UFRRJ
INSTITUTO DE ZOOTECNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

DISSERTAÇÃO

Near-infrared spectra, bromatological analyses, and *in vitro* digestibility for predicting indigestible neutral detergent fiber in tropical grasses

Catarina Fernandes de Oliveira

2025

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE ZOOTECNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

NEAR-INFRARED SPECTRA, BROMATOLOGICAL ANALYSES, AND *IN VITRO* DIGESTIBILITY FOR PREDICTING INDIGESTIBLE NEUTRAL DETERGENT FIBER IN TROPICAL GRASSES

Catarina Fernandes de Oliveira

Sob a Orientação do Professor
João Paulo Pacheco Rodrigues

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre(a),** no Curso de Pós-Graduação em Ciência Animal, Área de Concentração Zootecnia

Seropédica, RJ

Novembro de 2025

Universidade Federal Rural do Rio de Janeiro
Biblioteca Central / Seção de Processamento Técnico

Ficha catalográfica elaborada
com os dados fornecidos pelo(a) autor(a)

O48n

Oliveira, Catarina Fernandes de , 1998-
Near-infrared spectra, bromatological analyses, and in vitro digestibility for predicting indigestible neutral detergent fiber in tropical grasses / Catarina Fernandes de Oliveira. - Seropédica, 2025.
38 f.: il.

Orientador: João Paulo Pacheco Rodrigues.
Dissertação(Mestrado). -- Universidade Federal Rural do Rio de Janeiro, Programa de Pós Graduação em Ciência Animal, 2025.

1. Feed analysis. 2. in situ. 3. iNDF. 4. NIRS. 5. Spectroscopy. I. Rodrigues, João Paulo Pacheco, 1988 , orient. II Universidade Federal Rural do Rio de Janeiro. Programa de Pós Graduação em Ciência Animal III. Título.

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE ZOOTECNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

CATARINA FERNANDES OLIVEIRA

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre em Ciência Animal**, no Programa de Pós-Graduação em Ciência Animal, área de Concentração em Zootecnia.

DISSERTAÇÃO, APROVADA EM 28/11/2025

Documento assinado digitalmente
gov.br
JOAO PAULO PACHECO RODRIGUES
Data: 26/02/2026 10:33:45-0300
Verifique em https://validar.iti.gov.br

Assinatura

João Paulo Pacheco Rodrigues. Dr. UFRRJ
(Orientador)

Documento assinado digitalmente
gov.br
EDENIO DETMANN
Data: 26/02/2026 15:06:10-0300
Verifique em https://validar.iti.gov.br

Assinatura

Edenio Detmann. Dr. UFV

Assinatura

Tadeu Eder da Silva. Dr. UVM

Documento assinado digitalmente
gov.br
ANDRE MORAIS MOURA
Data: 26/02/2026 10:47:30-0300
Verifique em https://validar.iti.gov.br

Assinatura

André Morais Moura. Dr. UFRRJ

TERMO Nº 195/2026 - PPGZ (12.28.01.00.00.00.00.61)

(Nº do Protocolo: NÃO PROTOCOLADO)

(Assinado digitalmente em 09/04/2026 10:31)
TADEU EDER DA SILVA
ASSINANTE EXTERNO
CPF: ###.###.736-##

Visualize o documento original em https://sipac.ufrrj.br/documentos/ informando seu número: ***195****, ano:* ***2026****, tipo:* ***TERMO****, data de emissão:* ***01/04/2026*** *e o código de verificação:* ***f69c1b18ff***

DEDICATÓRIA

Dedico esse estudo à minha família, Etelberto Neto, Aline Fernandes e Carolina Fernandes, por acreditarem no meu potencial e sempre incentivarem meus estudos, minhas escolhas e meu trabalho.

AGRADECIMENTOS

À Deus, por sempre iluminar meus caminhos.

À Universidade Federal Rural do Rio de Janeiro (UFRRJ) e ao Programa de Pós-graduação em Ciência Animal (PPGCA), pelo ensino e infraestrutura, ciência e tecnologia disponibilizados durante minha pesquisa.

À Coordenação do PGCA, à Gislaine por sempre ser solicita e prestativa.

À FAPERJ, que através dos processos 200.124/2023 e 210.137/2023
forneceu apoio financeiro para a execução desse trabalho.

Ao CNPq pelo apoio ao laboratório via taxa de bancada, processo 312920/2023-7.

Aos meus pais, Etelberto e Aline Oliveira, por incentivarem meus estudos e me apoiar em todas as decisões que precisei tomar.

A minha irmã, Carolina, por sempre dizer que eu sou capaz de realizar os meus sonhos e por sonhar junto comigo.

Ao meu namorado, Nicolas, por me apoiar, incentivar e secar minhas lágrimas quando foi preciso.

À professora Joyce pelos conselhos, conversas e cafés, muito obrigada.

Aos professores João Paulo Pacheco e professora Luana Rufino, por me orientarem, pelos conhecimentos e opiniões compartilhados.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

As minhas amigas de laboratório, Laryssa, Lorhaine e Julia, por fazerem esse caminho mais divertido e leve. Obrigada pelas risadas e pela amizade.

Ao Hugo, Ximena, Paulla, Stella, Susy e a todos que colaboraram na condução do experimento. Meu mais sincero obrigada por todo apoio da equipe RuminaLab.

Muito obrigada a todos!

Por isso não tema, pois estou com você; Não tenha medo, pois sou o seu Deus. Eu o fortalecerei e o ajudarei; eu o segurarei com a minha mão direita vitoriosa.

Isaias 41:10

RESUMO

OLIVEIRA, Catarina Fernandes de. **Near-infrared spectra, bromatological analyses, and *in vitro* digestibility for predicting indigestible neutral detergent fiber in tropical grasses.** 2025. 38p. Dissertação (Mestrado em Ciência Animal). Instituto de Zootecnia, Programa de Pós-Graduação em Ciência Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2025.

O teor de FDN indigestível (FDNi) das forragens é uma informação relevante para a modelagem nutricional, formulação de dietas e avaliação de alimentos em ruminantes. O objetivo deste estudo foi avaliar o desempenho da predição de FDNi em *Urochloa* e *Megathyrsus spp*., utilizando combinações de espectros no infravermelho próximo (NIR) de um equipamento portátil com análises laboratoriais e/ou digestibilidade *in vitro* da FDN (DIVFDN). Um total de 242 amostras de *Urochloa spp* e *Megathyrsus spp*. coletadas a 50% da altura do dossel foram obtidas em três regiões. As análises laboratoriais de MS, MO, FDN e nitrogênio foram realizadas por via úmida. Os valores de referência de FDNi foram analisados por incubação *in situ* de 312 horas. As amostragens espectrais de 900 a 1700 nm foram obtidas por meio de um espectrômetro de infravermelho (NIR) portátil de reflectância. Quatro conjuntos de preditores foram definidos: espectros NIR (SPEC); SPEC com análises laboratoriais (SPEC_L); SPEC com DIVFDN (SPEC_D); e SPEC com DIVFDN e análises laboratoriais (SPEC_DL). Um total de 49 combinações de pré-processamento foi avaliado, e os modelos de calibração foram ajustados utilizando regressão de mínimos quadrados parciais (PLSR). Para cada conjunto de predição, o modelo de calibração com o menor erro quadrático médio (RMSE) da validação cruzada *leave-one-out* (LOOCV) foi selecionado para *bootstrap*. As métricas avaliadas incluíram RMSE, coeficiente de determinação (R^2), erro médio e razão entre desempenho e desvio (RPD). A comparação das métricas entre os conjuntos de preditores baseou-se em intervalos de confiança de 95% obtidos por 1000 interações *bootstrap*. As alturas das plantas e os teores de FDNi, e IVNDFD variaram de 15 a 215 cm, 81 a 426 e 382 a 837 g kg MS^{-1}, respectivamente. O aumento da complexidade dos conjuntos de preditores, de SPEC para combinações SPEC_D e SPEC_DL, reduziu o RMSE e aumentou R^2 e RPD, tanto no treino quanto na LOOCV. Maiores valores de RPD, de 3,04 em SPEC_D e 2,14 em SPEC_DL, foram observados. Os conjuntos SPEC_D e SPEC_DL resultaram em menores RMSE e maiores R^2 e RPD (IC95%). O erro médio foi próximo de zero para FDNi em todos os conjuntos de predição. Conclui-se que a combinação de IVNDFD com espectros obtidos por dispositivo NIR portátil melhora a predição de FDNi em gramíneas tropicais.

Palavras-chave: análise de alimentos, espectroscopia, FDNi, *in situ*, NIRS.

ABSTRACT

OLIVEIRA, Catarina Fernandes de. **Near-infrared spectra, bromatological analyses, and *in vitro* digestibility for predicting indigestible and potentially digestible neutral detergent fiber in tropical grasses.** 2025. 38p. Dissertation (Master of Science in Animal Science). Institute of Animal Science, Animal Science Graduate Program, Federal Rural University of Rio de Janeiro, Seropédica, RJ.

