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Near-Infrared Spectroscopy (NIRS) and Optical Sensors for Estimating Protein and Fiber in Dryland Mediterranean Pastures

João Serrano , Shakib Shahidian, Ângelo Carapau and Ana Elisa Rato

MED-Mediterranean Institute for Agriculture, Environment and Development,
Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94,
7006-554 Évora, Portugal; shakib@uevora.pt (S.S.); carapau_angelo@hotmail.com (Â.C.);
aerato@uevora.pt (A.E.R.)

* Correspondence: jmrs@uevora.pt; Tel.: +351-266-760-800

Abstract: Dryland pastures provide the basis for animal sustenance in extensive production systems in Iberian Peninsula. These systems have temporal and spatial variability of pasture quality resulting from the diversity of soil fertility and pasture floristic composition, the interaction with trees, animal grazing, and a Mediterranean climate characterized by accentuated seasonality and interannual irregularity. Grazing management decisions are dependent on assessing pasture availability and quality. Conventional analytical determination of crude protein (CP) and fiber (neutral detergent fiber, NDF) by reference laboratory methods require laborious and expensive procedures and, thus, do not meet the needs of the current animal production systems. The aim of this study was to evaluate two alternative approaches to estimate pasture CP and NDF, namely one based on near-infrared spectroscopy (NIRS) combined with multivariate data analysis and the other based on the Normalized Difference Vegetation Index (NDVI) measured in the field by a proximal active optical sensor (AOS). A total of 232 pasture samples were collected from January to June 2020 in eight fields. Of these, 96 samples were processed in fresh form using NIRS. All 232 samples were dried and subjected to reference laboratory and NIRS analysis. For NIRS, fresh and dry samples were split in two sets: a calibration set with half of the samples and an external validation set with the remaining half of the samples. The results of this study showed significant correlation between NIRS calibration models and reference methods for quantifying pasture quality parameters, with greater accuracy in dry samples ($R^2 = 0.936$ and $RPD = 4.01$ for CP and $R^2 = 0.914$ and $RPD = 3.48$ for NDF) than fresh samples ($R^2 = 0.702$ and $RPD = 1.88$ for CP and $R^2 = 0.720$ and $RPD = 2.38$ for NDF). The NDVI measured by the AOS shows a similar coefficient of determination to the NIRS approach with pasture fresh samples ($R^2 = 0.707$ for CP and $R^2 = 0.648$ for NDF). The results demonstrate the potential of these technologies for estimating CP and NDF in pastures, which can facilitate the farm manager's decision making in terms of the dynamic management of animal grazing and supplementation needs.



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1. Introduction

Natural or improved dryland pastures provide the basis of animal sustenance in extensive production systems in Portugal [1]. The characteristics of the Mediterranean climate, especially the hot and extremely dry summer, mean that during the summer months (July, August, and September) it is necessary to supplement the animals with concentrated feed [2]. The scarcity of pastures and the decrease in their quality may even extend into the autumn–winter months in dryer years [3]. This temporal (i.e., seasonal) variability resulting from climatic seasonality is compounded by an important spatial variability (i.e., within or between fields) [4], which is a consequence of the different fertility of the soils, the diversity of the floristic composition of the pastures and the influence of

trees and animal grazing [1], which interact with the climatic irregularity [5]. Understanding seasonal changes in pasture availability and nutrient content can enhance ruminant production systems and management [6]. However, relatively little is known about the nature and extent of spatial variability of pasture production in mixed farming systems [7], where the combination of spatial variability and temporal instability creates a certain level of unpredictability, making it difficult for farm managers to make decisions, namely in the timing and optimum grazing intensity [6] or animal supplementation [2]. Farmers and animal nutritionists require an accurate, precise, rapid, and cost-effective method of assessing the nutritive value of pastures and feeds [8]. A precise monitoring of pastures will (i) improve production system sustainability by enhancing feed utilization efficiency, (ii) improve productivity of livestock and conserved forages, and (iii) reduce the potential for wasting resources [6]. Timely information on supply and nutrient concentrations of the pasture and its associated variability will allow farmers to better match the nutrient supply with animal demand [6].

Understanding the spatial distribution of forage quality is important for addressing critical research questions in grassland science [9]. Our study focuses on a particular ecosystem, i.e., the montado, which is characteristic of the Mediterranean region; however, other studies have been carried out with the same purpose in other ecosystems with different characteristics, for example, in tropical rangeland [10], in semiarid rangelands [11], and in tallgrass prairie vegetation [9].

Grazing management decisions are dependent on assessing pasture availability and quality [1]. Pasture quality can be assessed by indicators such as crude protein (CP) or fiber (neutral detergent fiber, NDF) [12]. The traditional chemical analyses made using reference methods [13] for determining these parameters are destructive, requiring the cutting and collection of pasture samples, followed by an exhaustive set of laboratory procedures that involve costly material and human resources [14]. Another indirect cost that should not be overlooked has to do with the time needed to obtain laboratory results, which can take several weeks, and this reduces their utility for decision making. In this perspective, several technological proposals, integrated in the concept of precision agriculture (PA) and based on proximal and remote sensing, have been developed with the aim of providing a faster response at a lower cost.

The use of lab-based near-infrared spectroscopy (NIRS) for the analysis of fresh pasture samples has been commercially available for some years in many European animal feed analysis laboratories, while the use of satellites for this purpose is relatively new. The remote sensing (RS) of pasture quality is critical for a better understanding of livestock feeding patterns [9,10] and to support management decisions related to resource allocation [11]. The work of Serrano et al. [15] showed the importance of near-infrared spectroscopy (NIRS) in combination with satellite images (Sentinel-2) for estimating pasture quality in the Mediterranean montado ecosystem. There are several studies that show the relevance of NIRS associated with chemometrics methods to estimate the quality of pastures [16–19]. NIRS can be particularly useful in mixed natural pastures because their composition changes over time [16]. However, although this does not require chemical analysis and is normally considered a nondestructive method [8], it requires pasture cutting and some preprocessing of the samples before the spectroscopy analysis. Studies of NIRS evaluation conducted without prior sample preprocessing showed less accuracy due to the heterogeneous nature of the samples and the difficulty in obtaining a regular and suitable particle size [16,20]. In the case of fresh pasture samples, there is reference to the problem of water generating strong absorption signals, which overlap and obscure other spectral features and can cause nonlinear responses [20]. On the other hand, although the use of satellite imagery is a very promising, low-cost, and nondestructive technique [21,22], it has its own limitations [23]. The limitations of applying RS systems in farm management include the following: (a) the gathering and delivery of images in a timely manner; (b) the shortage of high spatial resolution (10 m × 10 m) images; (c) image interpretation and data extraction issues; and (d) the combination of these data with agronomic knowledge

into expert systems [24]. Handcock et al. [25] highlighted the difficulties of RS resulting from spatial resolution and the presence of clouds, as well as the spatial and temporal specificity of the associated algorithms. The presence of clouds is common during the autumn, winter, and spring seasons in temperate and rainy regions [15] which can lead to a low temporal resolution and make it impossible to obtain reliable information in critical periods of evolution of the pasture vegetative state. In the Montado, as in other forestry ecosystems, there is an added limitation due to inaccessibility of areas located under tree canopies [15]. These limitations of RS-based methods create an opportunity for the use of nondestructive proximal sensing (PS) to monitor the vegetation [26], namely, through real-time and portable near-infrared spectroscopy [6,27,28] or through proximal optical sensors [2]. The optic active sensor provides spectral vegetation indices, mainly the Normalized Difference Vegetation Index (NDVI) calculated by measuring the reflectance of the radiation emitted by the plants at certain wavelengths (Equation (1)), with values from -1.0 to $+1.0$; it is sensitive to changes in plant yield/maturity, drivers of nutrient concentrations changes and strongly correlated with pasture CP and NDF [2,29,30].

$$NDVI = \frac{NIR - Red}{NIR + Red} \tag{1}$$

where NIR is near-infrared radiation; and Red is visible red radiation

This study aims to characterize the spatial and temporal variability of the quality of dryland pastures in several regions of Portugal in the 2020 growing season, and on the other hand, to evaluate two types of technologies for estimating and monitoring the evolution of pasture crude protein (CP) and neutral detergent fiber (NDF): (i) near-infrared spectroscopy (NIRS) combined with a multivariate data analysis applied to fresh and dry samples and (ii) a proximal active optical sensor (AOS) applied in the field to calculate the Normalized Difference Vegetation Index (NDVI) (Figure 1).

