

## DETERMINATION OF NITROGEN LEVELS BASED ON SPECTRAL REFLECTANCE VALUES IN SHEEP FESCUE (*Festuca ovina* L.)

Yasar ÖZYİĞİT\*<sup>1</sup> Mehmet BİLGEN<sup>1</sup>

<sup>1</sup>Akdeniz University, Faculty of Agriculture, Department of Field Crops, Turkey.

\*Corresponding author: ozyigit@akdeniz.edu.tr

Received: 07.01.2011

### ABSTRACT

The objective of this study was to determine nitrogen levels in sheep fescue (*Festuca ovina* L.) using spectral reflectance data. Reflectance measurements were undertaken using a portable spectroradiometer measuring the wavelength range of 325-1075 nm of the electromagnetic spectrum. The treatments consisted of different concentrations of nitrogen, phosphorus and potassium for pots and plots. Spectral reflectance values were measured in both canopy level and single-leaf in the field and greenhouse studies. According to result of the study, the better results were obtained in greenhouse conditions. Nitrogen levels were affected reflectance values of blue region which located in the range of 400-500 nm. The results have shown that spectral reflectance data (especially blue region) could be used to estimate the N concentration in sheep fescue.

**Key Words:** Nitrogen, reflectance, remote sensing, sheep fescue,

### INTRODUCTION

Plants need many essential nutrients for growth, development and survival. Those nutrients required by plants in large quantities are called macronutrients (Newman et al., 2007). Nitrogen is one of the most important macronutrients for plant. Large amounts of N are needed by forage crops to produce maximum growth. Especially grasses require larger amount of nitrogen element to maximize yield. Also N applications increase protein content in grass forage (Malhi and McCartney, 2004). Based on the nitrogen concentration, the crude protein content can be determined using with a factor of 6.25 for N (Kawamura et al., 2008). Growers must know the status of nitrogen in forage crops for a successful animal production. For that purpose, chemical analysis are conducted in the laboratory.

But traditional laboratory analyses are expensive and time-consuming methods (Kokaly and Clark, 1999; Graeff et al., 2001; Li et al., 2006). Another problem for the analyst involves the large amount of sample must be taken from field to get reliable results and these may be difficult to obtain (Foley et al., 1998). Laboratory analysis may also result in environmental pollutants which are caused with the use of chemicals (Zhao et al., 2007). Therefore it is necessary to develop methods which give results in a shorter time and less expensive.

The efforts of obtaining information about plants using their optical characteristics have increased in recent years. Leaf optical properties are function of leaves structural water content and biochemicals (Asner, 1998). The interactions between leaf molecules (protein, cellulose, lignin) and solar radiation affect visible and near infrared reflectance.

Absorbance, transmittance and reflectance properties of short wavelength solar radiation (280-2800 nm) are affected by biochemical, morphological and anatomical components of plant's leaves. So that changes in the concentrations of absorptive chemicals provide a basis for changes in plant absorbance, transmittance, and reflectance (Ritchie, 2003). That features help determine nutrient content of plants with the use of remote sensing systems.

Remote sensing systems, as in many other areas, are also used in agriculture. The objective of this study was to determine nitrogen levels based on spectral reflectance values in sheep fescue which are used as fodder crop in many parts of the world.

### MATERIALS AND METHODS

This study was conducted in field and greenhouse conditions in Antalya-Türkiye. Sheep fescue which belongs to the grass family (*Poaceae*) was used as plant material. Field experiments were carried out in strong alkali, loam, saltless and low organic matter soil. Soil+perlite+peat (2:1:1) were mixed and the mixture of materials filled in to 22 cm-plastic pots in greenhouse experiment.

The field experiment was designed in randomized blocks with 3 replications. Plot size was 1.4\*1.4m (about 2 m<sup>2</sup>). Greenhouse experiment was designed in randomized plots with 3 replications. The treatments consisted of different concentrations (0, 20, 40 kg.da<sup>-1</sup>) of nitrogen, phosphorus and potassium doses for pots and plots. There were 81 pots and plots with 27 combinations of fertilizer doses (Table 1). 7 g and 0.21 g sheep fescue seeds were sown to each plot and pot, respectively.