Indigestible neutral detergent fiber (iNDF) content in forages is relevant information for nutritional modeling, diet formulation, and feed evaluation in ruminants. This study aimed to evaluate the predictive performance for iNDF in Urochloa and Megathyrsus spp. using combinations of near-infrared (NIR) spectra from a portable instrument with wet-chemistry analyses and/or in vitro NDF digestibility (IVNDFD). A total of 242 samples of *Urochloa spp*. and *Megathyrsus spp*., collected at 50% of canopy height, were obtained from three regions. Laboratory analyses of DM, OM, NDF, and nitrogen were performed using wet-chemistry methods. Reference iNDF values were determined by 312-h in situ incubation. Spectral measurements from 900 to 1700 nm were obtained using a portable NIR reflectance spectrometer. Four predictor sets were defined: NIR spectra (SPEC); SPEC plus laboratory analyses (SPEC_L); SPEC plus IVNDFD (SPEC_D); and SPEC plus IVNDFD and laboratory analyses (SPEC_DL). A total of 49 preprocessing combinations were evaluated, and calibration models were fitted using partial least squares regression (PLSR). For each predictor set, the calibration model with the lowest root mean square error (RMSE) from leave-one-out cross-validation (LOOCV) was selected for the bootstrap analysis. Evaluated metrics included RMSE, coefficient of determination (R^2), mean error (bias), and the ratio of performance to deviation (RPD). Comparisons among predictor sets were based on 95% confidence intervals obtained from 1,000 bootstrap iterations. Plant height and iNDF and IVNDFD concentrations ranged from 15 to 215 cm, 81 to 426 g kg^{-1} DM, and 382 to 837 g kg^{-1} DM, respectively. Increasing predictor-set complexity from SPEC to the SPEC_D and SPEC_DL combinations reduced RMSE and increased R^2 and RPD in both calibration and LOOCV. The highest RPD values (3.04 for SPEC_D and 2.14 for SPEC_DL) were observed. The SPEC_D and SPEC_DL sets resulted in lower RMSE and higher R^2 and RPD values (95% CI). The mean error was close to zero for iNDF across all prediction sets. In conclusion, combining IVNDFD with spectra acquired using a portable NIR device improves iNDF prediction in tropical grasses.Keywords: Feed analysis, iNDF, *in situ*, NIRS, spectroscopy.

Keywords: feed analysis, *in situ,* iNDF, NIRS, spectroscopy.

SUMÁRIO

1. INTRODUCTION

The digestibility of carbohydrates in ruminants is directly related to the characteristics of the forage and its interactions with the animal. The rate of digestion, rate of release from the non-escapable to the escapable pool, and retention time in the rumen can vary markedly according to neutral detergent fiber (NDF) characteristics (Allen and Mertens, 1988; Van Soest, 1994). Considering that the neutral detergent soluble fraction (NDS) is almost entirely degraded (Hall et al., 1998), the quantification of the indigestible fraction of NDF (iNDF) is of great importance in ruminant nutrition. Tpreprocessingon is a relevant nutritional entity because it allows the calculation of potentially digestible NDF (pdNDF) and plays an important role in rumen digesta load (Huhtanen et al., 2006). For instance, NDF, iNDF, pdNDF, and/or their ratios have been used in predictive equations of dry matter intake and ruminal or total gastrointestinal tract digestibility (Detmann et al., 2014; Huhtanen et al., 2006; Nousiainen et al., 2003).

The estimation of iNDF is primarily obtained from *in situ* incubations that require prolonged incubation times greater than 240 h (Casali et al., 2008; Krizsan and Huhtanen, 2013). *In situ* incubation requires the availability and maintenance of cattle fitted with rumen cannulas. Such dependence imposes significant limitations, such as high maintenance costs, ethical concerns regarding the use of surgically cannulated animals, and the lengthy analysis time. Thus, despite its importance and applications in ruminant nutrition, iNDF is still not included as a usual laboratory analysis component and has limited use as information at the field level. In this sense, methods to estimate iNDF without the need for in situ incubations could promote its use in ruminant nutrition and improve productive performance. Furthermore, they can increase the accuracy of intake equations, digestibility estimates, and the capacity of feedstuffs to be converted into animal products, among other examples.

Considering forages, the extent and rate of NDF digestibility are driven by the degree of lignification in the cell walls (Jung and Vogel, 1986). As lignification is determined by several factors, NDF digestion is influenced by forage species, stage of maturity, management, latitude, and climatic conditions (Van Soest et al., 1978). However, lignin analysis does not serve as a nutritional predictor as robust as iNDF analysis. This limitation occurs because lignin analysis does not account for all the lignocellulosic compounds which in fact are related to filling the gastrointestinal tract (Harper and McNeill, 2015). Additionally, lignin quantification is defined by its method and has several variants and interactions with sample matrices, which may reduce

reproducibility (Gomes et al., 2011). Consequently, iNDF cannot be accurately predicted based on lignin content, as lignin does not represent a uniform chemical entity within the composition of the cell wall (Detmann et al., 2004; Traxler et al., 1998a; Wilson, 1994). Thus, alternatives to lignin quantification may be more promising for predicting forage iNDF.

Near-infrared spectroscopy (NIRS) has been widely applied to evaluate various characteristics of forages. The ability of NIRS to predict the *in vivo* and *in vitro* digestibility of feeds and diets for cattle has been highlighted (Peters et al., 2023; Zahera et al., 2022), as well as to estimate the rate and extent of NDF degradation of grass silages (Wilman et al., 2000a). The utility of NIRS extends beyond digestibility, with studies showing its effectiveness in predicting the iNDF content in dried and ground samples (Fernández-Cabanás et al., 2023; Nousiainen et al., 2004; Refat; Yu, 2022; Zhang et al., 2021). Despite this progress, studies using NIRS to predict iNDF in forages have an important gap, as they do not address tropical pasture grasses such as *Urochloa* and *Megathyrsus spp*. In fact, the prediction of iNDF content using feed/forage composition and digestibility generates parameters that are entirely dependent on the forage type (Huhtanen et al., 2008; Palmonari et al., 2016). Furthermore, existing research is limited to using only the spectrum or the composition and *in vitro* digestibility as predictors, without measuring the impact of combining them. These variables are readily available in many laboratories and could improve the predictive capacity of NIRS calibrations for estimating iNDF content.

Based on the hypothesis that using spectra combined with chemical composition and *in vitro* digestibility data improves the prediction of iNDF, the objective of this study was to evaluate the performance of iNDF prediction in *Urochloa* and *Megathyrsus* samples, with combinations of NIRS with laboratory bromatological analysis and/or *in vitro* digestibility.

2. LITERATURE REVIEW

2.1 Neutral Detergent Fiber and its Indigestible Fraction

The assessment of the nutritive value of feedstuffs is based on an integrated analysis of intake, digestibility. However, since intake is highly influenced by various external factors unrelated to the feed itself, the evaluation primarily focuses on digestibility, aiming to identify the intrinsic properties of the feed (Cabral, 2002). The digestibility of forages by ruminants is largely determined by the NDF content, the rate and extent of NDF digestion, and the ruminal retention time (Van Soest, 1994).

The chemical and physical attributes of NDF are determinant factors in shaping its nutritional efects. It closely represents the insoluble feed fraction in aqueous medium, as found in the rumen, and refers to the fibrous fractions that exert a filling effect within the digestive tract, thereby regulating ruminal retention time and directly impacting voluntary intake (Detmann, 2010). The iNDF corresponds to the fraction of NDF that resists long digestive processes within the gastrointestinal tract of ruminants, being composed of complex lignin and highly lignified fractions of cellulose and hemicellulose (Detmann et al., 2012).

2.1.1 Importance of Indigestible Neutral Detergent Fiber

The evaluation of the fibrous fraction of forages constitutes an essential aspect for determining the nutritive value of ruminant diets. This fraction represents a cost-effective energy source, exhibits greater variability compared to other constituents of the cell wall, and should be considered the primary characteristic in assessing energy availability (Conrad et al., 1984). Within this fibrous component, the iNDF performs a crucial role in animal nutrition, as it is closely associated passage rate through the digestive tract. The presence of iNDF directly influences the rate at which feed is digested and transits through the gastrointestinal system, thereby affecting the efficiency of digestion and the utilization of nutrients provided by the diet (Valente, 2011).

Given its impact on both animal performance and nutritional modeling, the precise and accurate quantification of iNDF is critical, yet it faces methodological and practical challenges. Nutritionally, NDF comprises two distinct fractions: an indigestible fraction (iNDF) and a potentially digestible fraction (pdNDF). As established by Mertens (1993), pdNDF can be degraded in the rumen through the action of the ruminal microbiota, whereas the indigestible fraction is removed from the digestive system exclusively via passage. The quantification of

iNDF in forages is conventionally performed by analyzing the residual fibrous material remaining after standardized *in situ* or *in vitro* incubation periods.

Beyond defining the indigestible residue and its relevance to passage rate, the iNDF fraction is also widely employed to estimate digestibility in trials. In trials involving ruminants, the most employed internal markers are indigestible feed residues, represented by iNDF and indigestible acid detergent fiber (iADF) (Franco et al., 2021).

2.1.2 Challenges in Estimating iNDF

Owing to its indigestible nature and role in reducing the potentially digestible fibrous portion of the plant cell wall (Traxler et al., 1998), lignin is generally recognized as the primary factor limiting forage digestion (Van Soest, 1994). Consequently, it theoretically serves as the main information for estimating the iNDF content in two of the most widely adopted nutritional systems: the Cornell Net Carbohydrate and Protein System (CNCPS) and the National Research Council (NRC, 2001). It is worth noting that there are several methodological particularities regarding the determination of lignin content (Gomes et al., 2011),and limitations to the use of lignin analysis to predict iNDF are well established (Harper and McNeill, 2015).

