NIRS as a tool to determine the nutritional value of native pasture for small ruminants

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ABSTRACT: The design and adjustment of grazing animals feeding systems are laborious and time-consuming, mainly because the lack of quick and accurate tools to estimate forage grazed. Thus, it is crucial to develop technologies to allow quick and easy nutritional diagnosis in order to establish feeding strategies for animals at pastures. Fecal NIRS (FNIRS) is a remarkable technology due its precision, accuracy, and low cost of operation. Several parameters of diet quality have been reported be feasible using fecal NIRS, but the most has been done to crude protein (CP) and dry or organic matter digestibility (DMD or OMD). For dietary crude protein, R^2 ranged from 0.84 to 0.98, with mode of 0.98, while the mode of standard error of validation was 0.53 (0.06 to 3.21). On the other hand, for diet digestibility calibrations, the R^2 values ranged from 0.79 to 0.98, with more often number of 0.98, and standard error of validation, coming from 0.02 to 4.07, with average around 2.0, which can be considered a good performance for both. Besides diet quality, fecal NIRS has been also used as a tool to predict voluntary feed intake and diet botanical composition. This publication describe about the potential of this technology to nutrition of range small ruminants.

Key words: NIRS fecal, Caatinga, Nutrition precision, Goat, Sheep, Grazing

INTRODUCTION

Appropriate nutrition of the herds is fundamental to ensure animal efficiency. Besides supplying nutrients, nutrition implicates on animal health, economic efficiency, and environmental sustainability. However, the design and adjustment of grazing animals feeding systems are laborious and time-consuming, mainly because the lack of accurate tools to estimate forage grazed which, along of intake, is the background to nutrients balancing. This issue is still a challenge for ruminant raised at rangelands due the wide range of species and animal selectivity, requiring use of fistulated animals to access the quality of selected forage.

All those challenges to measure diet quality of grazing ruminants resulted in a contradictory scenario in the research of small ruminant nutrition in Brazil. If by one side, the most of animals are raised at rangelands (In the Northeast and South), less than 3% of Brazilian publications have been done with grazing animals. It demonstrates the demand for easier

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alternative techniques to fistulated animals to access quality of grazed forage to encourage new researchers working on range animals.

Therefore it is crucial to develop tools to allow quick and easy nutritional diagnosis in order to establish feeding strategies for animals at pastures, comprising the estimative of forage quality and identification of limiting nutrients to design more precise supplemental strategies. Among those technologies, the fecal NIRS (FNIRS) is a remarkable technology due its precision, accuracy, and low cost of operation.

The more intensive use of fecal NIRS technology to predict diet quality of grazing animals began 20 years ago at Texas A&M University with Dr. Jerry Stuth, who developed calibrations in which crude protein (CP) and organic matter digestibility (OMD) of grazed forage were accurately predicted from fecal spectra. Since that time, several publications in different countries have showed the reliability of fecal NIRS as a tool to monitoring nutrition of grazing animals. The use of fecal NIRS technology imply in a great advance on animal nutrition for forage-based production systems, allowing customized supplementation for a region, a herd or even individual animals.

The most exciting use of this approach is the association of the diet quality prediction with a tool to analyze the nutritional balance (i.e. NUTBAL PRO), allowing determining either a deficiency or an excess of nutrients (protein and or energy), predicting performance and developing low cost solutions of supplementation to correct a possible unbalancing.

This publication was assigned to describe the use of fecal NIRS technology as a tool to predict nutritional value of rangelands for small ruminants, addressing to results worldwide and aspects of building robust calibration

FECAL NIRS TECHNOLOGY AND NUTRITIONAL VALUE OF FORAGE GRAZED

Near infrared reflectance spectroscopy (NIRS) is an instrumental method for rapidly and reproducibly measuring the chemical composition of forage and feed samples. It is based on the fact that each of the major chemical components of a sample has near infrared absorption properties that can be used to 1) differentiate one component from another, and 2) determine nutrient concentration.