**Table 1.** Combinations of nitrogen, phosphorus and potassium doses

	N	P	K	N	P	K	N	P	K
1	0	0	0	10	20	0	19	40	0
2	0	0	20	11	20	0	20	40	0
3	0	0	40	12	20	0	21	40	0
4	0	20	0	13	20	20	22	40	20
5	0	20	20	14	20	20	23	40	20
6	0	20	40	15	20	20	24	40	20
7	0	40	0	16	20	40	25	40	0
8	0	40	20	17	20	40	26	40	20
9	0	40	40	18	20	40	27	40	40

Reflectance measurements were undertaken at the beginning of heading stage with using a portable spectroradiometer measuring the wavelength range of 325-1075 nm of the electromagnetic spectrum (Castro-Esau et al., 2006; Albayrak, 2008), but wavelength range of 400-900 nm was used to evaluate the results (Han and Rundquist, 2003, Lin and Liqun, 2006).

81 reflectance measurements were made in both canopy and single leaf in plots and pots. Canopy reflectance measurements were made during clear days between 10.00

and 11.30 am. in April. The optical sensor of the spectroradiometer was mounted at 1.5 m above plant surface in plots (Albayrak, 2008) and 25 cm above plant surface in pots. White reference panel measurement was made before the each three measurements and each measurement was made five replicated. Plant probe and leaf clip were used for single leaf measurement. Plants were clipped after the performance of the reflectance measurements and dried in 65°C for 48 hours (Brink et al., 2003; Halgerson et al., 2004). Nitrogen concentration was determined by Kjeldahl method.

All statistical analyses were conducted using stepwise regression analysis implemented in MINITAB statistical programme. In this method, wavelengths which associated with plant N levels were determined and regression equations were constituted using with the selected wavelengths.

## RESULTS

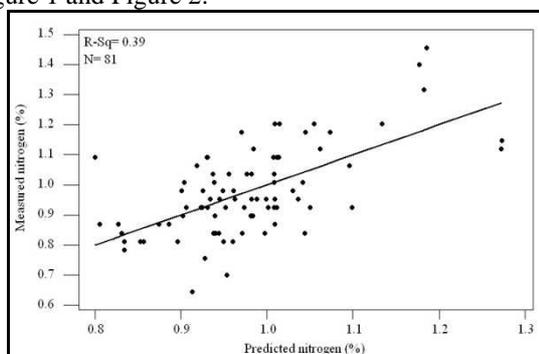
Result of canopy and single leaf measurements which were made in field conditions are shown in Table 2 (R letters which situated in the tables describe the reflectance. For example, R<sub>888</sub> shows value of reflectance in 888 nanometer.).

**Table 2.** Regression equations and R<sup>2</sup> values for nitrogen (N) levels in sheep fescue in field conditions

	Equations	R <sup>2</sup>
Canopy	$N = 1.02 + (28.6 \times R_{888}) + (22.0 \times R_{758}) + (-15.1 \times R_{744}) + (12.5 \times R_{833})$ $(-40.7 \times R_{900}) + (-61.3 \times R_{851}) + (52.8 \times R_{898})$	0.39**
Single leaf	$N = 1.55 + (6.36 \times R_{404}) + (-61.6 \times R_{547}) + (-7.82 \times R_{411}) + (60.0 \times R_{543})$ $(10.3 \times R_{420}) + (-8.18 \times R_{423})$	0.41**