In this context, the *in situ* technique is the gold standard to the estimation of iNDF by incubation periods equal to or longer than 288 hours (Casali et al., 2008; Detmann et al., 2025). However, it is important to highlight the requirement for rumen cannulated animals, which entails several limitations, including maintenance costs, prolonged analysis time, and ethical considerations associated with the use of cannulated animals.

2.1.3 Attempts to Assess iNDF Through *in vitro* Incubations

In vitro methods for determining feed digestibility are easier and more cost-effective, aiming to replicate rumen fermentation conditions through the use of inoculum. *In vitro* digestibility estimates enable reaserchers to predict the *in vivo* digestibility of these fractions by means of mathematical equations (Silva et al., 2017).

The pioneering technique was that of Tilley and Terry (1963), who developed it for forage prediction. The digestibility of 148 herbaceous species with known *in vivo* digestibility was evaluated, confirming a high correlation between *in vitro* and *in vivo* values determined in sheep. Over the years, modifications have been made to the original procedure; however, it still presents disadvantages, such as the lack of information regarding the kinetics of feed digestion,

it also involves the use of ruminal fluid collected from fistulated animals, which raises ethical concerns related to animal welfare, in addition to being labor-intensive (Suassuna et al., 2021).

An artificial rumen fermenter, developed by Ankom® and known as the Daisy^{II} Incubator, offers the advantage of allowing the simultaneous analysis of multiple samples, thereby reducing the labor required for digestibility assessments (Adesogan, 2002). Several studies comparing *in vitro* dry matter digestibility (IVDMD) values obtained using the Daisy^{II} artificial fermenter method with those derived from the traditional Tilley & Terry (1963) technique have indicated that both approaches have similar yield results (Mabjeesh; Cohen; Arieli, 2000; Santos et al., 2000). However, Silva et al. (2017) reported higher IVDMD values when determined using a Daisy^{II} incubator than using the traditional method.

Bender et al. (2016) conducted a comparative study between *in vitro* and *in situ* methodologies for estimating indigestible neutral detergent fiber (iNDF) in corn silage, evaluating two fermentation times (120 and 288 hours). The results showed that the *in vitro* 120 h incubation period provided a higher iNDF estimate (37.8% of total NDF) than *in situ* 120 h (32.1% of NDF), *in vitro* 288h (31.2%), and *in situ* 288 h (25.7%). The authors concluded that iNDF represents a consistent parameter with methodologically concordant results, regardless of the technique employed.

2.1.4 The *in situ* methods for estimating iNDF

The *in situ* technique allows for the incubation of a small amount of feed in porous, non-degradable bags suspended in the rumen of a fistulated animal for different periods (Ørskov and McDonald, 1979). This method assumes that the disappearance of the substrate within the bags represents the true degradation of the sample by ruminal microbes (Suassuna et al., 2021). However, this assumption is often debated, as controversies exist: as not all material exiting the bags was previously degraded (due to particle washout), and part of the residue is not truly undegradable matter (due to microbial contamination or potentially degradable matter remaining) leading to potential biases (Dijkstra et al., 2005). Despite these limitations, the *in situ* technique has been widely adopted in recent years, especially for quantifying iNDF through incubation periods equal or longer than 240 (Casali et al., 2008;Krizsan; Huhtanen, 2013; Detmann et al., 2025).

However, several factors can compromise the evaluation of *in situ* degradation of feedstuffs, such as the type of *filter bag* employed. Two standards are being used. Smaller filter

bags such as F57 *filter bag* from Ankom® and those manufactured with nonwoven fabric (NW, 100 g/m^2), provide similar iNDF results under the same incubation conditions (Valente, 2011). The Nordic feed evaluation system for ruminants (Volden, 2011) standardized the use of 38 µm nylon bags, whereas in updated recommendations, 11 µm nylon bags are recommended (Krizsan et al., 2015). Thus, variations in incubation techniques still exist.

The animal effect may be a variable to be considered; however, nutritionally, animals are unlikely to cause a difference regarding the iNDF value, what may occur is a difference in the rate of content degradation, which consequently affects the incubation time necessary to accurately estimate the undegradable residue (Sampaio et al., 2014).

Assunção et al. (2022) quantified the variability in degradation rates of potentially degradable fractions (DM, CP, non-wovenn among animals and established optimized sampling protocols for in situ assays using tropical feedstuffs. Utilizing five rumen-fistulated Nelore heifers, the authors evaluated the fermentation kinetics of four concentrates and three forages using both fixed and mixed model approaches. The inclusion of individual variation as a random effect within the mixed models substantially enhanced the accuracy of the adjusted degradation profiles. Their study demonstrated that employing fewer than three animals increases the likelihood of biased kinetic parameter estimates and inflates residual variance. Consequently, the authors recommended a minimum of three experimental units per treatment, combined with strategically distributed incubation schedules, to accurately characterize digestive dynamics under tropical conditions.

2.2 Near-Infrared Reflectance Spectroscopy (NIRS)

Near-infrared spectroscopy is a non-destructive analytical approach widely used for the qualitative and quantitative evaluation of diverse materials. The method is based on the interaction between electromagnetic radiation and samples, occurring in the spectral range between 780 and 2500 nanometers (nm), where characteristic molecular vibrational transitions are observed (Stuth et al., 2003; Ariza-Nieto et al., 2018).

It has been applied across various scientific fields, including chemistry, pharmacology, and the food industry. In forage evaluations, this technique has been applied to quantify nutritional characteristics since the pioneering research of Norris et al. (1976), demonstrating reliability and becoming an essential tool for obtaining relevant nutritional information (Andreu-Rodríguez et al., 2017).

The analytical mechanism is based on differentiated spectral responses resulting from the vibrational excitation of organic functional groups, enabling the precise characterization of qualitative and quantitative parameters in complex matrices (Ludwig; Khanna, 2001; Shenk; Workman Jr.; Westerhaus, 2007). Its reliability in analyzing the chemical composition of forages has been supported by previous studies (Molano et al., 2016). The Near-infrared spectroscopy technique, by combining spectral information with data from laboratory chemical analyses, allows for the estimation of the concentrations of both organic and inorganic components present in forages.

The absorption bands reveal information about the molecules present in the sample. These bands are associated with specific molecular vibrations, such as the stretching of chemical bonds and the deformation of functional groups (Ibáñez; D Alomar, 2009; Tassone et al., 2014). All organic bonds (e.g., CH, CC, C=C, CN, OH, and NH) exhibit absorption bands in the NIR region (Ludwig; Khanna, 2001), which allows NIRS to detect the nutrients in the analyzed sample, such as lipids, proteins, and carbohydrates (Ibáñez and D Alomar, 2009).

2.2.1 Application of NIRS in predicting of iNDF

The estimation of NDF and iNDF contents is a common practice in studies utilizing NIRS technology for forage analysis. In these studies, it is frequently observed that the calibration equations exhibit high reliability, with coefficients of determination (R^2) ranging from 0.62 to 0.99 for NDF, 0.75 to 0.97 for ADF, and 0.86 to 0.94 for lignin (Fernandes, 2015; Simeone et al., 2015; Molano et al., 2016; Ullmann et al., 2017; Durmic et al., 2017). This consistency evidences the capability of NIRS to provide accurate estimates for these essential forage components.

Nousiainen et al. (2004) evaluated the capability of NIRS to predict iNDF and the digestible NDF fraction (NDFd) in 94 silage samples derived from primary cuts and regrowth of temperate grasses. The researchers developed models and calibrations using spectral readings in the 1100 to 2498 nm range. The results from the calibrated equations showed high accuracy for iNDF prediction with a coefficient of determination (R^2) of 0.910, which was significantly greater for the regrowth than for the primary growth silages, outperforming models based on lignin or other chemical compounds. They concluded that NIRS is a viable tool compared to conventional methodologies, with potential applications in ruminant feed evaluation.

Nordheim et al. (2007) evaluated the applicability of NIRS in predicting the ruminal degradation characteristics of NDF, in dried or ensiled samples of perennial grasses and red clover. The authors considered the NDF residues after 144 hours of ruminal incubation as the indigestible fraction. The results demonstrated that the calibration equations for iNDF exhibited acceptable precision, with a Standard Error of Cross-Validation (SECV) of 17.7 g/kg of dry matter (DM), a coefficient of determination (R^2) of 0.92, and a ratio of performance to deviation (RPD) of 3.4. In contrast, the prediction of the NDF degradation rate (NDFkd) showed lower accuracy (SECV of 0.012/h; R^2 of 0.51; RPD of 1.7). The initial lag time could not be estimated using this technique. It was concluded that the calibration equations for aNDF are viable for application in practical evaluations.

2.2.2 Limitations of NIRS use in predicting iNDF

Despite the significant advances in utilizing NIRS as a tool for iNDF estimation, several gaps still exist that limit the full applicability of this technique either in research or field settings. One of the main challenges is the dependency on representative calibrations, as predictive models require a robust database that incorporates a wide range of variables. This method offers many advantages, such as rapid analysis, maintenance of sample integrity, and minimal invasiveness (Porep et al., 2015).

The main limitation of this methodology is its dependency on chemometric approaches in certain scenarios. As an indirect analytical technique that operates via calibration, its application requires comparative validation with reference methods. Consequently, the reliability of the results obtained depends on the accuracy and precision of the reference analysis (Carvalho, 2018).