NIRS is a calibration based technology, meaning that analysis is limited to only materials and nutrients for which calibrations have been developed. Differently from the traditional use of NIRS where spectral and chemical analyses are performed in the same matrix, Fecal NIRS is based on collecting spectra in one material (feces), while the reference analyses are performed in another (diet, i.e. extrusa or hand plucked forage) (Landau et al., 2008). The theory behind this is the assumption that fecal chemistry reflects widely the diet composition and, based on that, it is possible to calibrate equations to predict diet quality from fecal spectrum. This approach is not different from those previously used with fecal nitrogen and others indicators aiming develop

easy tools to monitoring nutritional status (Peripolli et al., 2011), but use much more indicators than only nitrogen, fiber or other single molecules.

Developing calibrations to predict diet quality from fecal NIRS

The use of fecal NIRS to predict diet quality of herbivores was first reported by Brooks et al. (1984), but more intensive works were developed by Dr. Jerry Stuth (Texas A&M University, USA) and Dr. David Coates (CSIRO, Australia). Several parameters of diet quality have been reported be feasible using fecal NIRS, as summarized by McCafferty et al. (2011) (Table 1). However, most of fecal NIRS calibration to predict diet quality has been done to crude protein (CP) and dry or organic matter digestibility (DMD or OMD), from which protein and energy status can be obtained. Other includes neutral and acid detergent fiber (NDF and ADF), tannins, and lignin. Dixon and Coates (2009) reviewed the publications about fecal NIRS use to herbivorous and present a very comprehensive database.

Tabela 1. Predictibility of diet parameters using fecal NIRS technology

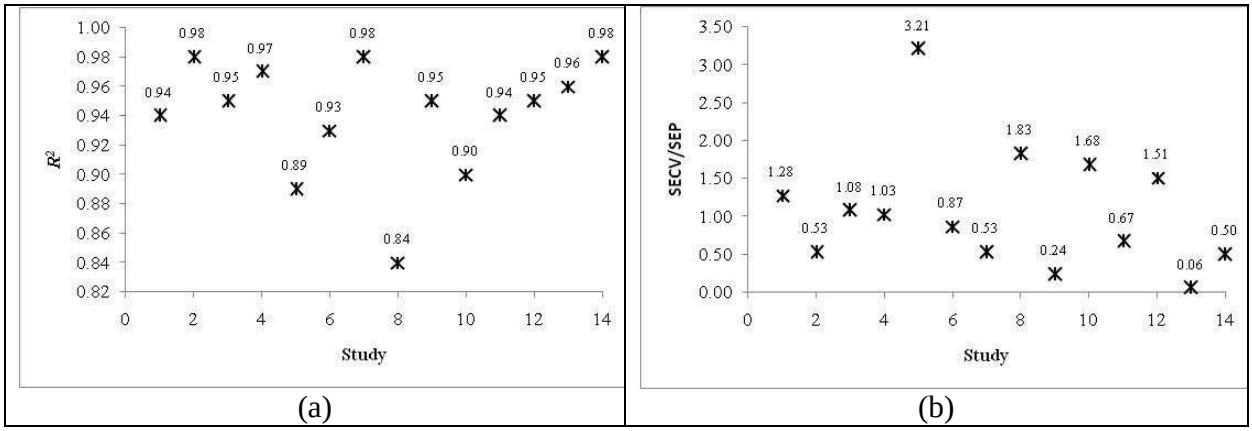
Parameters	R^2	Slope	Standard error or prediction
Crude protein	0.92	0.994	0.666
Crude protein digestibility	0.94	0.993	3.920
Metabolizable energy	0.92	0.960	0.352
Organic matter digestibility	0.81	0.952	2.580
Total phenolics	0.93	1.020	0.353
Total tannins	0.99	0.932	0.321

Source: Adapted from McCafferty et al. (2011)

In spite of fecal NIRS might be use to predict, potentially, any diet attributes, the performance of equations is not similar for all, varying depending on the attribute of interest. Crude protein equations, for example, usually have high predictability while organic or dry matter digestibility, shows more variable responses. Since fecal NIRS predictions depend on the accuracy and precision of the reference analysis, the higher experimental error observed for digestibility rather than crude protein, reflects in lower performance of equations. Wherefore, the decision about the model suitability should be based on the error of prediction and the desirable accuracy or precision. If the error is acceptable for the purpose for which the calibration was developed, certainly fecal NIRS will be a very helpful tool.