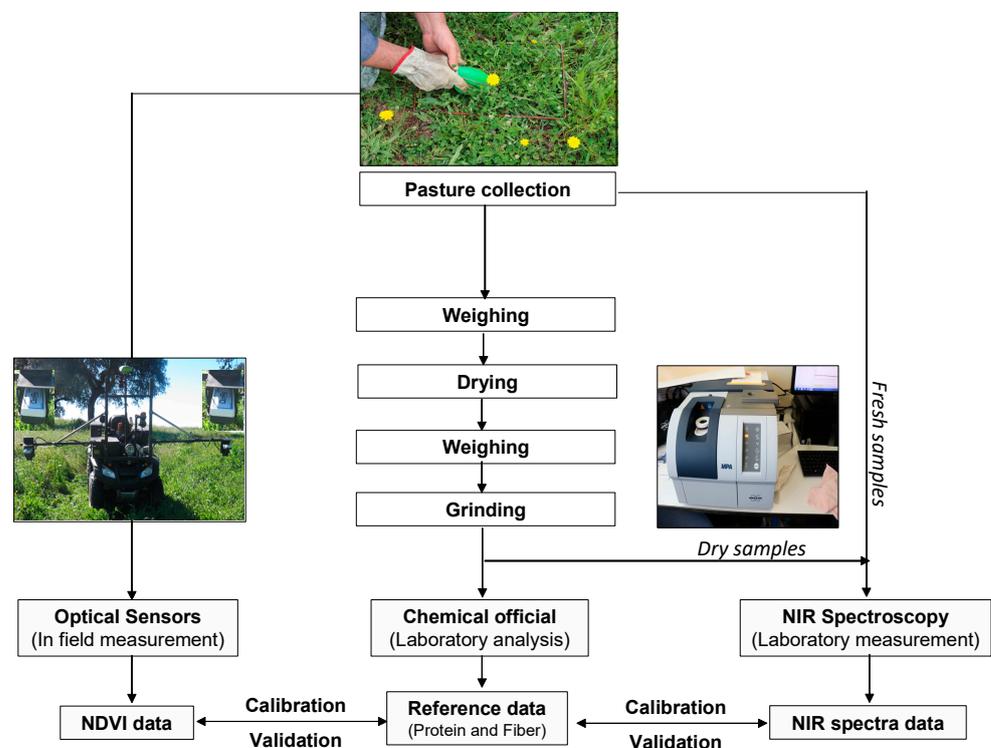


Figure 1. Schematic representation of the experimental approach used in this study.

2. Material and Methods

2.1. Characteristics of the Experimental Sites

The experiments were carried out in 8 fields (Azinhal, Cubillos, Grous, Mitra, Murteiras, Padres, Quinta França, and Tapada Números farms), each with an area of approximately 25 ha and located in 4 districts of Portugal (Beja, Évora, Portalegre, and Guarda; Figure 2). These annual or permanent biodiverse pastures (composed of different botanical species, e.g., legumes, grasses, composites, and other species) are representative of the regional dry-land pastures, with a common characteristic (such as the Holm oak or Cork oak montado) and grazing by sheep or cattle in a rotational or permanent system (further details on the characteristics of these sites are given in Serrano et al. [15]). The location of these fields are representative of the normal temperature and precipitation gradient in the country, with higher mean temperatures and smaller amounts of monthly rainfall in the southern districts (Beja and Évora in this case) and the reverse in the northern districts (Portalegre and Guarda in this case). Figure 3 illustrates the thermopluviometric graphs of these 4 districts between July 2019 and June 2020 (Source: Portuguese Institute of Sea and Atmosphere).

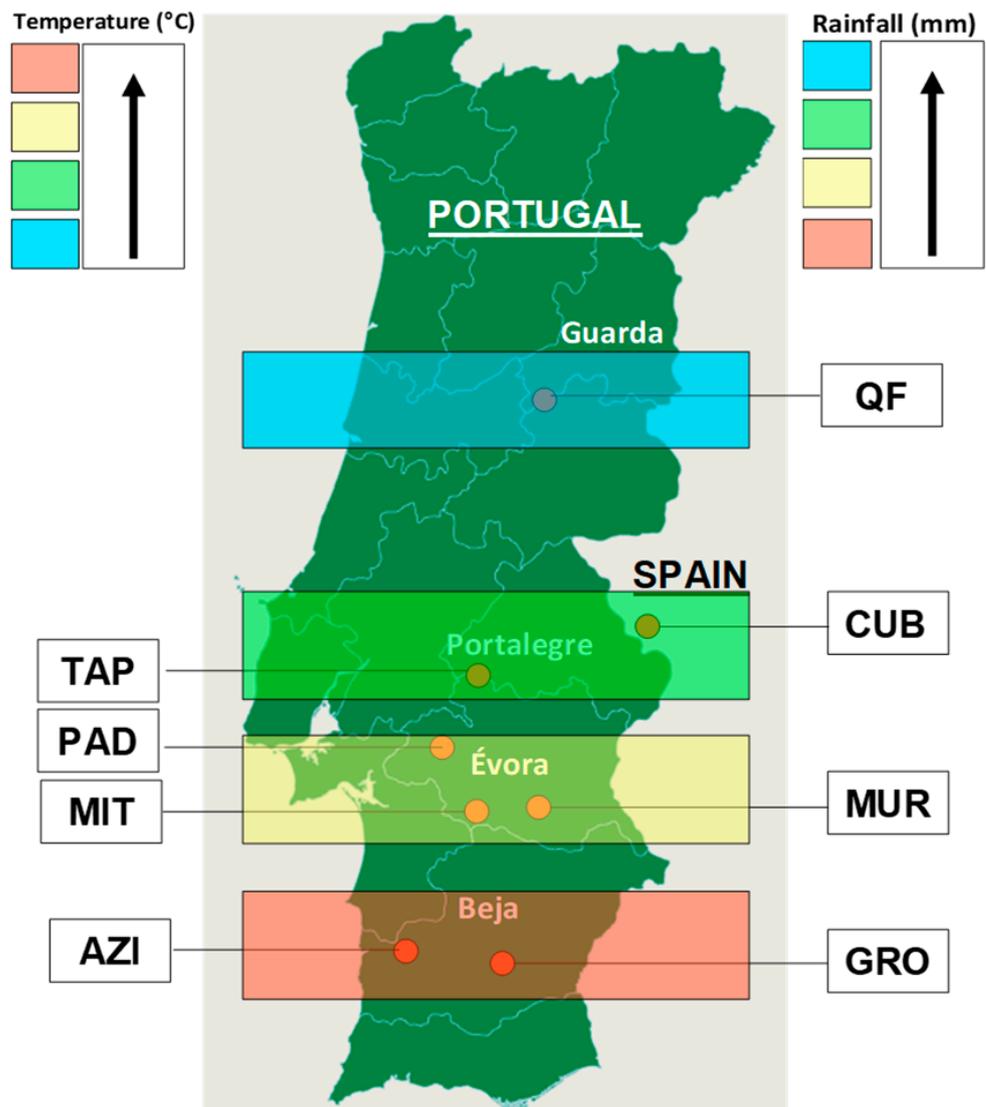


Figure 2. Location of experimental farms in Portugal.

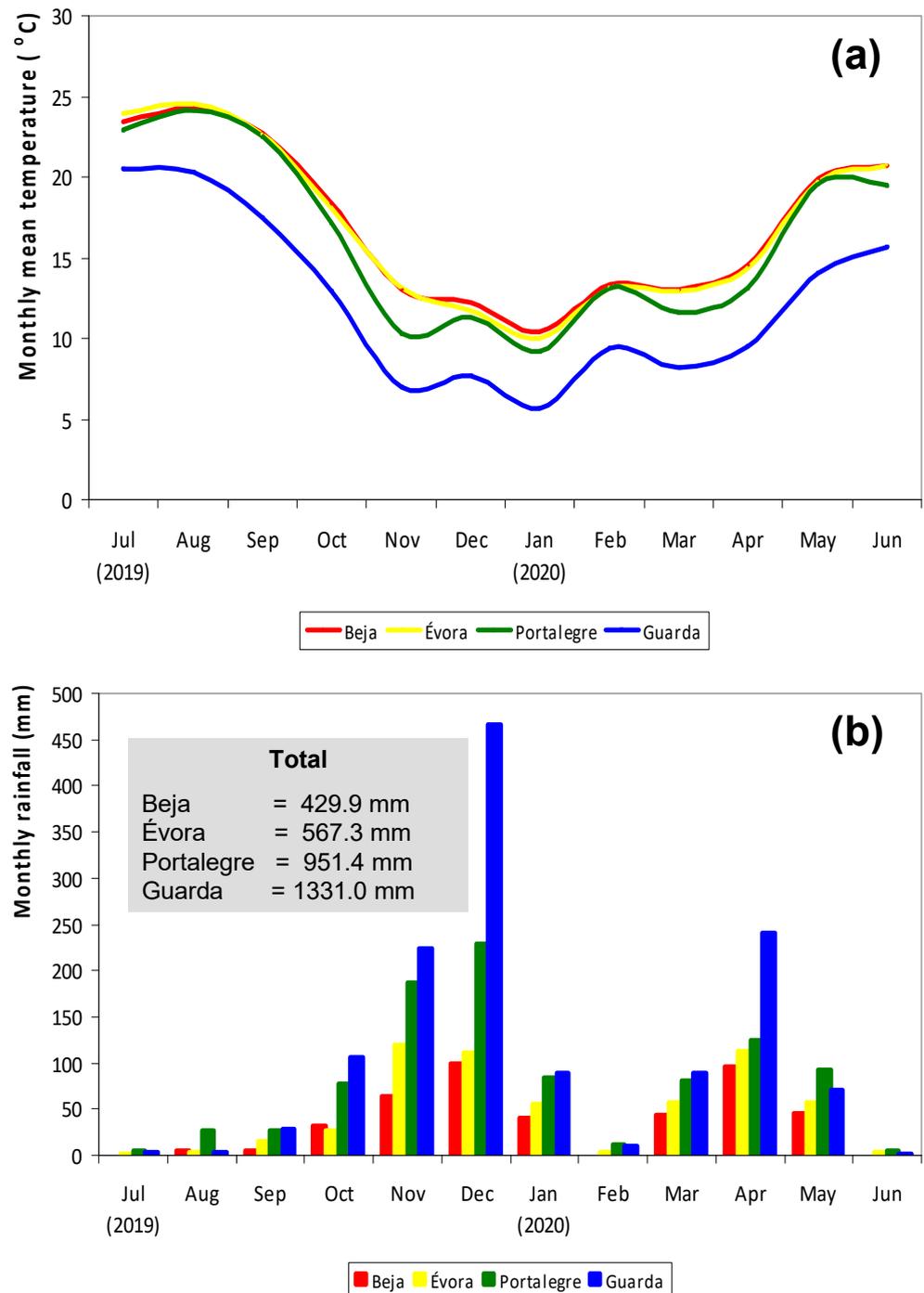


Figure 3. Thermopluviometric graphs of the Beja, Évora, Portalegre, and Guarda Portuguese districts between July 2019 and June 2020 (source: Portuguese Institute of Sea and Atmosphere). (a) monthly mean temperatures (b) monthly rainfall.