Furthermore, another limitation is the low sensitivity in estimating substances present in small quantities within the samples (Jamrógiewicz, 2012). Depending on the type of NIRS device, whether a portable or benchtop instrument, there may be difficulty in detecting and quantifying compounds present in trace amounts, as the spectral signal from these components is lost in the background noise generated during the reading. Regarding iNDF analysis, this limitation represents a significant hurdle for concentrate feeds, primarily due to the minute particle size and the inherently low signal-to-noise ratio during reflectance readings.

3. MATERIAL AND METHODS

All procedures involving the use of animals were approved by the Animal Ethics Committee of the Federal Rural University of Rio de Janeiro (protocol 0175-08-2022).

3.1 Forage samplings

From January to September 2024 a total of 120 samples of tropical grasses from the genus *Megathyrsus spp*. and 122 from *Urochloa spp*. were collected at the Universidade Federal Rural do Rio de Janeiro (22° 46' 26.6" S, 43° 41' 14.7" W), Universidade Federal de Viçosa (20° 46' 28.4" S, 42° 51' 29.9" W), and Universidade Federal de Lavras (21° 13' 49.8" S, 44° 58' 11.6" W). Samples were harvested at 50% of the canopy height, and the particle size was reduced with scissors to approximately 2–3 cm. At this point, each sample was distributed up to a 15 mm layer in a plastic tray and dried in a forced-air oven at 55°C for 48h, then ground to 2 and 1-mm sieve in a knife mill for iNDF analysis and bromatological analyses of dry matter, nitrogen, organic matter, and neutral detergent fiber, respectively.

3.2. Spectral samplings

Spectral data were collected using a reflective sampling portable spectrometer using dried and 1-mm milled samples (MYNIR Spectral Solutions, Embu das Artes, Brazil). Absorbance data were obtained in the wavelength range of 900–1700 nm, with an average interval of 4 nm (228 spectral bands), optical resolution from 10 to 12 nm, noise-to-signal ratio of 5000:1, and 20 scans per reading, using a 10 W halogen bulb as a light source and an InGaAs detector. For each sample and processing stage, six readings were randomly recorded. The average values of the six readings were used for calibration and statistical analyses.

3.3. Laboratory Analysis

Laboratory analyses were performed according to Dettmann et al. (2021): dry matter by oven-drying at 105°C for 16h (DM; method G-003/1), organic matter by burning in furnace at 550°C for 3h (OM; method M-001/2), nitrogen by Kjeldahl method (N; N-001/2), neutral detergent fiber using an autoclave (NDF; method F-002/2), with non-woven fabric (100g m^2) filter bags, without the use of sodium sulfite and α-amylase.

3.4. *In vitro* digestibility

In vitro DM (IVDMD) and NDF digestibility (IVNDFD) analyses were conducted using ANKOM DaisyII (Fairport, USA) artificial incubators with McDougall's buffer without urea

addition (Camacho et al., 2022; Detmann et al., 2025). In brief, 5×5 cm non-woven fabric (100 g/m2) filter bags were used with quadruplicates of 0.5 g of sample milled at 1 mm and 48 h incubations. The inoculum was obtained from three rumen-cannulated heifers averaging 430±20.4 kg BW and fed a roughage-to-concentrate ratio of 80:20 (12% CP on a DM basis). Rumen fluid was obtained by filtering with cheesecloth without blending and transported within 10 min in pre-heated (39°C) thermal bottles to the laboratory. A proportional pool of inoculum was prepared, and 400 mL was mixed with pre-heated 1600 mL of McDougall buffer solution per jar. Samples were randomly distributed among jars without replicates per sample in the same jar. After this step, each jar headspace was filled with CO_2, and the incubation time began. At the end of the incubation period, all filter bags were removed, washed with tap water, and dried in a forced air oven at 55°C for 24 h. Duplicates for IVDMD were dried in a conventional oven for 16 h at 105°C, and the remaining duplicates for IVNDFD were subjected to NDF analysis.

3.5. *In situ* iNDF procedure

For iNDF analysis, the same three rumen cannulated heifers used for *in vitro* incubations were used. To ensure pdNDF digestion, a rumen *in situ* incubation time of 312h was used, which is approximately 24h longer than that recommended in the literature (Krizsan and Huhtanen, 2013; Detmann et al., 2025). Filter bags were made of non-woven fabric (100 g/m²) with dimensions of 5×5 cm, and in each bag, 0.68 to 0.75 g of samples milled at 2 mm screen sieve were used (Valente et al., 2011). Two sequential runs were performed with one bag of each sample and eight blank bags per cow. Each cow received 144 bags per incubation, randomly divided into eight nylon bags (10×24 cm; 1×5 mm pore size) filled with 17 samples and one blank bag. Nylon bags were attached to a chain tied to the rumen cannula. At the end of each incubation, to remove excess digesta, the nylon bags were washed twice for 5 min in tap water in an automatic washing machine (Lavamax Neo Eco 12 kg; Suggar, Belo Horizonte, Brazil). The filter bags were removed from the nylon bags and washed again in a washing machine (Eletrolux Premium Care 11 kg, Curitiba, Brazil) three times for 15 min. The filter bags were then placed in plastic trays without overlapping and dried in a forced-air oven at 55°C. After this period, the filter bags were subjected to neutral detergent extraction, as mentioned in the NDF analysis. The iNDF fraction was determined following the protocol by Detmann et al. (2025), adapted by extending the incubation period to 312 h instead of the original 288 h, to ensure complete degradability exhaustion for tropical forages.

3.6. Treatments, spectral pre-processing and statistical analysis

Four predictor sets were defined: (i) near-infrared (NIR) spectra (SPEC); (ii) NIR spectra combined with laboratory analysis (SPEC_L); (iii) NIR spectra combined with IVNDFD (SPEC_D); and (iv) NIR spectra combined with both laboratory analyses and IVNDFD (SPEC_DL).

The average spectra of each sample were subjected to a set of 49 preprocessing combinations, including the raw data, using the *prospectr* package (Stevens and Ramirez-Lopez, 2013). These variations involved baseline correction, normalization, and the application of the Savitzky-Golay filter, with combinations of the following parameters: smoothing window of 7 and 11 points, second-order polynomial, derivatives of order 0 (no derivative), 1, and 2, and trimming wavelength range from 950 to 1650 nm. For each combpreprocessingdictor set, a calibration model was fitted using Partial Least Squares Regression (PLSR) using the SIMPLS algorithm and the *pls* package (de Jong, 1993; Liland et al., 1999). The predictors were mean-centered and scaled, and the number of latent components was limited to 15. For each prediction set, the calibration model with the lowest root mean square error (RMSE) of leave-one-out cross-validation (LOOCV) was selected and used in bootstrap analysis (Supplementary Figure 1).

The selected models in each predictor group were then subjected to 1000 bootstrap resampling iterations, with fixed number of components determined in model calibration using the *rsample* package (Frick et al., 2017). The metrics evaluated in bootstrap included: RMSE, coefficient of determination (R^2), bias (Bias), and ratio to performance and deviation (RPD; Williams; Sobering, 1993). The comparison between predictor sets was performed based on the confidence intervals from bootstrap iterations at the 95% confidence level (*i.e.*, 2.5^{th} and 97.5^{th} percentiles from the bootstrapped distribution). All data analyses were performed using R software version 4.5.1 (R Core Team, 2025).

4. RESULTS

4.1. Forage Chemical composition and spectra

The minimum, maximum, mean, and standard deviation of the chemical composition and digestibility of the forage samples are presented in Table 1. The samples covered a wide range of plant heights, ranging from 15 to 215 cm. The observed maximum values of IVDMD and

IVNDF of approximately 859 and 837 g kg-1, respectively, were for regrowth samples with low iNDF of approximately 81 g kg-1 DM and high N content of up to 32.1 g kg-1 DM. Low-nutritive value forages were also present in the dataset, with iNDF values up to 426 g kg-1 DM and low IVDMD and IVNDFD.

Table 1. Descriptive statistics of the chemical composition and in vitro digestibility of *Megathyrsus spp*. and *Urochloa spp*. grass samples (n=242).

Item[1]	Minimum	Maximum	Mean	SD
Plan height, cm	15	215	71	40.8
DM, g kg^{-1}	158	486	290	78.9
g kg DM^{-1}				
OM	871	954	916	17.6
Nitrogen	5.0	32.1	13.9	5.61
NDF	458	759	646	61.4
iNDF	81	426	190	82.5
g kg^{-1}				
IVDMD	483	859	680	86.3
IVNDFD	382	837	632	112.7

[1]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; iNDF = indigestible neutral detergent fiber; IVDMD = *in vitro* dry matter digestibility; IVNDFD = *in vitro* neutral detergent fiber digestibility.

The raw spectra of the evaluated samples are shown in Figure 1.