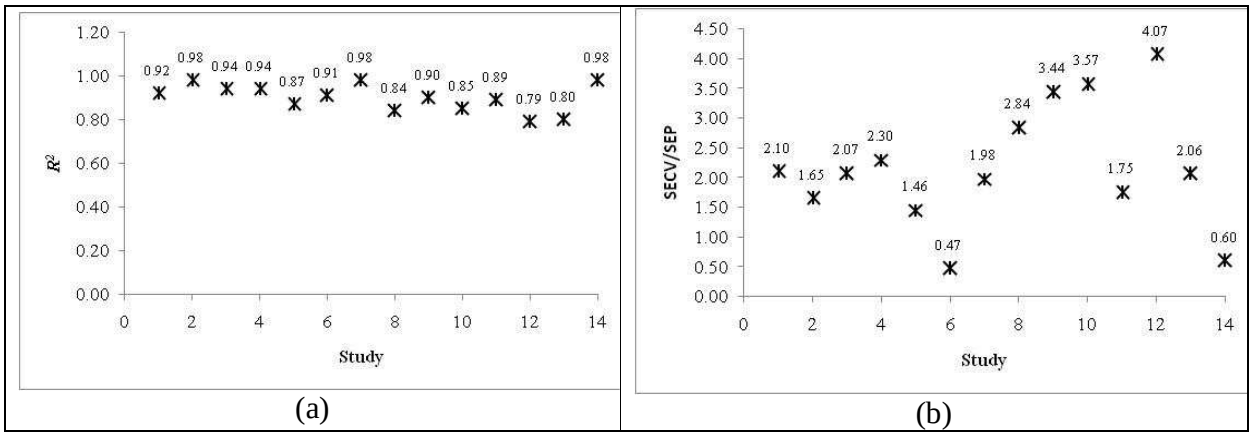
Performance of fecal NIRS models to predict diet of small ruminants is summarized for CP (Figures 1) and digestibility (Figures 2). These figures are based on reviews of Dixon and Coates et al. (2009) and Landau et al. (2006) and other individual newest publications. As can be seen, very good calibrations and validation parameters have been obtained. For dietary crude protein, R^2 ranged from 0.84 to 0.98, with mode of 0.98, while the mode of standard error of validation was 0.53 (0.06 to 3.21). On the other hand, for diet digestibility calibrations, the R^2 values ranged from 0.79 to 0.98, with more often number of 0.98, and standard error of

validation, coming from 0.02 to 4.07, with average around 2.0, which can be considered a good performance for both. Actually, based on reliability of fecal NIRS calibration to diet attributes, services for farmer or technicians has been provided by at least two institutions: Texas A&M University in USA, and Symbio Alliance in Australia. In these, customers can access report of diet quality or even complete advisory report, about feeding management.



(a) (b)

Figure 1 – Coefficient of determination (a) and standard error of cross-validation or prediction (b), of diet crude protein predicted by fecal NIRS with small ruminants. Adapted from Dixon and Coates et al. (2009) and Landau et al. (2006) updated with Keli et al. (2008), Decruyenaere et al. (2009), Mahipala et al. (2010), Cox et al. (2000) and Decantia (2009).



