Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
CMB Association Publisher

Original Research

Predicting some quality factors of annual ryegrass (*Lolium multiflorum* lam.) by means of spectral reflectance values

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Received August 31, 2018; **Accepted** October 25, 2018; **Published** October 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.13.19

Abstract: This study was conducted to predict some quality factors of the annual ryegrass (*Lolium multiflorum* Lam.) with spectral reflectance values in the Forage Crops Laboratory in the Department of Field Crops at the Agriculture Faculty, Akdeniz University, Turkey. In the study, variations resulting from different implementations (moisture level, bale density, propionic acid application and storage period) made during haymaking were determined with reflectance values. For reflectance measurements, a portable spectroradiometer and a contact probe (plant probe) were used and predicting models were created. Results of this study showed that quality factors of dried Annual ryegrass could be predicted with reflectance values, and that reflectances had higher efficacy in the red region for, the red and green, and the NIR region for crude protein, crude ash and crude cellulose, respectively. The results reveal that in dried annual ryegrass, there are significant relationships between feed quality factors such as crude protein, crude ash and crude cellulose, and reflectance values, and that especially crude protein levels can be rapidly and cheaply predicted by using reflectance values.

Key words: Ryegrass hay; Crude protein; Crude ash; Crude cellulose; Remote sensing.

Introduction

Annual ryegrass (*Lolium multiflorum* Lam.), also known as Italian ryegrass, is an annual herbaceous forage plant of the *Poaceae* family (1). Widely grown in many countries in the temperate zone of the world refrence is needed,

The performance of dairy animals in particular is directly linked to quality of feed, and as feed quality declines, reductions in yield are also was observed (2). Therefore, rapid and accurate determination of feed parameters has a key role both in nutritional diets of ruminants and in bioenergy conversion (3). But, the mainly chemical methods (Kjeldahl, Van Soest, Soxhlet, etc) used in quality (nutrients, crude protein, crude fat, crude cellulose, crude ash and nitrogen-free substances) determination are considerably expensive and time-consuming methods (4, 5). Moreover, the chemicals used in analyses may not only negatively affect the health of people working in laboratories (6) but will also give rise to the necessity of removing chemical waste in order to prevent environmental pollution following the analyses (7). This also incurs extra costs. To eliminate these disadvantages of chemical analyses, in recent years speedy and low-cost methods in which chemicals are not used have been examined. One of the most important of these methods is the remote sensing method in which reflectance values are used.

Remote sensing is described as a discipline that enables the determination of electromagnetic energy values of objects and bodies from a certain distance without coming into contact with them (8, 9). Remote sensing systems, which enable information to be gathered about signs that occur in objects and are invisible to the naked eye (10, 11), are widely used nowadays in many areas (for soil mapping, phenology, crop health, land usage, forest mapping, geological and hydrological purposes, drought and flood monitoring, etc.) (12, 13).

For quality determination in dried plant materials, the NIRS (near-infrared spectroscopy) system has been widely used in recent years. The NIRS system was used to determine quality factors in some grass types (14), in soybean (15), in sorghum (16), in Italian ryegrass (17) and in peas (18). However, in these studies the visible region of the spectrum (400-700 nm) was not given a great deal of attention. Although a large number of studies on green plants and green vegetation exist with regard to the visible region of the spectrum, an adequate number of studies on dried plant materials have not been conducted why? This the main question which should be answered.

In this study, the prediction of some quality factors (crude protein, crude ash and crude cellulose) in dry samples of annual ryegrass with the remote sensing method has been aimed at. In this way, an effective method for rapid prediction of nutritional values in dried forage crops will be revealed.

Materials and Methods

The study was carried out in the Forage Crops Laboratory in the Department of Field Crops at the Agriculture Faculty, Akdeniz University, Turkey. Plant materials (107 samples) were obtained from another study which was to determine effect of moisture level, bale density, propionic acid application and storage period on some quality factors in Annual ryegrass (*Lolium multiflorum* Lam.) hay and field experiment of study was performed in Antalya-Turkey conditions. Prior to the analyses, the samples were dried in a drying chamber at 60 °C for 72 hours and made ready for the chemical analyses by pulverising them in a mill. The crude protein, crude ash and crude cellulose rates of the samples were determined by the Weende analysis method (19).