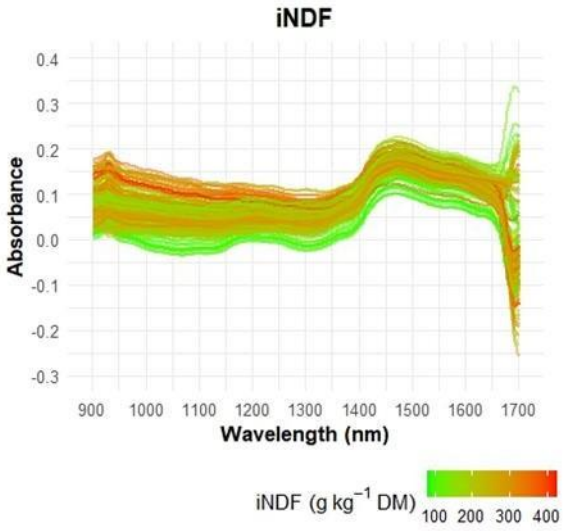


Figure 1. *Raw spectra according to indigestible (iNDF) of tropical grasses from the genera Megathyrsus spp. (n = 120) and Urochloa spp. (n = 122).*

4.2 Calibrations and model selection by LOOCV

Table 2 shows the metrics for the performance of the training and LOOCV of the calibrations according to the prediction sets for iNDF. Increasing the complexity of the SPEC to combined prediction sets decreased the RMSE and increased the R^2 and RPD in the training and LOOCV. Lower RMSE and greater RPD values in the LOOCV were observed in SPEC_D for iNDF.

Table 2. Accuracy and precision metrics of NIRS calibration models for predicting iNDF in tropical grasses using spectral data combined with laboratory results and *in vitro* NDF digestibility (n=242).

		Train[2]			Leave-one-out cross validation[2]							
	Pre-processing[1]	NLV	RMSE	R^2	RMSE	R^2	Bias	RPD	Intercept	SE	Slope	SE
SPEC	$B_{+}\|N_{-}\|W_{11}\|P_{2}\|M_{2}\|T_{-}$	10	31.2	0.856	36.5	0.804	0.232	2.25	9.3	6.88	0.948	0.033
SPEC_L	$B_{+}\|N_{-}\|W_{11}\|P_{2}\|M_{2}\|T_{-}$	10	29.7	0.870	35.6	0.814	0.162	2.31	11.2	6.69	0.937	0.032
SPEC_D	$B_{-}\|N_{-}\|W_{7}\|P_{2}\|M_{1}\|T_{-}$	15	22.3	0.926	27.1	0.892	-0.136	3.04	9.5	6.53	0.947	0.032
SPEC_DL	$B_{-}\|N_{-}\|W_{7}\|P_{2}\|M_{1}\|T_{-}$	15	22.5	0.926	27.7	0.887	-0.238	2.97	8.3	6.33	0.953	0.031

[1]B+ and B- are with or without baseline correction, respectively; N- means no normalization; W11 and W7 are Savitzky–Golay smoothing windows of 11 or 7 points; P2 means second order polynomial in Savitzky–Golay filter; M1 and M2 are derivatives of order 1 and 2, respectively; T- means no trimming.

[2]NLV is the number of latent variables; RMSE is the root mean squared error (g kg DM^{-1}); RPD is the ratio of performance to deviation. The RMSE values according to the NLV number are shown in Supplementary Figure 1. Scatterplots of observed and predicted values in LOOCV are shown in Supplementary Figure 2.

4.3 Bootstrap comparisons

The model performance metrics for each prediction set and target variable with 95%CI are presented in Figure 2. The prediction sets SPEC_D and SPEC_DL resulted in lower RMSE and higher R^2 and RPD ($P<0.05$) for predicting iNDF. Bias was close to zero in all prediction sets for iNDF. For iNDF the RPD values for SPEC_D and SPEC_DL had lower 95%CI limit greater than 3.

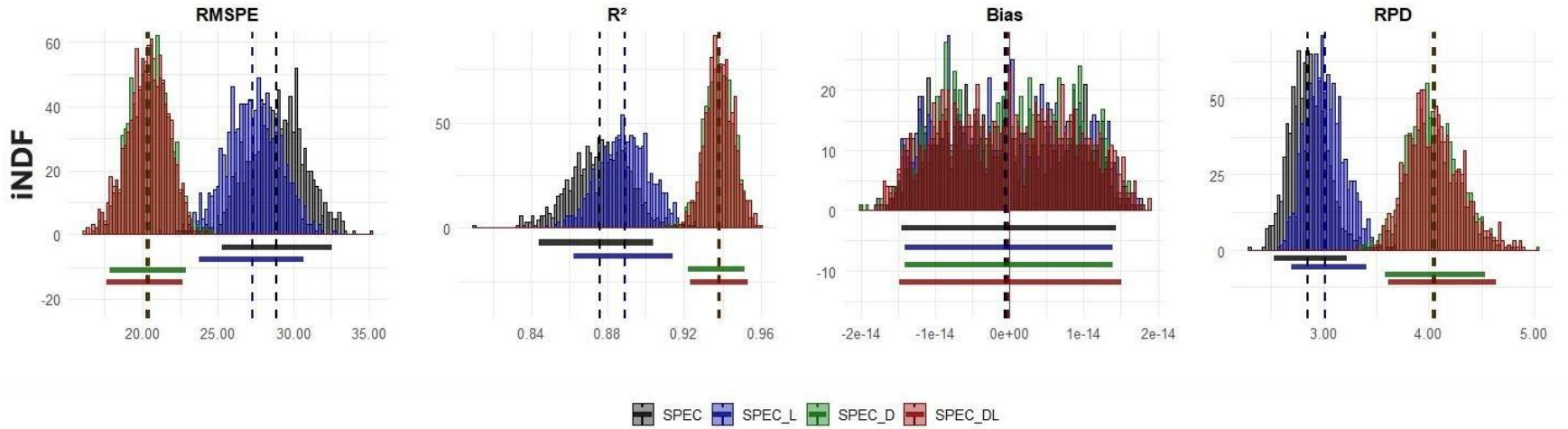


Figure 2. Empirical distributions of model's performance and its 95% confidence intervals (horizontal bars) by 1000 bootstrapping iterations of indigestible NDF (iNDF) using different prediction sets (SPEC = NIR spectra; SPEC_L = NIR spectra combined with laboratory analysis; SPEC_D = NIR spectra combined with IVNDFD; SPEC_DL = NIR spectra combined with both laboratory analyses and IVNDFD; RMSE = root mean squared error; R^2 = coefficient of determination; Bias = mean observed-predicted; RPD = ratio of performance deviation).

5 DISCUSSION

In general, the results indicate that combining spectra and IVNDFD can enhance the prediction of iNDF. Studies with external validation and field application are still necessary, but the potential use of combined spectra with IVNDFD appears promising and may support faster iNDF estimates for nutritional applications without the need of *in situ* incubations.

The range in DM, height, composition and digestibility of the samples used in this study comprises several management conditions, plant maturity and nutritive value of tropical grasses. Both *Urochloa spp*. and *Megathyrsus spp*. have several variations in cultivars, management practices and can be used at over different nutritive values in farm level. Thus, a sample matrix with wide variation in plant maturity and consequently in iNDF lignocellulosic bonds, as used in this study, is important to infer for tropical grasses. Regarding the iNDF method, it was based on a 2-mm particle size, incubation time longer than the minimum of 288h and small pore size filter bag recommended in the literature (Casali et al., 2008; S.J. Krizsan and Huhtanen, 2013; Valente et al., 2011). Therefore, the reference method can be considered appropriate for evaluating NIR and laboratory analysis capacity for predicting iNDF and pdNDF.

Relevant wavelength bands for predicting cell wall degradability in forages, including grasses, were identified from 1430-1620 and 1960-2230nm for high degradability and 1140 to 1430 for low degradability (Wilman et al., 2000b). The portable NIR spectrometer used in this study has been evaluated for forage analysis and is highlighted by its lower cost than usually evaluated portable and benchtop devices (Berzaghi et al., 2021). Its operational range from 900 to 1700 nm does not comprise all wavelength bands highlighted for the degradable fraction. Even with the narrowest wavelength window and simpler detectors and reading apparatus the portable device performed reasonably, achieving RPD values of 2.25. Acceptable RPD values for evaluating forage quality are intrinsically dependent on the analytical application, and for forage analysis the minimum values of 2.5 and 3 is used (Hsu et al., 1998; Williams, 2014). It highlights the promising results obtained using both SPEC_D and SPEC_DL, which achieved RPD values of 3.04 and 2.96, respectively.

Studies using spectroscopic models are mostly based on benchtop NIRS equipment. For instance, values of 3.60 for RPD in cross-validation were reported for predicting iNDF in Alfafa hay using benchtop NIRS from 400 to 2500nm and (Zhang et al., 2021b). For elephant grass

samples, calibrations with RPD values of 3.0 in were reported using benchtop NIRS from 400 to 2500nm (Fernández-Cabanás et al., 2023). The Nordic feed evaluation system is based on NIR calibrations using RPD values of 3.4 from independent validation (Nordheim et al., 2007) and used for applied nutritional models (Volden, 2011). Therefore, calibrations SPEC_D and SPEC_DL can be raised for external validation and have potential for routine iNDF prediction. The fact that our study achieves RPD values greater than 3.0 with a portable NIR demonstrates a significant strength related to lower costs and future on farm/field applicability.