(a) (b)

Figure 2 – Coefficient of determination (a) and standard error of cross-validation or prediction (b) of diet dry matter or organic matter digestibility predicted by fecal NIRS with small ruminants. Adapted from Dixon and Coates et al. (2009) and Landau et al. (2006) updated with Keli et al. (2008), Decruyenaere et al. (2009), Mahipala et al. (2010), Cox et al. (2000) and Decantia (2009).

Prediction of diet botanical composition

Shenk et al. (1979) were the first to show the potential of fecal NIRS to predict the diet composition in a mixed grass:legume diet with accuracy of approximately 0.10%. After that study several other works have tried to expand this approach for more complex pastures. Identification of plants in herbivorous diets is useful, not only to access selection and preference, but to find out about land degradation which is based on the appearance or disappearance of key plant species.

Currently, several publications have addressed to use of fecal NIRS to predict diet composition. We may highlight at least two approaches. First, Walker et al. (2007) have worked in fecal NIRS calibration to identify percentage of a weed, juniper (*Juniperus spp.*), in the diet of goats. Despite the fact that quantification showed low precision and accuracy, the magnitude of juniper participation in diets was predicted accurately. The intent with this work was to identify and select those animals genetically more able to consume high amount of this weed, which can help to control its population in rangelands or even to produce meat or milk based on intake of this non conventional roughage. Thereafter, the same group estimated the heritability (0.13) for this characteristic and suggested, based on publication of Utsumi et al. (2009), that the metabolism of phenolics and monoterpene are the main reason for this difference among animals for juniper voluntary intake.

Other approach is given by researchers from the Volcani Center, Israel, who is using fecal NIRS to predict intake of different native forage species by breeds of goats. They observed an adapted breed (Damascus goat) ingesting more *Pistacia lentiscus* when compared to Boer or Mamber breeds (Glasser et al., 2012). Interestingly, the intake of this plant was related to antihelmintic effect for goats (Landau et al., 2010), meaning that, besides the plant control at rangelands, fecal NIRS may help to understand foraging strategies of range animals to control internal parasites, helping to establish land management or even index to animal genetic selection.

Prediction of voluntary feed intake of grazing animals

Feed intake of grazing ruminants is a critical factor to establish appropriate strategies of animal management. Usually it has been estimated using empiric models, but several efforts trying to develop accurate methods to estimate forage intake of grazing animals have been made. Fecal nitrogen has repeatedly been shown to be a research target for predicting intake (Leite and Stuth, 1990). Despite these researches have begun as early as 1940s, recent works have still kept discussion about the use of fecal indexes to predict intake (Peripolli et al., 2011). As fecal NIRS had been showed to predict very accurately fecal nitrogen and much more other chemical elements related to diet of ruminants, it is reasonable to assume that it has a potential to be used for predicting intake in grazing animals.

Coleman et al. (1999) stated the prediction error associated to prediction of intake in ruminants by fecal NIRS technology are, generally, comparable or smaller the errors associated with prediction of voluntary dry matter intake from the NIR spectra of forage analyses or from conventional laboratory analysis. It agrees with the publication of Offer et al. (1998), who concluded that forage intake by sheep was more accurately predicted by NIRS than by any other classical chemical predictors.

However, fecal NIRS prediction would be expected to be an estimative of the potential intake, as limited by forage characteristics rather than necessarily the actual intake, which will be influenced by numerous physiological, pasture or environmental conditions, and others which could not affect fecal chemistry. It is suggested caution to apply these calibrations for the pasture systems and animal classes for which they were developed. In addition, possible constraints on voluntary intake due pasture availability and other animal or environmental influences on dry matter intake need to be considered (Coleman et al., 1999).

DEVELOPING ROBUST FECAL NIRS CALIBRATION

Fecal NIRS technology may be used successfully to predict diet quality of grazing animals only if a large database of fecal samples, from which spectra are collected, are plotted against a respective matrix of diet composition (reference value) using a multivariate statistics (Landau et al., 2008; Molle et al., 2008; Rothman et al., 2009).