2.2. Pasture Sampling and Laboratory Processing

Pasture sampling in each location was carried out at eight georeferenced areas measuring 10 m × 10 m. In each of these areas, composite pasture samples were obtained by collecting five subsamples with an electric shears at 1 to 2 cm above ground in a 0.5 m × 0.5 m area (defined with a metal quadrat). The sampling process was noted with the day of the year (DOY) and was performed in four different times through the growth cycle, i.e., between January 2020 (DOY 20) and June 2020 (DOY 161). However, in two

fields (Cubillos and Quinta França), due to road traffic restrictions imposed as a result of the COVID-19 pandemic, it was not possible to carry out some of the pasture collections (two at Cubillos and one at Quinta França). A total of 232 pasture samples were collected and subjected to reference laboratory analysis and NIRS analysis. These pasture samples were inserted into numbered plastic bags and transported to the MED-Animal Nutrition and Metabolism Laboratory at the University of Évora. Once in the laboratory, the pasture samples were weighed to obtain the fresh mass produced, then dried in an oven (72 h at 65 °C) and weighed again to establish the dry matter and pasture moisture content (PMC wet basis, in %). Next, these samples were ground using a Perten instrument mill equipped with a 1 mm sieve. The dehydrated samples were analyzed in order to determine the reference values of crude protein and neutral detergent fiber, expressed in percentage on a dry weight basis (CP and NDF, respectively, in %) using conventional methods of wet chemistry according to the Association of Official Analytical Chemists [31]: (i) nitrogen content was analyzed using the Kjeldhal method, i.e., a colorimetric determination in an autoanalyzer (Bran+Luebbe) with a factor of conversion to CP of 6.25 (method no. G-188-97 Rev 2, Bran+Luebbe, Analyser Division, Norderstedt, Germany); (ii) the NDF content was analyzed according to the Goering and Van Soest [13] method in a Fibertec digester (Foss Tecator AB, Hoganas, Sweden).

On two dates (in March and May), fresh pasture samples were immediately transported to a NIRS device for spectra acquisition, prior to the drying and screening processes. After the NIRS analysis, the CP and NDF reference values were determined by following the abovementioned process using these 96 samples.

2.3. Laboratory Spectra Acquisition and Processing

Spectroscopic measurements were carried out between 800 and 2778 nm on 96 fresh samples and 232 dry samples using a Bruker Fourier Transformation Near-Infrared (FT-NIR) spectrometer (MPA, Opus Bruker, Germany; Figure 4a). Fresh samples were placed in a sphere macrosample rotating channel, allowing for spectra collection from large areas of the samples, while dry and ground samples were placed in a Petri dish with a flat bottom and a 9 cm diameter. Spectra were collected in diffuse reflectance mode at a room temperature of 20 °C. Reflectance data (R) were measured as $\log 1/R$ (absorbance data) at 1 nm interval, and NIR spectra data were obtained. Five spectra were collected from each sample with a spectral resolution of 8 cm^{-1} and an average spectrum was used for further mathematical processing and chemometrics analysis.

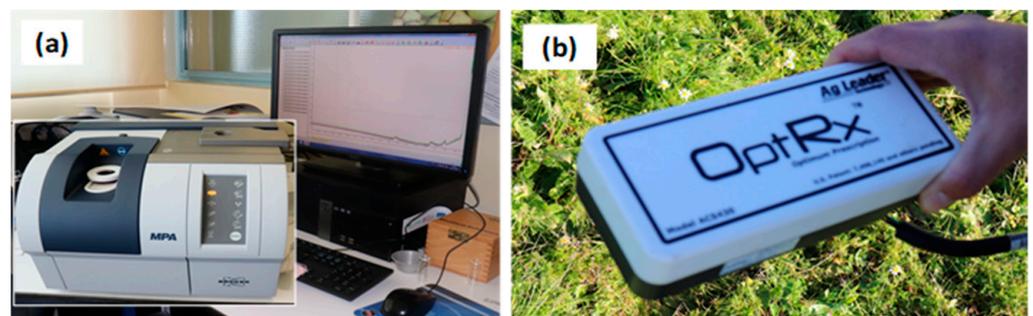


Figure 4. Near-infrared spectrometer (a) and active optical sensor (b) used in this study.

2.4. Field Optical Sensor Measurement

All pasture sampling areas were evaluated with an active optical sensor (AOS, OptRx, Ag Leader, Ames, IA, USA; Figure 4b) before cutting. The sensor (equipped with a small portable battery as the power source, which makes it independent of ambient light conditions) is placed 0.5 m above the pasture, and it measures simultaneously three visible and infrared bands: (i) red (670 nm), (ii) red edge (728 nm), and (iii) near infrared (NIR, 775 nm). With two of the abovementioned spectral bands, NDVI was calculated using

Equation (1). The operator stood still at the area of each georeferenced point and performed measurements for a five-minute period (approximately 300 records). The values of NDVI were organized in a spreadsheet and were matched with the coordinates of the respective sampling points to calculate the mean, standard deviation, and range.

2.5. Statistical Analysis

The statistical analysis of the results included a descriptive analysis with a calculation of the average and standard deviation (SD) of each dataset (PMC, CP, NDF, and NDVI). These data were organized graphically to show the evolution of pasture parameters over the pasture vegetative cycle (depending on the day of the year, DOY).

2.5.1. Statistical Analysis of Spectra

The Opus v. 7.5 software (Bruker Optik GmbH, Ettlingen, Germany) was employed for spectral data collection, and FT-NIR spectra were exported to the Unscrambler software (version 10.5.1, Camo, ASA, Oslo, Norway) for chemometric analysis; calibration and external validation models were obtained. Prediction models were developed using a partial least square regression (PLSR) algorithm, while considering an independent validation sample set for the chemometrics analysis [32]. In order to obtain the best predictive model, for PLSR, fresh and dry samples were split in two sets: a training set (calibration) with half of the samples and a test set with the remaining half of the samples, used as an external and independent validation set of the NIRS calibration models [15]. The selection of calibration and validation samples was based on the premise of a uniform distribution across the spectral space.

To find the most accurate model to quantify CP and NDF in pastures (and PMC in fresh samples), the calibration process was performed on the raw spectra data, and after the application of some preprocessing techniques (mathematical algorithms to remove any irrelevant information), the best prediction model was selected. Calibration and validation models were developed based on finding the latent factors on the data in order to maximize the covariance between the spectra and chemical analysis. The quantitative measure for the predictive accuracy from each model was evaluated using the coefficient of determination (R^2), root mean square errors (RMSE; Equation (2)), and the residual predictive deviation (RPD; Equation (3)) [15]. In the current study, in fresh pasture, the selected preprocessing methods included spectra derivative transformations and a range scaling method. The first derivative is very effective for removing baseline offset, and the second derivative is very effective for both baseline offset and for finding linear trends in the original spectra. The range scaling method used here was the standard normal variate (SNV) followed by the second derivative. The SNV technique is very useful when the total intensity in the spectra is sample-dependent [33]. In the absence of substantial adverse effects caused by undesired variations from light scattering, the original spectra may be used [34]. The original spectra were used for building the model to estimate the CP in dry pasture samples.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (E_i - M_i)^2}{n}} \quad (2)$$

$$\text{RPD} = \frac{\text{SD}}{\text{RMSE}} \quad (3)$$

where n is the number of observations, and E_i and M_i are the estimated and observed (measured) values, respectively.

2.5.2. Statistical Analysis of Optical Measuring

The statistical treatment of these results was performed using MSTAT-C software (MSTAT-C, Michigan State University, MI, USA) with a significance level of 95% ($p < 0.05$)

and consisted of an analysis of regression between the pasture reference values (PMC, CP and NDF) and NDVI measured by OAS.