The bootstrapping comparisons aimed to provide hypothesis testing among differences within prediction sets. Regarding RMSE, R^2 and RPD, groups of better performance were clearly defined, with SPEC_D and SPEC_DL. Considering the digestion process in the rumen and the sequential extraction of NDS, the iNDF is mainly composed of lignocellulosic biomass. Lignocellulosic biomass is rigidly structured by the presence of the phenolic *p*-coumaric and ferulic acids which establish crucial bonds for cell wall cross-linking (Hatfield et al., 1999). The density and degree of polymerization of these aromatic linkages determine the cell wall indigestible fraction, as these bonds act as a barrier that prevents the access of fibrolytic enzymes from rumen microbes (Jung and Allen, 1995; Raffrenato et al., 2017). Considering that the IVDNDF accounts for both microbial degradation of pdNDF and is limited by these aromatic linkages on iNDF, its combination with SPEC is clearly positive and logical for improving iNDF prediction. However, because the performances of SPEC_D and SPEC_DL were similar, a parsimonious prediction can be recommended using SPEC_D.

Several limitations regarding the need for IVNDFD analysis can be raised, such as the time for incubation and analysis, the need for donor animals, and costs. However, it is important to state that the potential replacement of long time *in situ* incubations, in which rumen cannulated animals are mandatory, by potential use of lower costs NIR devices and *in vitro* incubations which can be made using stomach probe sampling or slaughtered animals opens perspective of easier and faster iNDF evaluation (Fortina et al., 2022; Terré et al., 2013). Future studies with lower incubation time (*e.g.* 24h), integration with other *in vitro* methods such as gas kinetics, and inclusion of other feed analysis may support integrating NIRS and laboratory results to replace *in situ* 288h incubation and improve the use of nutritional information of iNDF in forages.

6 CONCLUSIONS

This study demonstrated that combining IVNDFD with NIR spectroscopy using a portable device improved the prediction of iNDF in tropical grasses. The spectra combined with laboratory results and IVNDFD performed similarly to NIR spectra combined with IVNDFD, and by parsimonious, the last-mentioned prediction strategy is recommended with promising performance.

7 REFERENCES

Adesogan, A.T., 2002. What are feeds worth? A critical evaluation of selected nutritive value methods. Proceedings, in: 13th Annual Florida Ruminant Nutrition Symposium. Florida, pp. 33–46.

Allen, M.S., Mertens, D.R., 1988. Evaluating Constraints on Fiber Digestion by Rumen Microbes. J Nutr 118, 261–270. https://doi.org/10.1093/jn/118.2.261

Andreu-Rodríguez, J., Pérez-Espinosa, A., Pérez-Murcia, M.D., Moral, R., Agulló, E., Ferrández-Villena, M., Ferrández-García, M.T., Bustamante, M.A., 2017. Near infrared reflectance spectroscopy (NIRS) for the assessment of biomass production and C sequestration by Arundo donax L. in salt-affected environments. Agric Water Manag 183, 94–100. https://doi.org/10.1016/j.agwat.2016.10.005

Ariza-Nieto, C., Mayorga, O., Mojica, B., Parra, D., Afanador-Tellez, G., 2018. Use of Local algorithm with near infrared spectroscopy in forage resources for grazing systems in Colombia. J Near Infrared Spectrosc 26, 44–52. https://doi.org/10.1177/0967033517746900

Assunção, A. de S., Silva, T.E. da, Quirino, D., Franco, M. de O., Detmann, E., 2022. Variability among Animals and Incubation Protocols for Ruminant In Situ Degradation Studies with Tropical Feeds. Animals 12, 1901. https://doi.org/10.3390/ani12151901

Bender, R.W, Cook, D.E, Combs, D.K., 2016. Comparison of in situ versus in vitro methods of fiber digestion at 120 and 288 hours to quantify the indigestible neutral detergent fiber fraction of corn silage samples. J Dairy Sci 99, 7, 5394-5400. doi: 10.3168/jds.2015-10258.

Berzaghi, P., Cherney, J.H., Casler, M.D., 2021. Prediction performance of portable near infrared reflectance instruments using preprocessed dried, ground forage samples. Comput Electron Agric 182, 106013. https://doi.org/10.1016/j.compag.2021.106013

Cabral, L.S., 2002. Avaliação de alimentos para ruminantes por intermédio de métodos in vivo e in vitro: validação. Universidade Federal de Viçosa, Viçosa-MG.

Camacho, L.F., Silva, T.E., Rodrigues, J.P., Franco MO., Detmann, E., 2022. A Standard Procedure for In Vitro Digestion Using Rumen Fermenters: A Collaborative Study. Animals 12, 2842. https://doi.org/10.3390/ani12202842

Carvalho, J.C. de et al, 2018. Novos desafios da pesquisa em nutrição e produção animal. Pirassununga, SP.

Casali, A.O., Detmann, E., Valadares Filho, S. de C., Pereira, J.C., Henriques, L.T., Freitas, S.G. de, Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. Revista Brasileira de Zootecnia 37, 335–342. https://doi.org/10.1590/S1516-35982008000200021

Conrad, H.R., Weiss, W.P., Odwongo, W.O., Shockey, W.L., 1984. Estimating Net Energy Lactation from Components of Cell Solubles and Cell Walls. J Dairy Sci 67, 427–436. https://doi.org/10.3168/jds.S0022-0302(84)81320-X

de Jong, S., 1993. SIMPLS: An alternative approach to partial least squares regression. Chemometrics and Intelligent Laboratory Systems 18, 251–263. https://doi.org/10.1016/0169-7439(93)85002-X

Detmann, E., Rodrigues, J.P.P., Da Silva, T.E., BRITO NETO, A. de S., FRANCO, M. de O., 2021. Methods for feed analysis, 2rd ed. Viçosa.

Detmann, E., Rodrigues, J.P.P., Da Silva, T.E., BRITO NETO, A. de S., FRANCO, M. de O., 2025. Methods for feed analysis, 3rd ed. Viçosa.

Detmann E, Souza MA, Valadares Filho SC, BERCHIELLI TT, CABRAL LS, LADEIRA MM, et al, 2012. Métodos para Análise de Alimentos. Viçosa.

Detmann, E., Gionbelli, M.P., Huhtanen, P., 2014. A meta-analytical evaluation of the regulation of voluntary intake in cattle fed tropical forage-based diets1. J Anim Sci 92, 4632–4641. https://doi.org/10.2527/jas.2014-7717

Detmann, E., Zervoudakis, J.T., Cabral, L. da S., Rocha Júnior, V.R., Valadares Filho, S. de C., Queiroz, A.C. de, Ponciano, N.J., Fernandes, A.M., 2004. Validação de equações preditivas da fração indigestível da fibra em detergente neutro em gramíneas tropicais. Revista Brasileira de Zootecnia 33, 1866–1875. https://doi.org/10.1590/S1516-35982004000700026

Dijkstra, J., Forbes, J.M., France, J., 2005. Quantitative aspects of ruminant digestion and metabolism. CABI Publishing, UK. https://doi.org/10.1079/9780851998145.0000

Durmic, Z., Ramírez-Restrepo, C.A., Gardiner, C., O'Neill, C.J., Hussein, E., Vercoe, P.E., 2017. Differences in the nutrient concentrations, *in vitro* methanogenic potential and other fermentative traits of tropical grasses and legumes for beef production systems in northern Australia. J Sci Food Agric 97, 4075–4086. https://doi.org/10.1002/jsfa.8274

Fernandes, A.M.F., 2015. Uso da espectroscopia de reflectância do infravermelho próximo (NIRS) para previsão da composição bromatológica de vagens de algaroba e palma forrageira.

Fernández-Cabanás, V.M., Pérez-Marín, D.C., Fearn, T., Gonçalves de Abreu, J., 2023a. Optimisation of the predictive ability of NIR models to estimate nutritional parameters in elephant grass through LOCAL algorithms. Spectrochim Acta A Mol Biomol Spectrosc 285, 121922. https://doi.org/10.1016/j.saa.2022.121922

Fernández-Cabanás, V.M., Pérez-Marín, D.C., Fearn, T., Gonçalves de Abreu, J., 2023b. Optimisation of the predictive ability of NIR models to estimate nutritional parameters in elephant grass through LOCAL algorithms. Spectrochim Acta A Mol Biomol Spectrosc 285, 121922. https://doi.org/10.1016/j.saa.2022.121922

Fortina, R., Glorio Patrucco, S., Barbera, S., Tassone, S., 2022. Rumen Fluid from Slaughtered Animals: A Standardized Procedure for Sampling, Storage and Use in Digestibility Trials. Methods Protoc 5, 59. https://doi.org/10.3390/mps5040059

Franco, M.O., Detmann, E., Batista, E.D., Rufino, L.M.A., Paulino, M.F., Valadares Filho, S.C., 2021. Nutritional performance and metabolic characteristics of cattle fed tropical forages with protein and starch supplementation. An Acad Bras Cienc 93. https://doi.org/10.1590/0001-3765202120190487

Frick, H., Chow, F., Kuhn, M., Mahoney, M., Silge, J., Wickham, H., 2017. rsample: General Resampling Infrastructure. CRAN: Contributed Packages. https://doi.org/10.32614/CRAN.package.rsample

Gomes, D.I., Detmann, E., Valadares Filho, S. de C., Fukushima, R.S., de Souza, M.A., Valente, T.N.P., Paulino, M.F., de Queiroz, A.C., 2011b. Evaluation of lignin contents in tropical forages using different analytical methods and their correlations with degradation of insoluble fiber. Anim Feed Sci Technol 168, 206–222. https://doi.org/10.1016/j.anifeedsci.2011.05.001

Hall, M.B., Pell, a. N., Chase, L.E., 1998. Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. Anim Feed Sci Technol 70, 23–39. https://doi.org/10.1016/S0377-8401(97)00068-0

Harper, K., McNeill, D., 2015. The Role iNDF in the Regulation of Feed Intake and the Importance of Its Assessment in Subtropical Ruminant Systems (the Role of iNDF in the Regulation of Forage Intake). Agriculture 5, 778–790. https://doi.org/10.3390/agriculture5030778

Hatfield, R.D., Ralph, J., Grabber, J.H., 1999. Cell Wall Structural Foundations: Molecular Basis for Improving Forage Digestibilities. Crop Sci 39, 27–37. https://doi.org/10.2135/cropsci1999.0011183X003900010005x

Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R., Nørgaard, P., 2006. Digestion and passage of fibre in ruminants, in: Sejrsen, K., Hvelplund, T., Nielsen, M.O. (Eds.), Ruminant Physiology Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, Wageningen.