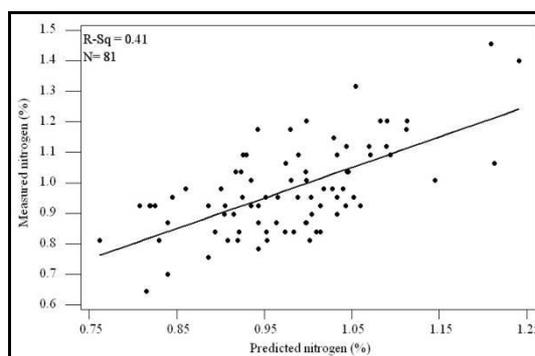
\*\* : P < 0.01

In field-canopy measurements, 7 wavelengths in 700-900 nm NIR (Near Infrared Reflectance) state, were selected for nitrogen levels with using stepwise regression. Regression equation was developed with selected wavelength and equation R<sup>2</sup> value was determined as 0.39. Also, 6 wavelengths (4 blue and 2 green) were selected with stepwise regression analysis in field-single leaf reflectance measurement and R<sup>2</sup> value of regression equation was calculated 0.41. Regression graphics of field-canopy and field-single leaf reflectance measurements are shown in Figure 1 and Figure 2.



**Figure 1.** Relationships between laboratory-measured nitrogen (N) concentrations and predicted values based on reflectance data in field-canopy measurements

Significant relationships were determined between nitrogen levels and reflectance value in the greenhouse experiments. Regression coefficient was calculated as 0.93 both canopy and single leaf reflectance measurements (Table 3).



**Figure 2.** Relationships between laboratory-measured nitrogen (N) concentrations and predicted values based on reflectance data in field-single leaf measurements.

While five wavelengths (4 blue and 1 green) were used in equation for estimated nitrogen level in canopy measurements, seven wavelengths (5 blue, 1 green and 1 NIR) were determined in single leaf measurements.

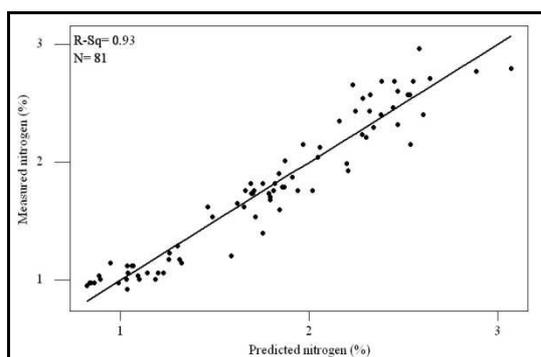
Regression graphics of greenhouse-canopy and greenhouse single leaf reflectance measurements are shown in Figure 3

and Figure 4. Strong correlation between predicted and measured N are shown in these figures.

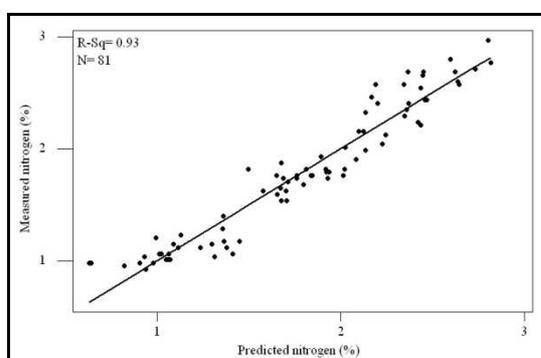
**Table 3.** Regression equations and  $R^2$  values for nitrogen (N) levels in sheep fescue in greenhouse conditions

	Equations	$R^2$
Canopy	$N=2.30+(-43.5 \times R_{584}) + (-172 \times R_{423}) + (-313 \times R_{432}) + (277 \times R_{435}) + (320 \times R_{419})$	0.93**
Single leaf	$N=1.85+(-12.5 \times R_{548}) + (22.6 \times R_{400}) + (-21.0 \times R_{402}) + (94.6 \times R_{430}) + (-30.0 \times R_{415}) + (-58.9 \times R_{445}) + (1.94 \times R_{898})$	0.93**

\*\* :  $P < 0.01$



**Figure 3.** Relationships between laboratory-measured nitrogen (N) concentrations and predicted values based on reflectance data in greenhouse-canopy measurements



**Figure 4.** Relationships between laboratory-measured nitrogen (N) concentrations and predicted values based on reflectance data in greenhouse-single leaf measurements.