3. Results and Discussion

3.1. Spatial and Temporal Variability Pattern of the Measured Parameters

Table 1 shows the mean, SD, and range of reference pasture parameters (PMC, CP, and NDF) and the NDVI obtained by the AOS in the 8 fields and on different dates. These pastures started their vegetative cycle between September and October 2019, depending on the rainfall accumulated in the beginning of autumn. Between November and January, air temperatures reached low values (with a mean of approximately 5 °C in the Guarda district and approximately 10 °C in the Portalegre, Évora, and Beja districts; Figure 3a), and so the pasture remained in a dormant phase, with practically no vegetative growth [1]. It is therefore normal for pasture quality to remain relatively high and stable in this period (high PMC and CP, low NDF), corresponding to a peak in NDVI (near DOY 60). From March and until the last sampling date (May–June), the rise in temperature and the lower rainfall (Figure 3a,b, respectively) triggered a decrease in pasture quality, with a significant decrease in CP (Figure 5a), which is reflected in a clear decrease in NDVI (Figure 5b). This pattern is similar in the various districts, with higher values of CP and NDVI in the most northern region (“QF”, Guarda district) and lower values of CP and NDVI in the south (“AZI” and “GRO”, Beja district), reflecting the evolution of the temperature–rainfall pair. In Figure 5a,b, it is possible to verify that the trend line resulting from all samples collected in all locations shows an intermediate behavior between the warmest zone and the least rainfall zone (Beja district) and the coldest and rainiest zone (Guarda district). In the most southern areas (Beja district), the period of greatest vegetative vigor of the pasture (greater NDVI) is shorter, which may reflect the combined effect of high temperatures and decreased rainfall, both important drivers in dryland crops, as is the case of biodiverse permanent pastures. The vegetative cycle of pastures in warmer areas can mean anticipating the animal supplementation needs in extensive grazing. With reference to the maintenance needs of adult sheep in terms of CP (9.4% DM [2,35]; Figure 5a), it is possible to verify that in 2020, in the warmer areas (Beja district) the animal supplementation needs in terms of protein occurred right at the beginning of May (DOY 125; Figure 5a), while in the colder areas (Guarda district) these needs occurred only in early June (DOY 155; Figure 5a).

In addition to the seasonality or temporal variability that these data reflect, which is characteristic of climatic influence, there is also an important spatial variability, especially at the CP level, which is required for the development FT-NIR calibration models. The average CV across the four dates and eight sampling sites was 5.1% for PMC (range of 1.7–12.8%), 17.0% for CP (range of 4.0–39.8%), 8.0% for NDF (range of 3.3–20.7%), and 7.9% for NDVI (range of 2.5–18.8%). This spatial variability, especially CP, reflects the diversity of species that characterize these pastures (grasses, legumes, composites, and other species) with their different rates of growth and development in response to the evolution of temperature and rainfall and also to the spatial variability of soil fertility. Spatial variability is a good predictor of the suitability of employing differentiated management technologies and techniques [23], for example, for the differentiated management of pasture, animal grazing and fertilization, or soil amendment [29], which is an important component of the PA concept [23].

Table 1. Mean, standard deviation (SD), and range of pasture parameters in the 8 fields and on different dates for dry (D) and fresh (F) samples.

| Field Code | Date | DOY | D/F | PMC, % | | CP, %DM | | NDF, %DM | | NDVI | |
|------------|--------|-----|-----|----------------|-----------|----------------|-----------|----------------|-----------|-------------------|-------------|
| | | | | Mean \pm SD | Range | Mean \pm SD | Range | Mean \pm SD | Range | Mean \pm SD | Range |
| "AZI" | 21-JAN | 21 | D | 72.1 \pm 4.7 | 62.5–77.3 | 11.5 \pm 1.8 | 9.2–13.8 | 58.7 \pm 5.3 | 47.3–62.9 | 0.636 \pm 0.055 | 0.529–0.708 |
| | 02-MAR | 62 | D/F | 78.2 \pm 3.7 | 72.7–83.1 | 15.8 \pm 1.1 | 14.4–17.7 | 53.2 \pm 3.7 | 48.8–59.9 | 0.698 \pm 0.045 | 0.607–0.748 |
| | 21-APR | 112 | D | 83.1 \pm 1.9 | 79.8–86.1 | 12.4 \pm 1.8 | 8.9–14.4 | 56.5 \pm 3.5 | 51.8–63.6 | 0.724 \pm 0.035 | 0.660–0.768 |
| | 28-MAY | 149 | D/F | 55.7 \pm 6.9 | 46.9–65.8 | 7.3 \pm 1.9 | 5.2–11.6 | 62.5 \pm 2.3 | 60.0–67.2 | 0.310 \pm 0.026 | 0.281–0.358 |
| "CUB" | 29-JAN | 29 | D | 87.1 \pm 1.5 | 84.7–88.4 | 20.4 \pm 2.7 | 16.2–25.3 | 43.2 \pm 4.3 | 35.0–49.6 | 0.778 \pm 0.058 | 0.679–0.833 |
| | 10-MAR | 70 | D/F | 80.9 \pm 2.0 | 78.0–83.1 | 16.8 \pm 1.6 | 15.4–20.3 | 41.4 \pm 1.9 | 37.9–44.1 | 0.740 \pm 0.032 | 0.702–0.789 |
| "GRO" | 21-JAN | 21 | D | 75.9 \pm 4.6 | 68.3–80.0 | 17.6 \pm 2.3 | 15.4–21.7 | 48.9 \pm 4.2 | 41.5–55.6 | 0.651 \pm 0.072 | 0.543–0.728 |
| | 02-MAR | 62 | D/F | 78.2 \pm 3.7 | 73.8–84.1 | 15.0 \pm 0.6 | 13.8–15.7 | 45.0 \pm 3.3 | 40.3–50.4 | 0.738 \pm 0.042 | 0.660–0.788 |
| | 21-APR | 112 | D | 72.2 \pm 2.9 | 68.1–76.3 | 7.8 \pm 0.9 | 6.3–9.0 | 66.2 \pm 3.6 | 59.8–70.6 | 0.561 \pm 0.051 | 0.453–0.610 |
| | 28-MAY | 149 | D/F | 53.7 \pm 6.9 | 41.9–64.2 | 7.0 \pm 1.2 | 5.8–9.6 | 65.9 \pm 3.1 | 59.8–69.4 | 0.288 \pm 0.019 | 0.264–0.329 |
| "MIT" | 20-JAN | 20 | D | 79.5 \pm 5.8 | 68.5–84.7 | 17.1 \pm 3.1 | 10.8–21.4 | 43.9 \pm 9.1 | 32.9–57.5 | 0.734 \pm 0.092 | 0.628–0.831 |
| | 03-MAR | 63 | D | 87.6 \pm 1.8 | 85.0–90.1 | 17.6 \pm 2.4 | 14.8–20.3 | 45.4 \pm 3.3 | 41.2–50.4 | 0.793 \pm 0.020 | 0.776–0.837 |
| | 14-APR | 105 | D | 87.1 \pm 2.2 | 83.6–89.2 | 15.2 \pm 3.5 | 10.8–19.2 | 44.7 \pm 6.3 | 36.2–52.9 | 0.814 \pm 0.046 | 0.731–0.853 |
| | 26-MAY | 147 | D/F | 67.4 \pm 7.4 | 58.8–75.5 | 9.5 \pm 2.1 | 6.7–11.8 | 59.9 \pm 5.7 | 53.2–67.4 | 0.457 \pm 0.086 | 0.323–0.557 |
| "MUR" | 22-JAN | 22 | D | 76.8 \pm 3.4 | 73.1–83.6 | 11.0 \pm 3.1 | 7.7–17.8 | 63.9 \pm 3.2 | 59.0–67.2 | 0.530 \pm 0.062 | 0.422–0.617 |
| | 09-MAR | 69 | D/F | 79.9 \pm 2.8 | 75.8–83.6 | 15.7 \pm 5.8 | 8.7–25.8 | 51.3 \pm 3.8 | 45.0–56.7 | 0.600 \pm 0.046 | 0.521–0.668 |
| | 20-APR | 111 | D | 83.2 \pm 1.4 | 81.6–85.8 | 15.2 \pm 3.1 | 11.2–21.0 | 54.2 \pm 3.7 | 49.1–59.9 | 0.649 \pm 0.049 | 0.575–0.713 |
| | 29-MAY | 150 | D/F | 75.1 \pm 4.3 | 68.3–79.4 | 8.6 \pm 1.2 | 7.0–10.3 | 61.8 \pm 3.3 | 57.3–66.0 | 0.446 \pm 0.057 | 0.366–0.534 |
| "PAD" | 20-JAN | 20 | D | 77.7 \pm 3.7 | 70.8–82.3 | 16.1 \pm 2.0 | 13.3–20.1 | 50.6 \pm 3.7 | 45.5–56.5 | 0.690 \pm 0.028 | 0.640–0.733 |
| | 09-MAR | 69 | D/F | 78.1 \pm 2.0 | 74.9–80.8 | 16.6 \pm 2.2 | 13.0–19.9 | 45.2 \pm 2.5 | 40.4–47.7 | 0.739 \pm 0.020 | 0.716–0.764 |
| | 20-APR | 111 | D | 86.8 \pm 1.5 | 84.6–89.1 | 19.0 \pm 2.6 | 13.9–21.9 | 47.4 \pm 1.9 | 44.5–49.8 | 0.820 \pm 0.029 | 0.777–0.856 |
| | 29-MAY | 150 | D/F | 67.6 \pm 3.1 | 63.8–72.8 | 9.7 \pm 1.1 | 7.2–10.9 | 60.6 \pm 2.0 | 56.4–62.7 | 0.488 \pm 0.042 | 0.432–0.550 |
| "QF" | 30-JAN | 30 | D | 84.9 \pm 2.2 | 82.0–87.7 | 20.3 \pm 3.1 | 15.4–26.1 | 50.1 \pm 6.1 | 40.4–60.4 | 0.789 \pm 0.025 | 0.760–0.837 |
| | 23-APR | 114 | D | 80.6 \pm 2.2 | 77.0–83.4 | 16.7 \pm 1.6 | 15.0–18.9 | 45.1 \pm 1.9 | 42.7–47.8 | 0.744 \pm 0.065 | 0.662–0.817 |
| | 09-JUN | 161 | D | 63.8 \pm 3.8 | 57.9–68.4 | 9.2 \pm 1.6 | 6.8–11.7 | 56.4 \pm 3.6 | 52.3–61.8 | 0.369 \pm 0.032 | 0.328–0.423 |
| "TAP" | 22-JAN | 22 | D | 74.5 \pm 7.5 | 62.4–83.1 | 10.8 \pm 4.3 | 6.2–17.8 | 56.2 \pm 9.4 | 41.4–66.1 | 0.620 \pm 0.058 | 0.552–0.733 |
| | 10-MAR | 70 | D/F | 76.1 \pm 4.6 | 68.5–81.4 | 15.0 \pm 3.3 | 11.8–22.1 | 45.8 \pm 4.0 | 41.7–53.1 | 0.640 \pm 0.053 | 0.548–0.700 |
| | 24-APR | 115 | D | 79.4 \pm 2.2 | 75.7–82.5 | 9.0 \pm 1.1 | 7.5–11.2 | 56.7 \pm 5.5 | 49.2–63.7 | 0.656 \pm 0.055 | 0.594–0.754 |
| | 01-JUN | 153 | D/F | 70.0 \pm 6.5 | 55.7–76.3 | 8.0 \pm 1.4 | 5.7–10.0 | 58.7 \pm 7.0 | 48.4–71.1 | 0.431 \pm 0.051 | 0.360–0.504 |