The spectral reflectance measurements were taken under laboratory conditions in a dark environment admitting no light from outside. For the measurements, a spectroradiometer (ASD Inc., Boulder, CO, USA) (20-22) that can make reflectance measurements between the wavelengths of 325-1075 nm of the electromagnetic spectrum and a contact probe (plant probe) were used. Contact probe was attached to the spectroradiometer (23) and contain a 100 W halogen lamp as an artificial light source (24). Also measurement area of this device is 1 cm in diameter. Prior to the measurements, each plant sample was placed in a glass petri dish 11 cm in diameter and 2 cm in height. Next, the light of the contact probe component was turned on and a calibration measurement was taken with a white reference panel (Spectralon® , Labsphere Inc., North Sutton, USA) (25). Following this measurement, the contact probe was placed at the upper part of the sample and the results of measurements taken in different areas of each sample in 5 repetitions were recorded on the computer. During the measurements, the reference panel measurements were repeated on each new sample.

For statistical analysis of data, firstly; the mean of the 5 repetitive measurements were taken for the reflectance value at each wavelength for each samples. Stepwise regression analysis in MINITAB statistical software was used for statistical analysis. After analysis, wavelengths which was associated with the crude protein, crude ash and crude cellulose levels of the samples were determined. Later, predicted models containing 10 wavelengths were created for each quality factor with using these wavelengths. In the reflectance measurements, since there were too many oscillations at wavelengths below 400 nm, wavelengths of 325-399 nm were not included in the statistical analysis. Therefore, wavelengths were classified as bands of 400-500 nm for blue, 500-600 nm for green, 600-700 nm for red and 700-1075 nm for near infra-red were evaluated (26, 27).

Results

In the study, significant relationships were determined between the quality factors (crude protein, crude ash and crude cellulose) of the Annual ryegrass samples and the reflectance values. The prediction models created using the spectral reflectances and r^2 values are given in Table 1 (The letter "R" in the equations represents the reflectance value at the wavelength next to which it is written (e.g. "R670 nm" is the reflectance value at 670 nm). As can be seen in the table, the r^2 value of crude protein prediction model was determined as 89.34%. When wavelengths of crude protein prediction model are classified according to their places in the electromagnetic spectrum, it is seen that 3 of them (422, 428, 446 nm) are in the blue region, 1 of them (527 nm) is in the green region and 6 of them (611, 631, 642, 646, 661, 670 nm) are in the red region of the spectrum. The graph showing the relationship between the crude protein level predicted by using the prediction model and the crude protein level measured in the laboratory analyses is shown in Figure 1A. When this figure is examined, it can be seen that there are very significant relationships between the predicted levels and the measured levels.

As seen in the Table 1, significant relationships between the reflectance values and crude ash values were found, and r^2 value was determined as 72.21%. While 1 (486 nm) of the wavelengths forming the prediction model appear in the blue region of the spectrum, 3 of them (548, 581, 596 nm) appear in the green region, 4 of them (605, 639, 647, 648 nm) appear in the red region and 2 of them (701 and 1012 nm) appear in the near-infrared region. The relationships between the crude ash level predicted by using the prediction model and the crude ash level measured in the laboratory analyses can be seen in Figure 2B.

In Table 1, the crude cellulose prediction models and r^2 values are shown. According to the table, the r^2 value of crude cellulose prediction model was determined as 56.43%. When the wavelengths in the prediction model were classified according to their locations in the spectrum, it is seen that 3 of them (405, 411, 425 nm) are located in the blue region, 2 of them (666, 674 nm) were located in the red region, and 5 of them (1034, 1051, 1054, 1074, 1075 nm) were located in the near-infrared region of the spectrum in the model. The graph showing the relationship between the predicted crude cellulose level determined in this prediction model and the crude cellulose level determined in the laboratory analyses is shown in Figure 1C.

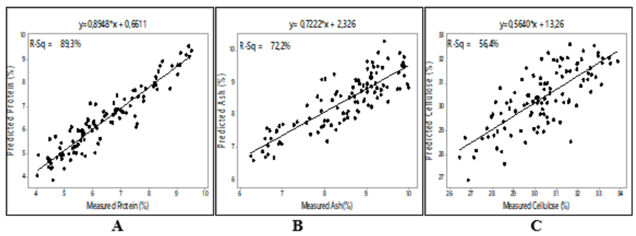

Figure 1. Relation between measured contents in laboratory and predicted content based on prediction models (A: Crude Protein, B: Crude Ash, C: Crude Cellulose).

Table 1. The prediction models and r^2 values of quality factors.