## DISCUSSIONS

According to results of this research, the best results were obtained in greenhouse experiments for both canopy and single leaf. Regression equations consisted of a lot of wavelengths of blue band (400-500 nm). Also important relationships were determined between nitrogen levels and NIR band wavelengths in field-canopy measurements. Reflectance values of plants in the region of the visible and near-infrared region were related to nitrogen availability. Strong reflectance occurs in blue and near infrared region in leaves which contain high rates of nitrogen. While red

reflectance increased under the limited nitrogen concentrations due to amount of chlorophyll reduction, infrared reflectance reduced (Serrano et al., 2000).

The results of this research were not according to other study on the basis of wavelength, but similar results were obtained on the basis of bands (blue, green, red and NIR). Starks et al. (2007) determined strong relationships (0.82) between nitrogen concentrations and reflectance values of NIR region ( $R_{705}/R_{1685}$ ) in bermudagrass (*Cynodon dactylon* L.). Blackmer et al. (1996), reported that reflectances of green (550 nm) and NIR (710 nm) were useful for detecting maize (*Zea mays* L.) plant N deficiencies. In another study, Xue et al. (2004) determined that reflectance value of NIR wavelength regions of spectrum was effected by nitrogen applications. Results of the same study were shown that reflectance value in 560 nm was sensitive for nitrogen levels and reflectance value of this wavelength had negative relationships with leaf nitrogen content. Lamb et al. (2002) reported that leaf reflectance in red-edge range of wavelengths (690–740 nm) could be used to estimate leaf N concentration and total N content of Italian ryegrass (*Lolium multiflorum* Lam.). In the same study, reflectance increased in the NIR wavelength regions with increasing leaf nitrogen content. Yang et al. (2010) determined the existence of high negative correlation ( $r^2=0.77$ ) between chlorophyll content and reflectance values in 549 nm (green) and 713 nm (NIR) in fescue (*Festuca arundinacea*).

## CONCLUSIONS

The better results were obtained in greenhouse experiments to estimate the N concentration. Reflectance values of blue region which located in range 400 and 500 nm were affected by nitrogen levels. Also the results have shown that NIR and green region could be used to estimate N concentration. The results of this study demonstrated the potential of using spectral reflectance data to estimate the N concentration and consequently protein level in sheep fescue.

## ACKNOWLEDGEMENT

This article is a part of the doctoral dissertation of Yaşar ÖZYİĞİT. The authors would like to thank the Akdeniz University Scientific Research Projects Coordination Unit of Türkiye (Project number: 2005.03.0121.014) for funding this research.