Note: Pasture moisture content, PMC; crude protein, CP; neutral detergent fiber, NDF; and Normalized Difference Vegetation Index, NDVI.

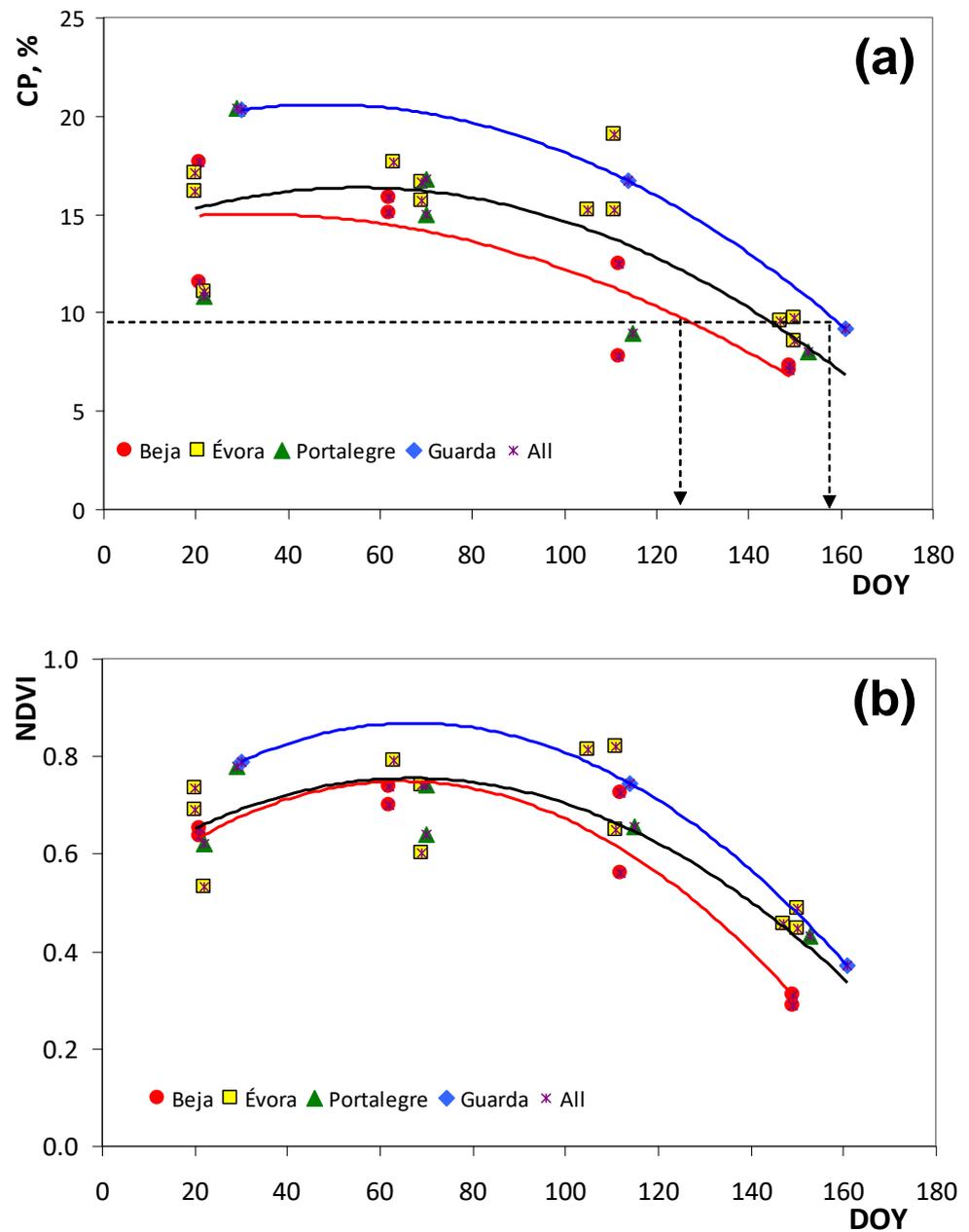


Figure 5. Crude protein (CP; (a)) and normalized difference vegetation index (NDVI; (b)) patterns of the pasture by districts, between January and June 2020.

3.2. Evaluation of Near-Infrared Spectroscopy (NIRS)

Statistics regarding calibration and external validation of prediction models developed using PLSR to correlate the NIRS absorbance spectra with the quality parameters obtained by chemical reference processing are presented in Table 2 for fresh pasture samples (PMC, CP, and NDF) and in Table 3 for dry pasture samples (CP and NDF).

For each parameter, as several preprocessing methods were considered, only the selected pretreatment is shown based on the criteria presented above; for each parameter, the pretreatment with higher values of R^2 and RPD and with lower values of RMSE and bias was selected [28,36]. According to Fagan et al. [37], a model is considered good when the R^2 is around 0.90 and the RPD is greater than 3. In this case, the best results in fresh pasture samples were obtained using (i) the standard normal variate with first derivative preprocessing for PMC ($R^2 = 0.834$; RPD = 2.72; RMSE = 3.517; bias = 0.101) of the external validation model (Table 2); (ii) the first derivative preprocessing for CP ($R^2 = 0.702$;

RPD = 1.88; RMSE = 2.303; bias = -0.049) and NDF ($R^2 = 0.720$; RPD = 2.38; RMSE = 3.241; bias = -0.045) of the external validation models (Table 2). In dry pasture samples, the best results were obtained using (i) the “raw data” for CP ($R^2 = 0.936$; RPD = 4.01; RMSE = 1.174; bias = 0.031) of the external validation (Table 3); (ii) the first derivative preprocessing for NDF ($R^2 = 0.914$; RPD = 3.48; RMSE = 2.752; bias = -0.048) of the external validation model (Table 3).

Table 2. Statistics regarding calibration and external validation models of pasture moisture content (PMC), crude protein (CP) and neutral detergent fiber (NDF) in fresh samples using near-infrared spectroscopy (NIRS) spectra and partial least squares regression (PLSR).

| Pasture Parameter (Spectral Pre-Processing) | LV | Slope | Intercept | R ² | RMSE | Bias | RPD |
|---|----|-------|-----------|----------------|-------|----------|------|
| <i>Calibration Model</i> | | | | | | | |
| PMC (SNV + 2nd derivative) | 5 | 0.948 | 3.729 | 0.948 | 1.975 | - | - |
| CP (1st derivative) | 7 | 0.833 | 2.112 | 0.833 | 1.720 | - | - |
| NDF (1st derivative) | 6 | 0.824 | 10.045 | 0.824 | 3.020 | - | - |
| <i>External Validation Model</i> | | | | | | | |
| PMC (SNV + 2nd derivative) | 5 | 0.849 | 10.839 | 0.834 | 3.517 | 0.101 | 2.72 |
| CP (1st derivative) | 7 | 0.745 | 3.165 | 0.702 | 2.303 | -0.049 | 1.88 |
| NDF (1st derivative) | 6 | 0.774 | 12.905 | 0.720 | 3.241 | -0.045 | 2.38 |

SNV, standard normal variate; LV, latent variables; R², coefficient of determination; RMSE, root mean square error; Bias, average difference between predicted and actual values; RPD, residual predictive deviation.

Table 3. Statistics for calibration and external validation models of crude protein (CP) and neutral detergent fiber (NDF) in pasture dry samples using near-infrared spectroscopy (NIRS) spectra and partial least squares regression (PLSR).

| Pasture Parameter (Spectral Pre-Processing) | LV | Slope | Intercept | R ² | RMSE | Bias | RPD |
|---|----|-------|-----------|----------------|-------|----------|------|
| <i>Calibration Model</i> | | | | | | | |
| CP (raw data) | 6 | 0.941 | 0.855 | 0.941 | 1.250 | - | - |
| NDF (1st derivative) | 6 | 0.948 | 2.662 | 0.948 | 2.293 | - | - |
| <i>External Validation Model</i> | | | | | | | |
| CP (raw data) | 6 | 0.983 | 0.260 | 0.936 | 1.174 | 0.031 | 4.01 |
| NDF (1st derivative) | 6 | 0.951 | 2.094 | 0.914 | 2.752 | -0.438 | 3.48 |

LV, latent variables; R², coefficient of determination; RMSE, root mean square error; Bias, average difference between predicted and actual values; RPD, residual predictive deviation.