Huhtanen, P., Nousiainen, J., Rinne, M., 2008. Recent developments in forage evaluation with special reference to practical applications. Agricultural and Food Science 15, 293. https://doi.org/10.2137/145960606779216317

Jamrógiewicz, M., 2012. Application of the near-infrared spectroscopy in the pharmaceutical technology. J Pharm Biomed Anal 66, 1–10. https://doi.org/10.1016/j.jpba.2012.03.009

Jung, H.G., Allen, M.S., 1995. Characteristics of Plant Cell Walls Affecting Intake and Digestibility of Forages by Ruminants', J. h i m. Sci.

Jung, H.G., Vogel, K.P., 1986. Influence of Lignin on Digestibility of Forage Cell Wall Material. J Anim Sci 62, 1703–1712. https://doi.org/10.2527/jas1986.6261703x

Krizsan, S.J., Huhtanen, P., 2013. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. J Dairy Sci 96, 1715–1726. https://doi.org/10.3168/jds.2012-5752

Krizsan, S.J., Rinne, M., Nyholm, L., Huhtanen, P., 2015. New recommendations for the ruminal in situ determination of indigestible neutral detergent fibre. Anim Feed Sci Technol 205, 31–41. https://doi.org/10.1016/j.anifeedsci.2015.04.008

Liland, K.H., Mevik, B.-H., Wehrens, R., 1999. pls: Partial Least Squares and Principal Component Regression. CRAN: Contributed Packages. https://doi.org/10.32614/CRAN.package.pls

Ludwig, B., Khanna, P.K., 2001. Use of near infrared spectroscopy to determine inorganic and organic carbon fractions in soil and litter, in: Assessment Methods for Soil Carbon.

Mabjeesh, S.J., Cohen, M., Arieli, A., 2000. In Vitro Methods for Measuring the Dry Matter Digestibility of Ruminant Feedstuffs: Comparison of Methods and Inoculum Source. J Dairy Sci 83, 2289–2294. https://doi.org/10.3168/jds.S0022-0302(00)75115-0

Molano, M.L., Cortés, M.L., Ávila, P., Martens, S.D., Muñoz, L.S., 2016. Ecuaciones de calibración en espectroscopía de reflectancia en el infrarrojo cercano (NIRS) para predicción de parámetros nutritivos en forrajes tropicales. Tropical Grasslands-Forrajes Tropicales 4, 139. https://doi.org/10.17138/TGFT(4)139-145

Nordheim, H., Volden, H., Fystro, G., Lunnan, T., 2007. Prediction of in situ degradation characteristics of neutral detergent fibre (aNDF) in temperate grasses and red clover using near-infrared reflectance spectroscopy (NIRS). Anim Feed Sci Technol 139, 92–108. https://doi.org/10.1016/j.anifeedsci.2006.11.024

Nousiainen, J., Ahvenjärvi, S., Rinne, M., Hellämäki, M., Huhtanen, P., 2004. Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. Anim Feed Sci Technol 115, 295–311. https://doi.org/10.1016/j.anifeedsci.2004.03.004

Nousiainen, J., Rinne, M., Hellämäki, M., Huhtanen, P., 2003. Prediction of the digestibility of primary growth and regrowth grass silages from chemical composition, pepsin-cellulase solubility and indigestible cell wall content. Anim Feed Sci Technol 110, 61–74. https://doi.org/10.1016/S0377-8401(03)00206-2

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci 92, 499–503. https://doi.org/10.1017/S0021859600063048

Palmonari, A., Gallo, A., Fustini, M., Canestrari, G., Masoero, F., Sniffen, C.J., Formigoni, A., 2016. Estimation of the indigestible fiber in different forage types. J Anim Sci 94, 248–254. https://doi.org/10.2527/jas.2015-9649

Peters, J.F., Swift, M.L., Penner, G.B., Lardner, H.A., McAllister, T.A., Ribeiro, G.O., 2023. Predicting fecal composition, intake, and nutrient digestibility in beef cattle consuming high forage diets using near infrared spectroscopy. Transl Anim Sci 7. https://doi.org/10.1093/tas/txad043

Porep, J.U., Kammerer, D.R., Carle, R., 2015. On-line application of near infrared (NIR) spectroscopy in food production. Trends Food Sci Technol 46, 211–230. https://doi.org/10.1016/j.tifs.2015.10.002

R Core Team, 2025. R: A Language and Environment for Statistical Computing.

Raffrenato, E., Fievisohn, R., Cotanch, K.W., Grant, R.J., Chase, L.E., Van Amburgh, M.E., 2017. Effect of lignin linkages with other plant cell wall components on in vitro and in vivo neutral detergent fiber digestibility and rate of digestion of grass forages. J Dairy Sci 100, 8119–8131. https://doi.org/10.3168/jds.2016-12364

Refat, B., Yu, P., 2022. Evaluation of prediction of indigestible fiber fraction (iNDF) of whole-crop barley silage by using non-destructive spectroscopic techniques as a fast-screening method: comparison between FTIR vs. NIR. Canadian Journal of Plant Science 102, 1130–1138. https://doi.org/10.1139/cjps-2022-0054

Sampaio, C.B., Gomes, D.Í., Regadas Filho, J.G.L., Detmann, E., Valadares Filho, S.C., 2014. Variations among animals when estimating the undegradable fraction of fiber in forage samples. Semin Cienc Agrar 35, 2739. https://doi.org/10.5433/1679-0359.2014v35n5p2739

Santos, G.T., A.M.A., G.G.D., M.E.C., C.U., J.C.C., & D.J.C., Assis, M.A., Gonçalves, G.D., Modesto, E.C., Cecato, U., Jobim, C.C., Damasceno, J.C., 2000. Determinação da digestibilidade in vitro de gramíneas do gênero Cynodon com uso de diferentes metodologias. Acta Sci.

Shenk, J.S., Workman Jr., J.J., Westerhaus, M.O., 2007. Application of NIR Spectroscopy to Agricultural Products. Handbook of Near-Infrared Analysis 347–386.

Silva, T.E., Detmann, E., Camacho, L.F., Saliba, E.O.S., Palma, M.N.N., Valadares Filho, S.C., 2017. Comparação de métodos in vitro para a quantificação da digestibilidade da matéria seca e da fibra em detergente neutro de forragens e concentrados. Arq Bras Med Vet Zootec 69, 1635–1644. https://doi.org/10.1590/1678-4162-9096

Stevens, A., Ramirez-Lopez, L., 2013. prospectr: Miscellaneous Functions for Processing and Sample Selection of Spectroscopic Data. CRAN: Contributed Packages. https://doi.org/10.32614/CRAN.package.prospectr

Stuth, J., Jama, A., Tolleson, D., 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crops Res 84, 45–56. https://doi.org/10.1016/S0378-4290(03)00140-0

Suassuna, J.A., de Andrade, A.P., Rufino, M.O.A., Fernades, B.D.O., Menezes, D.R., de Medeiros, A.N., 2021. Métodos in situ e in vitro utilizados para avaliação de alimentos e dietas de ruminantes. Pubvet 15. https://doi.org/10.31533/pubvet.v15n10a938.1-15

Tassone, S., Masoero, G., Peiretti, P.G., 2014. Vibrational spectroscopy to predict in vitro digestibility and the maturity index of different forage crops during the growing cycle and after freeze- or oven-drying treatment. Anim Feed Sci Technol 194, 12–25. https://doi.org/10.1016/j.anifeedsci.2014.04.019

Terré, M., Castells, L., Fàbregas, F., Bach, A., 2013. Short communication: Comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. J Dairy Sci 96, 5290–5294. https://doi.org/10.3168/jds.2012-5921

Traxler, M.J., Fox, D.G., Van Soest, P.J., Pell, A.N., Lascano, C.E., Lanna, D.P., Moore, J.E., Lana, R.P., Vélez, M., Flores, A., 1998a. Predicting forage indigestible NDF from lignin concentration. J Anim Sci 76, 1469. https://doi.org/10.2527/1998.7651469x

Ullmann, I., Herrmann, A., Hasler, M., Taube, F., 2017. Influence of the critical phase of stem elongation on yield and forage quality of perennial ryegrass genotypes in the first reproductive growth. Field Crops Res 205, 23–33. https://doi.org/10.1016/j.fcr.2017.02.003

Valente, 2011. Utilização De Tecidos Na Avaliação De Compostos Fibrosos E Na Degradação Ruminal In Situ De Alimentos Para Ruminantes.