## LITERATURE CITED

- Albayrak, S., 2008. Use of reflectance measurements for the detection of N, P, K, ADF and NDF contents in sainfoin pasture. *Sensors*, 8: 7275-7286.
- Asner, G.P., 1998. Biophysical and biochemical sources of variability in canopy reflectance. *Remote Sensing of Environment*, 64: 134-53.
- Blackmer, T.M., J.S. Schepers, G.E. Varvel, E.A. Walter-Shea, 1996. Nitrogen deficiency detection using reflected shortwave radiation from irrigated corn canopies. *Agronomy Journal*, 88: 1-5.
- Brink, G.E., D.E. Rowe, K.R. Sistani, A. Adeli, 2003. Bermudagrass cultivar response to swine effluent application. *Agronomy Journal*, 95: 597-601.
- Castro-Esau, K.L., G.A. Sánchez-Azofeifa, B. Rivard, 2006. Comparison of spectral indices obtained using multiple spectroradiometers. *Remote Sensing of Environment*, 103: 276-288.
- Foley, W.J., A. McIlwee, I. Lawler, L. Aragonés, A.P. Woolnough, N. Berding, 1998. Ecological Applications of Near Infrared Spectroscopy - A Tool For Rapid, Cost Effective Prediction of The Composition of Plant and Animal Tissues and Aspects of Animal Performance. *Oecologia*, 116: 293-305.
- Graeff, S., D. Steffens, S. Schubert, 2001. Use of reflectance measurements for the early detection of N, P, Mg, and Fe deficiencies in corn (*Zea mays* L.). *Journal of Plant Nutrition and Soil Science*, 164: 445-450.
- Halgerson, J.L., C.C. Sheaffer, N.P. Martin, P.R. Peterson, S.J. Weston, 2004. Near-infrared reflectance spectroscopy prediction of leaf and mineral concentrations in alfalfa. *Agronomy Journal*, 96: 344-351.
- Han, L., D.C. Rundquist, 2003. The spectral responses of *Ceratophyllum demersum* at varying depths in an experimental tank. *International Journal of Remote Sensing*, 24(4): 859-864.
- Kawamura, K., N. Watanabe, S. Sakanoue, Y. Inoue, 2008. Estimating forage biomass and quality in a mixed sown pasture based on partial least squares regression with waveband selection. *Grassland Science*, 54: 131-145.
- Kokaly, R.F., R.N. Clark, 1999. Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. *Remote Sensing of Environment*, 67(3): 267-287.
- Lamb, D.W., M. Steyn-Ross, P. Schaare, M.M. Hanna, W. Silvester, A. Steyn-Ross, 2002. Estimating leaf nitrogen concentration in ryegrass (*Lolium* spp.) pasture using the chlorophyll red-edge: theoretical modelling and experimental observations. *International Journal of Remote Sensing*, 23(18): 3619-3648.
- Li, B., O.W. Liew, A.K. Asundi, 2006. Pre-visual detection of iron and phosphorus deficiency by transformed reflectance spectra. *Journal of Photochemistry and Photobiology. B: Biology*, 85: 131-139.
- Lin, Y., Z. Lique, 2006. Identification of the spectral characteristics of submerged plant *Vallisneria spiralis*. *Acta Ecologica Sinica*, 26(4): 1005-1011.
- Newman, Y.C., C. Mackowiak, R. Mylavarapu, M. Silveira, 2007. Fertilizing and liming forage crops. EDIS publication SS-AGR-76.
- Malhi, S.S., D.H. McCartney, 2010. Fertilizer management for forage crops in the Canadian Great Plains: a review. Available at: [http://www1.foragebeef.ca/\\$foragebeef/frgebeef.nsf/all/frg90/\\$FILE/fertilizermanagementofforagecropscanadiangreatplains.pdf](http://www1.foragebeef.ca/$foragebeef/frgebeef.nsf/all/frg90/$FILE/fertilizermanagementofforagecropscanadiangreatplains.pdf) (Accessed: 20.12.2010)
- Ritchie, G.L. 2003. Use of ground-based canopy reflectance to determine radiation capture, nitrogen and water status, and final yield in wheat. M.S. thesis. (Unpublished) Utah State University, Logan. 122 pp.
- Serrano, L., I. Fillela, J. Penuelas, 2000. Remote sensing of biomass and yield of winter wheat under different nitrogen supplies. *Crop Science*, 40: 723-731.
- Starks, P.J., D. Zhao, M.A. Brown, 2007. Estimation of nitrogen concentration and in vitro dry matter digestibility of herbage of warm-season grass pastures from canopy hyperspectral reflectance measurements. *Grass and Forage Science*, 63: 168-178.
- Xue, L., W. Cao, W. Luo, T. Dai, Y. Zhu, 2004. Monitoring leaf nitrogen status in rice with canopy spectral reflectance. *Agronomy Journal*, 96: 135-142.
- Yang, F., J. Li, X. Gan, Y. Qian, X. Wu, Q. Yang, 2010. Assessing nutritional status of *Festuca arundinacea* by monitoring photosynthetic pigments from hyperspectral data. *Computers and Electronics in Agriculture*, 70: 52-59.
- Zhao, D., P.J. Starks, M.A. Brown, W.A. Phillips, S.W. Coleman, 2007. Assessment of forage biomass and quality parameters of bermudagrass using proximal sensing of pasture canopy reflectance. *Grassland Science*, 53: 39-49.