Tables 2 and 3 show that the PLSR models selected for CP and NDF quantification in dry pasture samples (Table 3), compared to those of the fresh pasture samples (Table 2), exhibited (i) a small difference in R² and RMSE between calibration and external validation models; and (ii) a higher R² and RPD and lower RMSE, both indicators of model accuracy [36]. Alomar et al. [20] and Parrini et al. [16] justified this advantage of dried samples for two reasons: Samples subjected to laboratory physical preprocessing (drying, grinding) are more homogeneous in terms of particle size, and they do not have water interference, which generates strong absorption signals that overlap and obscure other spectral features. According to Alomar et al. [20], dehydration extends the conservation of samples that cannot be immediately analyzed in the laboratory. It also standardizes the water content of tissues of variable moisture content and facilitates grinding, thus improving homogenization and decreasing sampling errors.

The optimized spectra of NIRS (with several preprocessing methods considered) for PMC, CP, and NDF in fresh pasture samples, and for CP and NDF in dry pasture samples are presented in Figures 6 and 7, respectively.

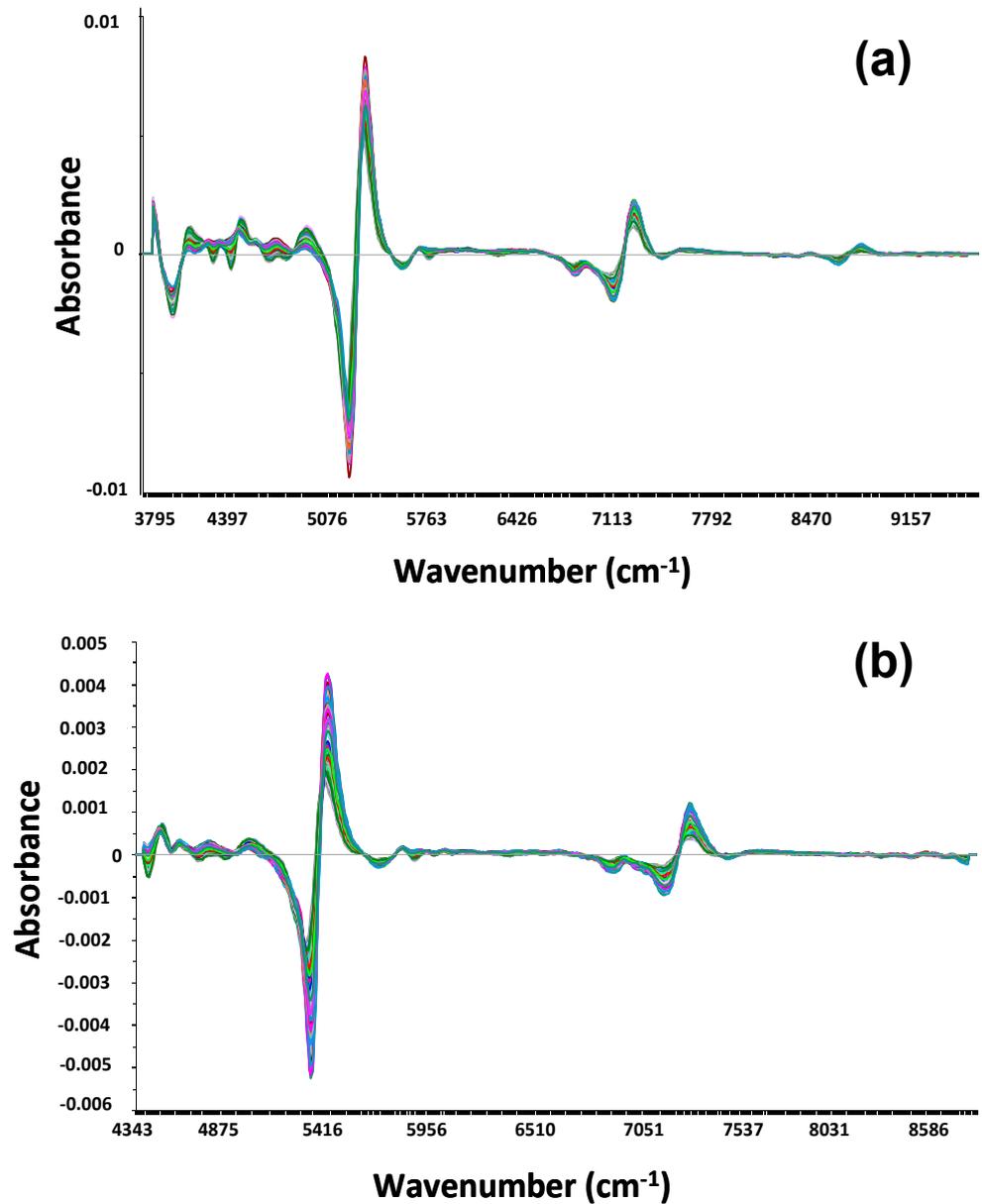


Figure 6. Optimized near-infrared (NIR) spectra in fresh pasture samples: (a) for pasture moisture content (PMC) using SNV with 2nd derivative spectral preprocessing; and (b) for crude protein (CP) and near detergent fiber (NDF) using 1st derivative spectral preprocessing.

In fresh pasture samples (Figure 6), as was expected, the best correlations happened with PMC. NIRS spectroscopy is well-established technology used for moisture testing in many food products, as water display characteristic absorption peaks in a NIR spectrum. The raw spectra region selected was defined within the wavenumber range $3795\text{--}9157\text{ cm}^{-1}$ ($2635\text{--}1092\text{ nm}$), with absorbance peaks (critical spectra zones) in the region of $5100\text{--}5700\text{ cm}^{-1}$ ($1960\text{--}1750\text{ nm}$).

In dry pasture samples (Figure 7), the raw spectra region selected was defined within the wavenumber range $3865\text{--}9000\text{ cm}^{-1}$ ($2600\text{--}1110\text{ nm}$), with successive absorbance peaks in the region of $3865\text{--}5550\text{ cm}^{-1}$ ($2600\text{--}1800\text{ nm}$) for CP and in the region of $4070\text{--}6000\text{ cm}^{-1}$ ($2450\text{--}1670\text{ nm}$) for NDF. According to Souza et al. [19], this behavior is due to the interference of the functional groups' connections between hydrogen atoms and carbon, nitrogen, and oxygen atoms (C-H, N-H, and O-H, respectively).

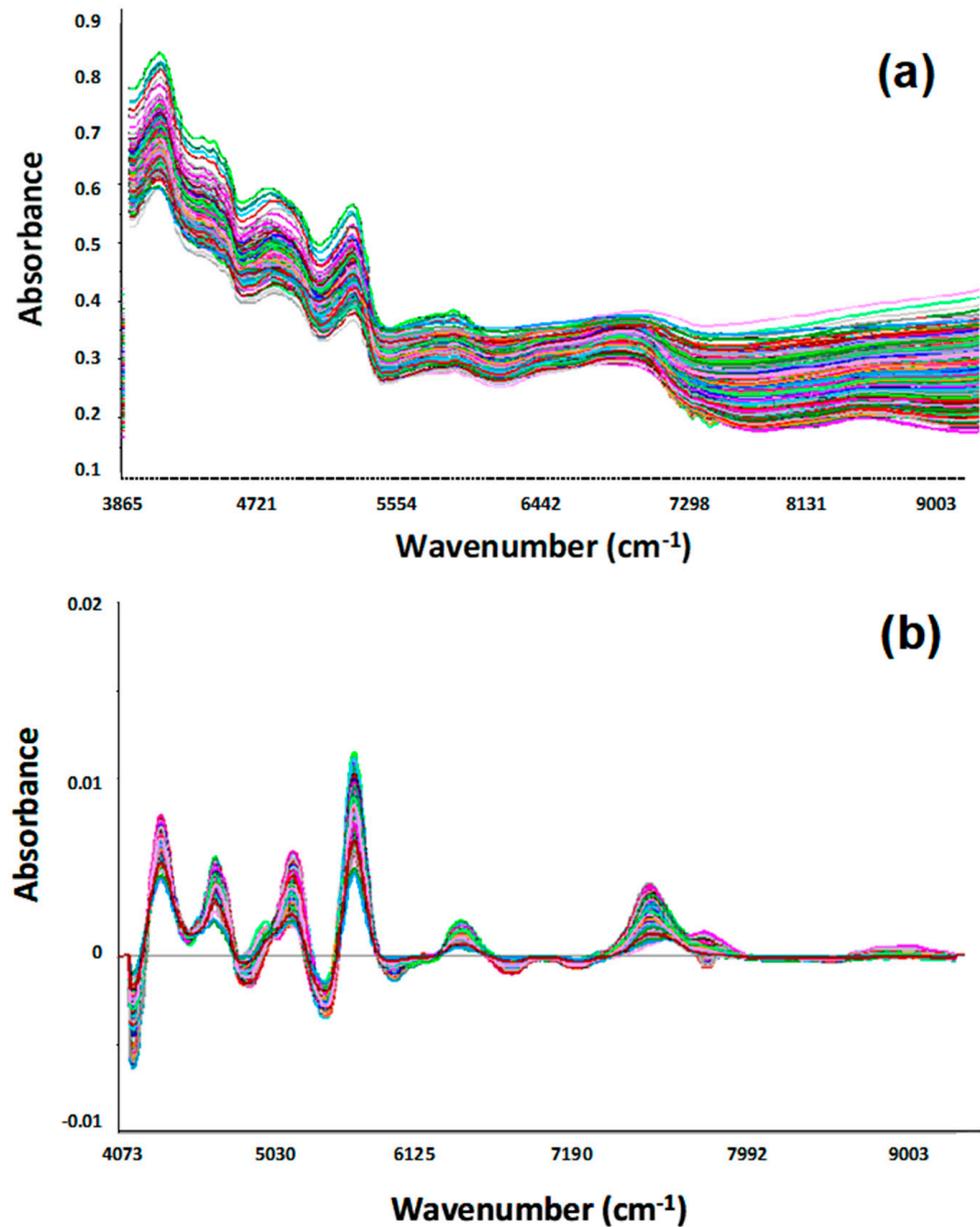


Figure 7. Optimized near-infrared (NIR) spectra in dry pasture samples; (a) for crude protein (CP) using raw data spectral processing; and (b) for near detergent fiber (NDF) using 1st derivative spectral preprocessing.