	Prediction models	r^2
Crude Protein	=9,10+(101,6*R670nm)+(-140,6*R661nm)+(121,5*R631nm)+(-54,9*R446nm)+(-171,9*R646nm) +(151,0*R642nm) +(26,84*R428nm)+(12,01*R422nm)+(-84,2*R611nm)+(32,4*R527nm)	89.34
Crude Ash	=11,98+(51,2*R701nm)+(-150,4*R647nm)+(-3,87*R1012nm)+(48,2*R605nm)+(-126,5*R581nm) +(138,8*R596nm) +(-103,3*R639nm)+(120,8*R648nm)+(-51,5*R486nm)+(58,2*R548nm)	72.21
Crude Cellulose	=35,98+(-20,41*R1075nm)+(-457,9*R674nm)+(9,18*R411nm)+(-37,84*R1034nm)+(23,16*R1054nm) +(-57,7*R425nm) +(491,8*R666nm)+(16,30*R1051nm)+(7,70*R1074nm)+(8,83*R405nm)	56.43

Discussion

The lack of chlorophyll and water in dried, photosynthetically inactive plants, together with the accumulation of crude cellulose and lignin result in differences in reflectances in the SWIR (short-wave infrared) region (28). In dried plants, due to lack of water, absorption is not observed at wavelengths of 1733, 2100 and 2300 nm in the near-infrared region. The absorption features here are defined by organic bonds that plant biochemical as crude protein, lignin and crude cellulose content. The C–H, N–H and C–O bonds in these molecules have overtone and combination bands that absorb the near-infrared region of the spectrum (29). According to Elvidge (30), however, although dried plant material does not contain chlorophyll or water, absorption can take place in the visible region between 400-900 nm. Patel et al. (31) performed correlation analysis on the dried vegetation measurements. While they found positive relationship in the visible region of the spectrum, negative relationship were determined in the infrared region. In a study conducted on dried plants in pasture areas, Beeri et al. (32) reported that reflectance values between wavelengths of 670–690 nm (red) and 990–1300 nm (infrared) were affected by the quality status of the plants.

Guo et al. (33) performed spectral measurements on grass-type pasture plants in a laboratory environment and determined that reflectances between wavelengths of 550-750 nm containing the green, red and infrared regions of the spectrum were closely correlated with the crude protein content of the samples. In the same study, they determined that crude ash content affected reflectances between 1116-1284 nm in the infrared region. Also the crude cellulose-like cell-wall substance ADF affected reflectances between 470-518 nm, 550-750 nm and 1116-1284 nm. Another cell-wall substance NDF affected reflectances between 470-518 nm. There were significant statistical relationships between these quality factors and the wavelengths specified. In our study, the wavelengths appearing in the prediction models created for crude protein, crude ash and crude cellulose show similarity with the wavelengths stated by Guo et al.

The crude ash values of feeds are closely correlated with their mineral matter content (34). In fact, Hoffman (35) names crude ash simply as the total mineral matter content of feeds. When considered from this viewpoint, while examining the relationship between crude ash content and reflectance values in plants, their mineral matter content also needs to be considered. In a number of studies conducted, it has been revealed that mineral matter contents of plants affect reflectances and that it is possible to predict mineral matter levels by using spectral reflectance values (36-38). In these studies, it was determined that reflectances varied according to the plants' mineral matter contents particularly in the visible region of the spectrum. This situation shows that crude ash contents that vary depending on mineral matter content can also be predicted by means of spectral reflectance values. According to the results obtained in our study, the fact that the 10-wavelength prediction model created for crude ash values has a relatively high regression coefficient (72.21%) confirms this situation.

Stark et al. (39) reported that in a laboratory environment, the nitrogen (the most important indicator of crude protein), ADF and NDF values of mixtures containing different pasture plants could be predicted with great accuracy by using wavelength reflectance values between 400-1100 nm. In another study, Wu et al. (40) stated that in sorghum, near-infrared reflectances appearing at wavelengths of 1180-2492 nm were correlated with crude cellulose content (r^2=0.96). Pu et al. (41) stated that since water was not present in dried vegetation, at a wavelength of 1780 nm appearing in the near-infrared region of the spectrum, absorption was generated by plant chemicals such as crude cellulose, sugar and starch. Ustin and Gamon (42) reported that in dried plants, factors such as sugar, nitrogen and cell-wall substances that increase densities through water loss defined the characteristics of absorption and that wavelengths of 1750, 2150 and 2300 nm appearing in the near-infrared region of the spectrum was very important in this respect. The results obtained in our study show a similarity with these results, and, 5 near-infrared wavelengths (1034, 1051, 1054, 1074 and 1075 nm) appear in the cellulose prediction model.

In conclusion, significant relationships were identified between the reflectance values and the crude protein and crude ash contents of the samples. In the model created for crude protein, a greater number of wavelengths appeared in the red region of the spectrum. Nevertheless, in the model created for crude ash, wavelengths appeared entirely in the visible region of the spectrum, although most wavelengths were used in the red (four) and green (three) regions. In the prediction crude cellulose model, however, 5 of the 10 wavelengths used appeared in the NIR region of the spectrum.

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