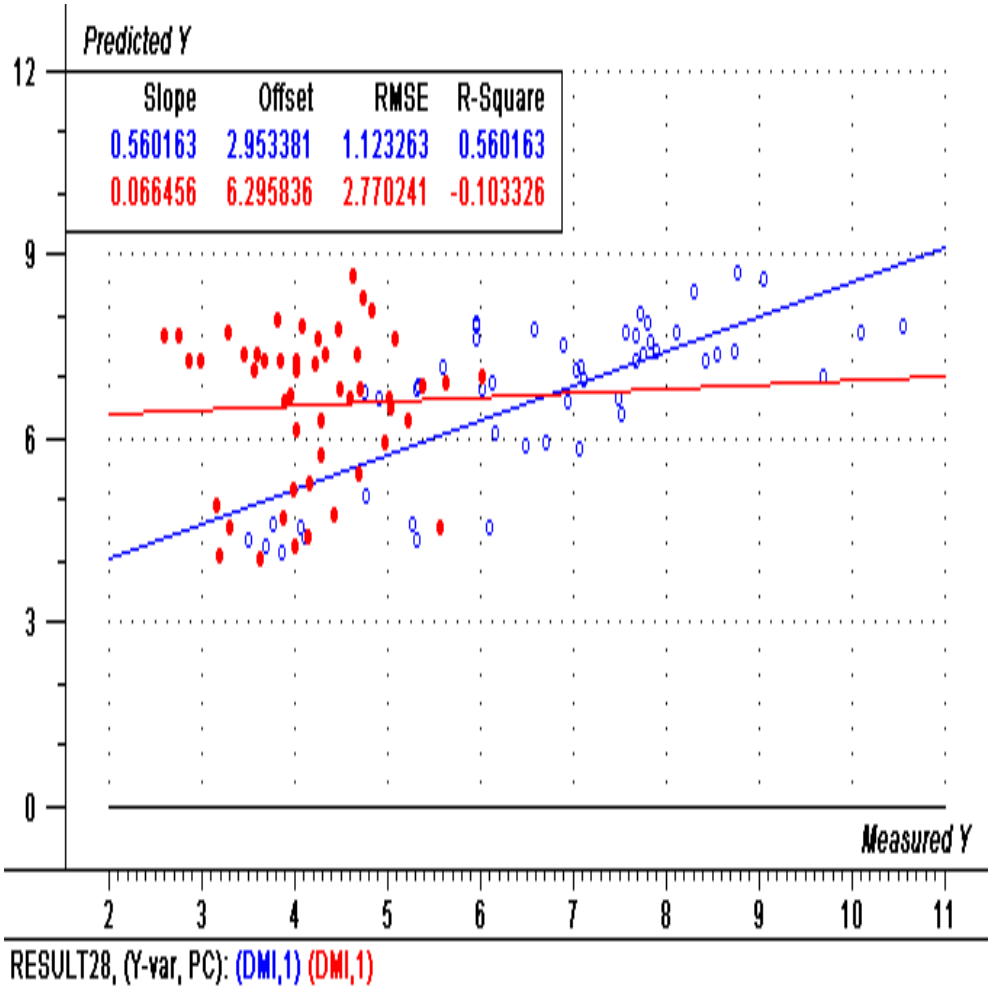


Even with better $R^2c = 0.96$ and $R^2cv = 0.77$, compared to results previously reported for the prediction of DMI by the n-alkane technique (R^2v ranging from 0.18 to 0.72; Oliván et al., 2007; Ferreira et al., 2007), our results are limited and would be viable only to be used at the same circumstances of our study. Considering the $R^2p = -0.10$ found while using the test-set validation (Table 4 and Figure 6), it can be determined that the current database lacks the size necessary to predict DMI of an independent data set or that the restricted treatment just led to poorer calibrations, taking into account that DMI values are less uniform and may not be as clearly defined chemically by constituents such as CP. In addition, modeling voluntary DMI is hard because it is a complex system not only affected by the animal, forage and environment (including management), but also by the interactions among them (Pulina et al., 2013). However, in comparison with other methods, F.NIRS appeared to be accurate enough for estimating the voluntary intake. For instance, Mayes and Dove (2000) confirmed that F.NIRS were as accurate as the n-alkanes ratio technique for estimating the dietary nutrient intake of different ruminants, such as cattle, sheep and goats but it still containing considerably errors.

The variation of DMd in the dataset can be found in Table 1, while the calibration curves for DMd are presented in Table 5. Using the dataset "PERIOD MEAN", $R^2c = 0.93$ and RPD = 2.9 were slightly better than previous results reported in a review by Dixon and Coates (2009),

especially considering that we have a restricted treatment in our study. The calibration (R^2c = 0.93; RMSEC = 2.67) and cross-validation (R^2cv = 0.88; RMSECV = 3.44) statistics for predicting DMd were worse than results reported for any other parameter in this study. However, according to recommendations by Williams (2004), this equation may be viable for some applications, since R^2c and R^2cv are higher than 0.80 and RPD close to 3, but with caution as RMSEC and RMSECV are quite different (2.67 and 3.44).

Figure 6. F.NIRS calibration of dry matter intake using test-set validation (external). The figure represent measured (x axis) v. predicted (y axis) values for the calibration data set (50 AL samples, blue circles) and the external validation data set (50 RE samples, red circles) for dry matter intake.



6. CONCLUSION

Reported calibrations equations in this study illustrate the existence of significant correlations between diet characteristics and fecal spectra, even without robust validation of independent sets. F.NIRS showed its potential for prediction of % CP and % NDF of the diet consumed, requiring just one day of sampling, even when intake is limited by non-nutritional factors. Further research is needed to develop more robust and larger calibration equations for the prediction of DMI, however, our results provides good evidence that improved F.NIRS calibrations can be used as an alternative method to monitor diet composition of grazing animals. Such results, opens the way to the development of decision-making tools for supplementation of grazing animals, reducing production costs and improving animal performance.

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