Figures 8 and 9 show the measured versus predicted values for PMC, CP, and NDF in calibration and validation phases in fresh pasture samples (Figure 8), and for CP and NDF in dry pasture samples (Figure 9). These results show that the range of the calibration and validation sets was similar for all parameters, which contributed to a good representativeness of the whole group of samples. The coefficient of determination obtained with dry pasture samples (0.936 to CP and 0.914 to NDF; $p < 0.01$) suggests the potential of NIRS to estimate these two parameters, which are important indicators of pasture quality. This excellent potential as a predictor is confirmed by RPD values (4.01 to CP and 3.48 to NDF) [38–40] and can even be used as routine analysis [41]. These values also indicate a greater accuracy of predictor model for CP than for NDF, which is in agreement with other studies [15,42].

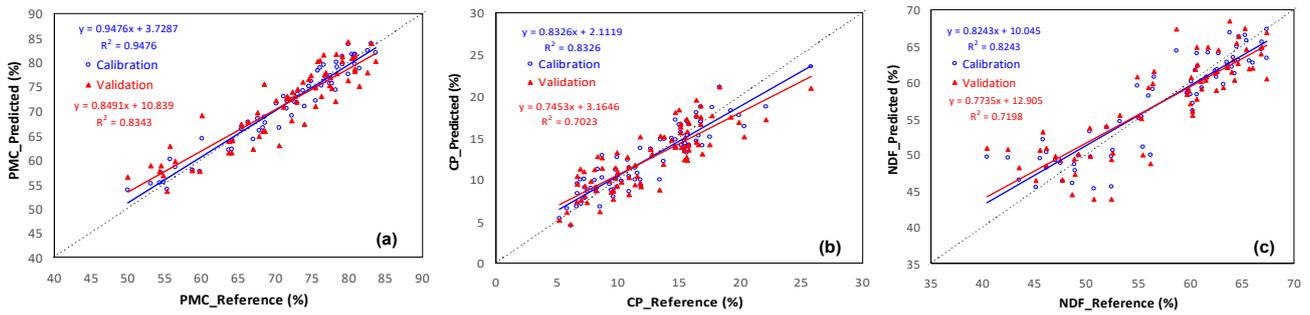


Figure 8. Reference values versus predicted values for calibration and validation phases in fresh pasture samples: (a) pasture moisture content (PMC), (b) crude protein (CP), and (c) neutral detergent fiber (NDF).

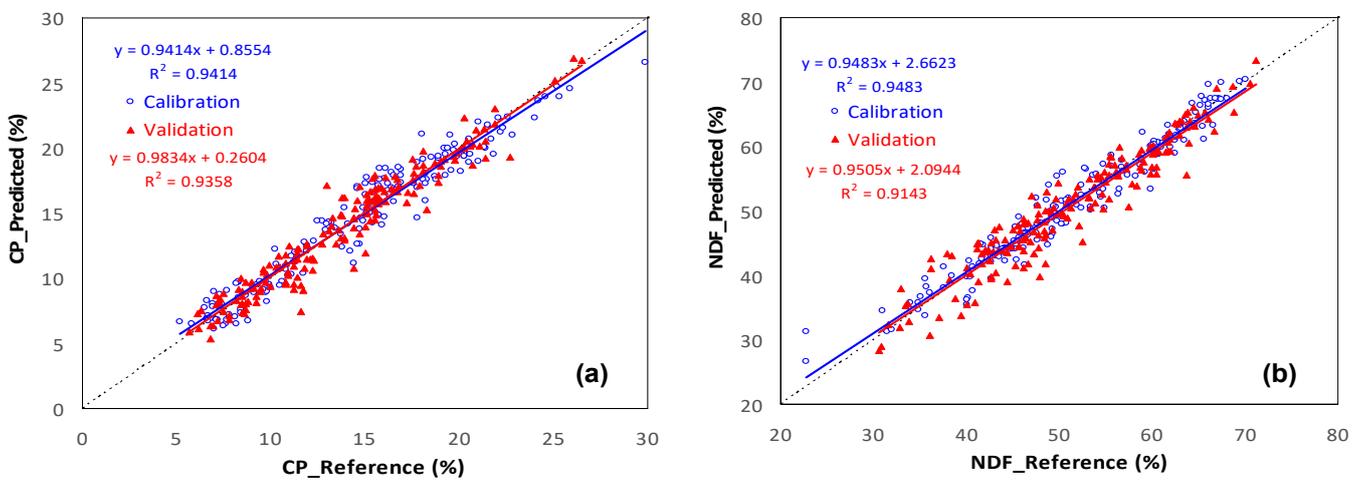


Figure 9. Reference values versus predicted values for calibration and validation phases in dry pasture samples: (a) crude protein (CP) and (b) neutral detergent fiber (NDF).

The results obtained with fresh pasture samples (without sample preparation and homogenization) gave us some reservations regarding the use of real-time, portable, and nondestructive spectrometers to monitor the vegetation quality due to the variability of external environmental factors, such as the moisture content, which is one of the most critical factors that degrade the prediction accuracy [6,26–28]. In view of the small number of publications on the use of fresh samples and on the effect of sample preparation on the accuracy of the pasture quality estimate through NIR spectroscopy [16], the results obtained in this work confirm the importance of this type of studies.

3.3. Evaluation of Field Optical Sensor

The limitations revealed by NIR spectroscopy combined with the PLS regression approach for determining fresh pasture quality parameters (CP and NDF) led to the field testing of the optical sensor (OptRx). The analysis of regression between the average pasture reference values of PMC, CP, and NDF, provided by traditional laboratory methods at each of the eight experimental fields and each of the four sampling dates, and the nondestructive and in-field NDVI measurements carried out by the proximal optical sensor result in calibration models with significant ($p < 0.05$) and similar coefficient of determination ($R^2 = 0.836$ for PMC, $R^2 = 0.707$ for CP and $R^2 = 0.648$ for NDF; Figure 10) to those obtained by NIRS combined with the PLS regression in fresh pasture samples. According to Jackson and Ash [43] and Gu et al. [44], higher NDVI values are indicative of greater vigor and photosynthetic activity; this is directly connected to the presence of chlorophyll in the leaves. The significant correlation between NDVI and PMC

or CP, and the significant and negative correlation between NDVI and NDF was confirmed in previous studies [2,15,29].

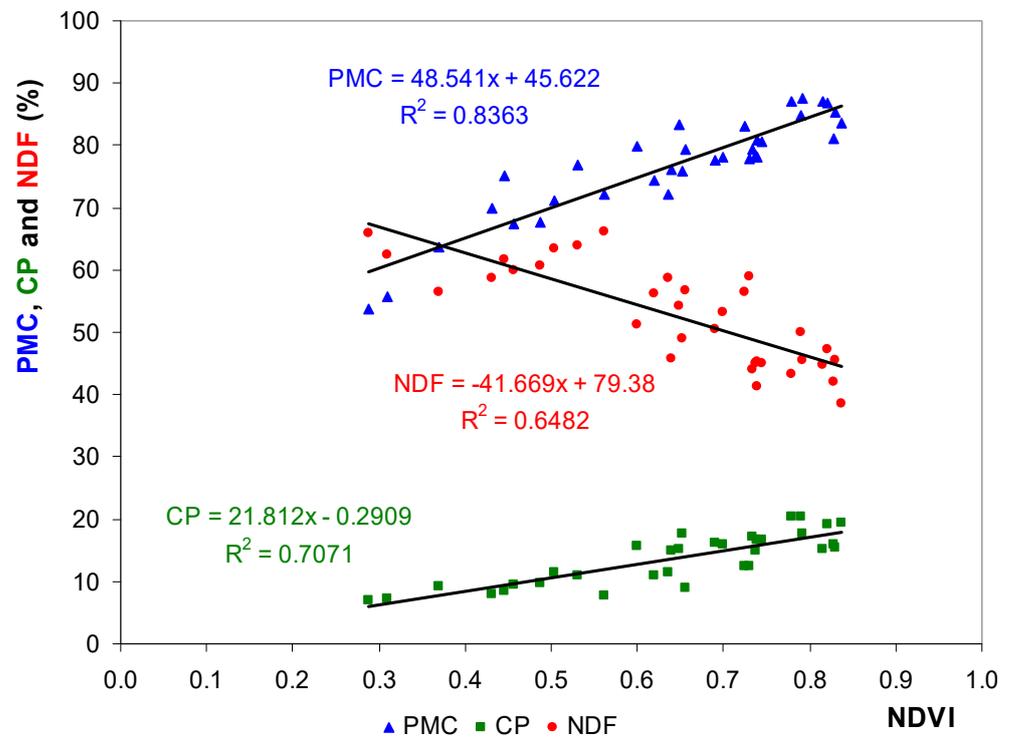


Figure 10. Reference values of pasture moisture content (PMC), crude protein (CP) and neutral detergent fiber (NDF) obtained in laboratory versus normalized difference vegetation index (NDVI) measurements carried out by the optical sensor OptRx.