Generally, the development of NIRS calibration follows the same steps: database preparation, calibration of equation, validation and prediction of unknown samples. The first step is probably the more time-consuming and laborious, but critical to achieve good results. It involves building a database of diet:feces pairs of samples from which the equations will be developed. After database preparation, mathematical pretreatments and multivariate statistics are used as a tool do develop calibrations. The least and mandatory step is the validation which will ensure the validity of model to predict unknown samples. Thereafter, fecal scanning may be use to predict diet quality, becoming easier and dynamics to monitor diet at low cost, using a chemical-free and non-destructive technology.

Preparing a database

Several methods have been used to obtain a representative sample of diet grazed by ruminants which include hand plucking, exclusion cages or fistulated animals (esophagus or rumen) (Holechek et al., 1982). For fecal NIRS calibration, despite all criticism, esophageal fistulated animals, over the years, was extensively used to collect sample representing more closely the diet actually selected by grazing animals, especially in rangelands, allowing a great advance in the background about quality of diet and supplementation strategies of ruminants at pastures.

The most important factor in the database preparation is to collect the pair diet:feces in an interval where the indigestible matter in the feces represent the diet. Thus, it must take into account the time required for undigested forage to reach the feces. The general protocol for small ruminants is to collect feces 24h after the diet sampling. This protocol has been showed to be useful for animals at rangelands and it was already used to develop the only Brazilian fecal equation for small ruminant in the Northeast semiarid rangelands by Cox et al. (2000) at the Embrapa Goats and Sheep. However, it could not be the same when the animals graze tropical grasses. Estimates of Bueno et al. (2007) using chromium mordant showed that ewes grazing a tropical grass excrete indigestible forage matter as long as 48h after intake. It clearly raise the need to determine the optimum interval to collect pair diet:fecal sample for small ruminants at tropical grasses.

Despite all development based on oesophageal fistulated animals in the past, keeping and managing them is hard, discouraging the preparation of database using this kind of surgically prepared animals. Rumen-fistulated animals have been suggested as an alternative approach to collect extrusa in free-ranging small ruminant. Recently, Santos et al. (2008) has showed that rumen-fistulated animals can be used to predict selected in rangelands as accurately as esophageal ones. The procedure includes a rumen evacuation technique prior a 1-hour grazing time. After this time, extrusa are collected directly from the rumen and the original rumen content is replaced. It can make easier to obtain diet sample, since handle rumen fistulated animals is much easier than the oesophageal fistulated ones. Based on this evidences, the research groups working with grazing small ruminants in Brazilian Northeast region have agreed to use rumen-fistulated in their experiments to collect extrusa as a preferential technique.

Although the hand pucking technique is not generally recommended for collection of the diet selected by ruminants grazing rangelands, some publications have used this approach. Landau et al. (2008) collected the diet apparently grazed with reconstituted diets based on bite counts and on the simulated bite method for reference values, using observation in 2 stages. The first stage comprised direct and continuous observation of individual animals to determine the number of bites removed, for each plant species and bite-type category. The second stage comprised collection of representative samples of each specie and bite-type category for the determination of their mass and quality. Bite like samples were clipped so that the sample collection combined species and bite-type categories, according to the recorded foraging behavior. The model developed using this approach performed well according to statistics.

According to Landau et al. (2008), comparing with the use of fistulated animals bite count methodology has three advantages: 1) information is obtained for the entire grazing days; 2) the same animal is used for diet estimation and fecal sampling; and 3) diets selected by fistulated animals may be different from those of non fistulated residents.

A third methodology to collect pairs diet:feces, is using pen-fed animals simulating, at trough, the complexity of diet available at pasture. Landau et al. (2005) prepared diets comprised

of concentrate in combination with legume hay (n=60) or four species of browse (n=91) (*Pistacia lentiscus* L., *Phyllirea latifolia* L., *Calicotome villosa*, and *Pinus brutia*. Browse branches were cut daily. Diets were weighed and distributed. The study consisted of twelve 10-day tests once every morning. The performance of calibration for CP (R^2 = 0.98, SEC = 0.42 and SECV = 0.50) and IVDMD (R^2 = 0.97, SEC = 1.72 and SECV = 2.14) were within the expected range. Those calibrations should be used with need careful when extrapolated to grazing situation. Adequate validation needs to be conducted to ensure this expansion will be right.