Valente, T.N.P., Detmann, E., Valadares Filho, S. de C., Cunha, M. da, Queiroz, A.C. de, Sampaio, C.B., 2011. In situ estimation of indigestible compounds contents in cattle feed and feces using

bags made from different textiles. Revista Brasileira de Zootecnia 40, 666–675. https://doi.org/10.1590/S1516-35982011000300027

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Ithaca: Cornell University Press.

Van Soest, P.J., Mertens, D.R., Deinum, B., 1978. Preharvest Factors Influencing Quality of Conserved Forage. J Anim Sci 47, 712–720. https://doi.org/10.2527/jas1978.473712x

Volden, H., 2011. NorFor - The Nordic feed evaluation system. Brill | Wageningen Academic. https://doi.org/10.3920/978-90-8686-718-9

Williams, P., 2014. The RPD Statistic: A Tutorial Note. NIR news 25, 22–26. https://doi.org/10.1255/nirn.1419

Williams, P.C., Sobering, D.C., 1993. Comparison of Commercial near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds. J Near Infrared Spectrosc 1, 25–32. https://doi.org/10.1255/jnirs.3

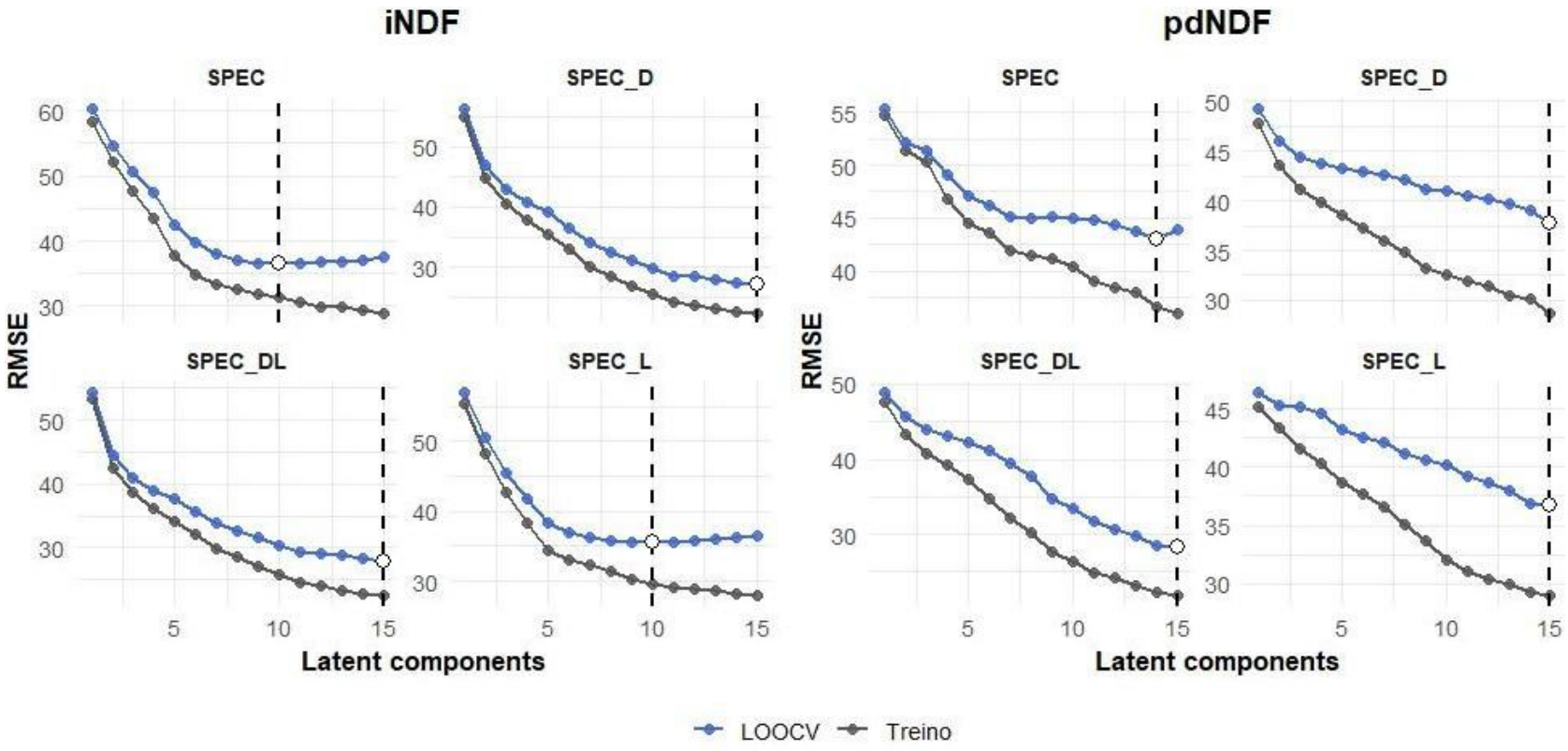
Wilman, D., Field, M., Lister, S.J., Givens, D.I., 2000. The use of near infrared spectroscopy to investigate the composition of silages and the rate and extent of cell-wall degradation. Anim Feed Sci Technol 88, 139–151. https://doi.org/10.1016/S0377-8401(00)00224-8

Wilson, J.R., 1994. Cell wall characteristics in relation to forage digestion by ruminants. J Agric Sci 122, 173–182. https://doi.org/10.1017/S0021859600087347

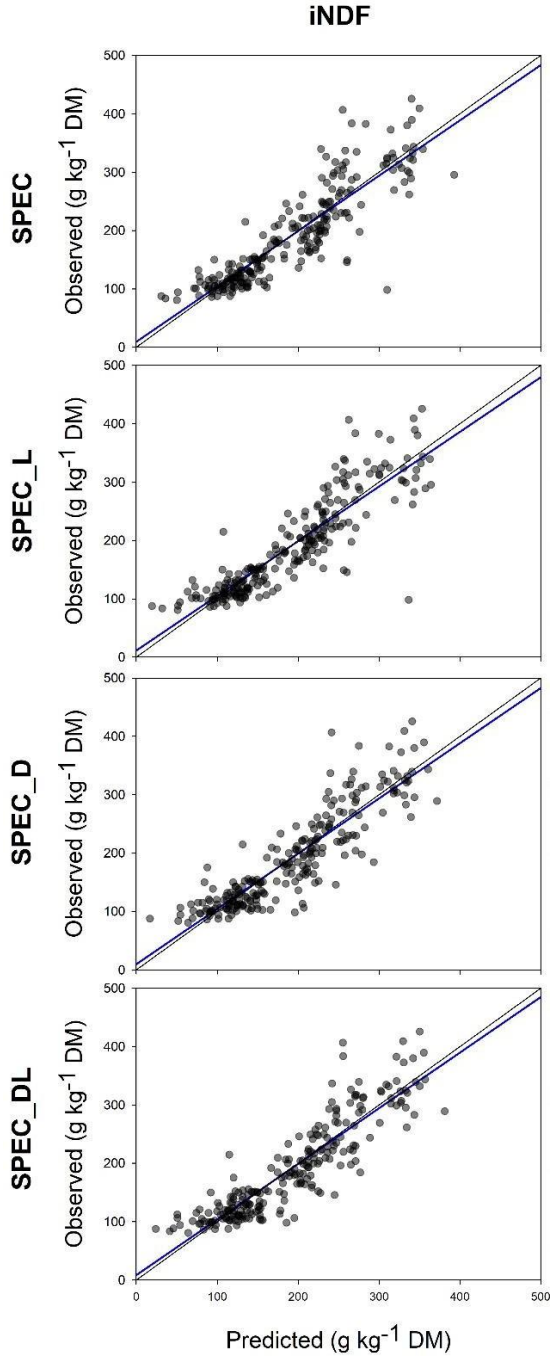
Zahera, R., Sari, L.A., Permana, I.G., Despal, 2022. The use of near-infrared reflectance spectroscopy (NIRS) to predict dairy fibre feeds in vitro digestibility. IOP Conf Ser Earth Environ Sci 951, 012100. https://doi.org/10.1088/1755-1315/951/1/012100

Zhang, G.J., Wang, Y., Yan, Y.H., Hall, M.H., Undersander, D.J., Combs, D.K., 2021. Comparison of two in situ reference methods to estimate indigestible NDF by near infrared reflectance spectroscopy in alfalfa. Heliyon 7, e07313. https://doi.org/10.1016/j.heliyon.2021.e07313

Supplementary Figures



Supplementary Figure 1: Root mean square error (RMSE) of calibration (Training) and leave-one-out cross-validation (LOOCV) as a function of the number of latent components for prediction models of indigestible (iNDF) and potentially digestible (pdNDF) neutral detergent fiber in tropical grasses. Models represent different predictor sets: NIR spectra (SPEC); SPEC with laboratory analyses (SPEC_L); SPEC with *in vitro* digestibility (SPEC_D); and SPEC with both *in vitro* digestibility and laboratory analyses (SPEC_DL). The vertical dashed line indicates the number of components selected for each model.



Supplementary Figure 2: Relationship between observed and predicted values of indigestible neutral detergent fiber (iNDF, g kg^{-1} DM) in tropical grasses for different predictor sets: NIR spectra (SPEC); SPEC with laboratory analyses (SPEC_L); SPEC with *in vitro* digestibility (SPEC_D); and SPEC with both *in vitro* digestibility and laboratory analyses (SPEC_DL). The solid black line represents the identity line, and the blue line represents the regression line.