Table 4 shows (a) for each one of the eight experimental fields and (b) for two seasons (winter and spring), the linear correlation coefficients (r) obtained between NDVI, measured by active optical sensor in each sampling point in four dates (three in “QF” and two in “CUB”), and the correspondent reference values of PMC, CP and NDF. These results show that the correlation between NDVI and different pasture quality parameters is significant in all locations and in both seasons. This is a good indicator in view of the spatial (location) and temporal (season) variability. However, some locations have lower correlation coefficients (for example, “TAP” or “MUR”). Figure 11 shows this model variability for CP prediction, taking as an example the experimental fields with higher (“PAD”) and lower (“TAP”) correlation coefficients (Table 4). A comparison of the reference values versus predicted values (based in the general model of Figure 10) presents a very good coefficient of determination (R^2 ; approximately 0.80) in the “PAD” field and very low (approximately 0.30) in the “TAP” field. These spatial variability raises the need to include in future works other variables that help to characterize the specificity of each field, namely in relation to the pasture floristic composition. The difference in spatial versus temporal variation in structural attributes of key plant functional groups appears to be the primary driver of differences among regression models [11], specifically, for example, in dryland pastures, legumes with prostrate canopies versus grasses with more vertically canopies [1]. This general model (Figure 10) also includes, in each location (except “CUB” and “QF”), four dates throughout pasture vegetative cycle (between January and June), which may have an effect on its reliability [11]. Table 4 confirms, for all parameters, better correlation coefficients in spring than in winter, which helps to explain part of the error associated with the proposed general model (Figure 10). These results suggest that more investigation is required to assess the capabilities of this proximal sensor in the field, in a context of great spatial and temporal variability

Table 4. Linear correlation coefficients (r) obtained in each field and season between NDVI, measured by active optical sensor in each sampling point and four dates (three in “QF” and two in “CUB”), and the correspondent reference values of pasture moisture content (PMC), crude protein (CP) and neutral detergent fiber (NDF).

| Experimental Field/Season | NDVI vs. PMC | NDVI vs. CP | NDVI vs. NDF |
|---------------------------|--------------|-------------|--------------|
| Field (n) | | | |
| “AZI” (32) | 0.9095 * | 0.7823 * | −0.6672 * |
| “CUB” (16) | 0.5785 * | 0.7460 * | −0.7667 * |
| “GRO” (32) | 0.8887 * | 0.7342 * | −0.7376 * |
| “MIT” (32) | 0.9411 * | 0.8012 * | −0.8662 * |
| “MUR” (32) | 0.6353 * | 0.6406 * | −0.6055 * |
| “PAD” (32) | 0.9444 * | 0.8712 * | −0.8777 * |
| “QF” (24) | 0.9718 * | 0.8534 * | −0.6246 * |
| “TAP” (32) | 0.7090 * | 0.6275 * | −0.6513 * |
| Season (n) | | | |
| Winter (120) | 0.6264 * | 0.6072 * | −0.6735 * |
| Spring (112) | 0.9008 * | 0.7830 * | −0.7390 * |

Note: n = Number of samples; * = Statistically significant at the 95% confidence level ($p < 0.05$).

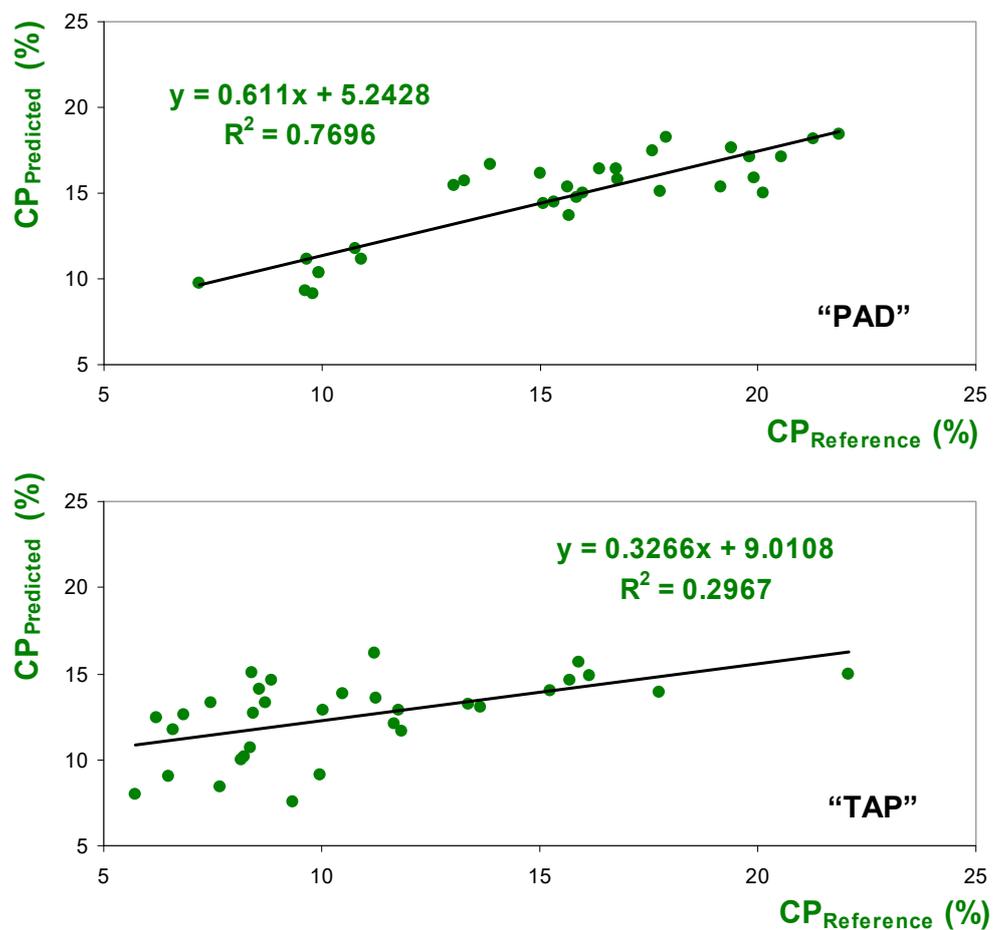


Figure 11. Reference values versus predicted values of pasture crude protein (CP) in two experimental fields (“PAD” and “TAP”).

The advantage of measurements with optical sensor relative to laboratory NIRS approach is that it is nondestructive, and it reads directly in the field without disturbing the sample, which allows the farmer to collect a much higher sampling density in less time. Although measuring NDVI from optical sensors installed on satellites is also a

very interesting alternative [15] due to the ease of obtaining the data, it does have some limitations in comparison with proximal optical sensors, as is the case with the OptRx sensor, namely, a lower spatial resolution (pixel of 10 m × 10 m), intermittent temporal availability (aggravated by the presence of clouds), and the inaccessibility of the areas under tree canopy, which is a preponderant element in the montado ecosystem. For this reason, the current trend is towards the use of proximal, portable, and mobile sensors as a complementary tool for remote sensing [29].

4. Conclusions

Grazing management decisions in extensive livestock systems are based on the evaluation of pasture availability and quality. The results of this study showed a significant correlation between NIRS calibration models or NDVI obtained by optical proximal sensing and reference methods for quantifying pasture crude protein and fiber. The most accurate indicators were obtained with NIRS models applied to pasture samples that had undergone a drying and screening process ($R^2 = 0.936$ for CP and $R^2 = 0.914$ for NDF). The excellent potential of NIRS spectra as a predictor is confirmed by RPD values (4.01 for CP and 3.48 for NDF). Nevertheless, the NIRS approach applied to fresh pasture samples shows lower accuracy ($R^2 = 0.702$ for CP and $R^2 = 0.720$ for NDF), similar to the NDVI measured in the field by optical proximal sensing ($R^2 = 0.707$ for CP and $R^2 = 0.648$ for NDF). These issues justify carrying out more studies to evaluate the effect of pasture sample preparation on their spectral response. On the other hand, the use of proximal sensors (portable spectrometers or optical sensors) to estimate the quality of pasture throughout the growing season can be very interesting as a complement to remote sensing from satellite images. This complementarity is essential in the montado ecosystem for accessing the pasture under tree canopy, and in general for resolving the negative effect of clouds on the information supplied by satellite images.

Author Contributions: J.S. ≈ 40%: Conceptualization; formal analysis; funding acquisition, investigation, methodology, supervision, writing. S.S. ≈ 20%: Conceptualization, investigation, review and editing. Â.C. ≈ 10%; A.E.R. ≈ 30%; Conceptualization, formal analysis, investigation, methodology. All authors have read and agreed to the published version of the manuscript.

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