Finally, as to any equation developed, for robust calibration it is mandatory to ensure that the maximum variability in the material must be enclosed into the database. Thus, the recommendation is to collect diet:feces pairs sample throughout the year, ensuring that samples will be collected in wet, dry, and transitions seasons (wet to dry and dry to wet). There is not a general rule to estimate the number of samples required to develop calibrations. Obviously as much variability in the composition or in the chemical analysis, as many samples will be required. Reported calibrations have showed databases ranging from 36 to 951 samples. However, we need to take into account that it is a final calibration dataset. Probably the original dataset were larger than that, allowing the deletion of outliers and other actions that reduce the database during the calibrations. In anyway, fecal NIRS technology is comparable to breeding programs. As larger your database, more accurate will be your predictability. It is also strongly recommended a periodical update in the database with new pairs of diet:feces samples increasing variability and becoming the calibration more robust.

Calibrating equations

Once the database of spectra and chemical analyses has been prepared, chemometric techniques are required to extract chemical information from physical data (spectra). Chemometrics is an area of science where mathematic tolls are used (especially statistics) to analyze and explain chemical data. The steps comprising NIRS calibrations are: observation of data, pretreatment (when necessary), and the development of multivariate model, evaluation of results, validation and evaluation of predictions.

Usually some pretreatment are required before equation calibration, aiming to reduce the ratio signal:noise of data. In other words, we need to reduce non informative areas of spectra to enhance those containing chemical information. Below, the most common pretreatments in chemometrics applied to spectroscopy are issued.

Scatter correction

This tool was originally developed to reduce the disturbing effect of light scattering. Powders, aggregates of grains of different particle sizes often display light scattering effects. Also, different path lengths in solid samples (i.e. differences in package of cells) increase scatter effect. The methods commonly applied are Multiplicative Scatter Correction (MSC), Standard normal variate (SNV) alone or plus detrend.

Smoothing and differentiation

Smoothing tries to reduce random noise and thus removes narrow spikes in a spectrum. Differentiation extracts relevant information (but increase noise). In the first derivative an additive baseline is removed and therefore spectra that are shifted in parallel to other absorbance values will have identical first derivative spectra. A second derivative removes a constant and a linear baseline. The most used techniques for smoothing and differentiation are Savitzky-Golay filters.

Validation

As previously stated, validation is a crucial step to ensure reliability of prediction of unknown samples. It is vital to clarify some concepts about validation, a mandatory step of model development. There are at least two validation methods usually reported in NIRS calibration: cross-validation and independent validation. In the first, data are taken from the calibration database and predicted through the equation developed with remain data. Several segments are predicted by the developed equation in this approach. Independent validation implies in evaluating the model by predicting samples collected in a different place or time from those used for calibration. The last is the recommended approach if the interest is to expand the use of equations to different situations. However, in both approaches, predicted and observed values are comparable statistically.

Unfortunately, despite the very wide database developed with fecal NIRS worldwide, mostly report only the performance of calibration and internal validation. In this case, if the intent is to use the calibration to predict unknown samples in wide cases, a further external validation procedure is required. However, those publications reporting external validation leave no doubt this technology is robust and can be widely used to support farmers and technicians to monitor nutrition of grazing animals. The lack of large databases, with external robust validation is probably the reason for the few initiatives of providing services based on fecal NIRS to farmers and extension people. As stated before, basically the Grazing Animal Nutrition Laboratory (GANLAB) from Texas A&M University in USA and the Symbio Aliance in Australia, are currently providing this service as routine, despite the great potential of this technology.

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