

**CHLOROPHYLL CONCENTRATION (SPAD VALUES) AS AN INDICATOR OF -  
CRUDE PROTEIN CONTENT AND AS A SELECTION CRITERION IN GRASS  
BREEDING**

N. G á b o r è í k

Grassland and Mountain Agriculture Research Institute, Mládežnícka 36, 974 21 Banská

Bystrica, Slovakia, gaborcik@nmost.vutphp.sk

**Abstract**

The main aim of the study was to analyse chlorophyll a + b content ( SPAD values) determined by portable chlorophyllmeter (SPAD- 502, Minolta, Japan) and crude protein content in leaves of timothy, cocksfoot, perennial ryegrass and meadow fescue ( total 24 cultivars). Differences between both parameters were confirmed and a close relationship between chlorophyll content ( SPAD values) and crude protein concentration was found. Correlation coefficient between SPAD and crude protein varied from 0.541<sup>++</sup> for ryegrass to 0.906<sup>++</sup> for timothy. This fact should be used for selection of grasses with higher crude protein content and/or better use of mineral soil nitrogen.

**Keywords:** Grasses, chlorophyll, crude protein, prediction , selection

**Introduction**

A hand-held chlorophyll meter is a useful device for indirect detection of chlorophyll a + b content in plant leaves (Yadava, 1986) and can accelerate determination of chlorophyll using acetone ( Arnon et al., 1956) or another extractants ( Moran, 1982, Hiscox and Israelstam,

1979). This equipment should be used *in situ* and also allows to determine nitrogen status in plant leaves ( Wood et al., 1994) and optimize nitrogen plant nutrition including forage grasses ( Kantety et al., 1996) and/or seed grass crop ( Virtanen and Peltoten, 1996). It was indicated that chlorophyllmeter may be used in the selection for higher nitrogen content in grasses (Gáborčík, 1998a).

The main objective of the experiments was to investigate a feasibility of the chlorophyll meter use for determination of crude protein in forage grasses.

### **Material and Methods**

In 1999 in a field experiment (DUS-test, Plant Testing Station, Spišské Vlasy, 389 m elevation) SPAD –values ( chlorophyll a + b concentration ) and nitrogen content of timothy ( *Phleum pratense* L.), cocksfoot ( *Dactylis glomerata* L. ), perennial ryegrass ( *Lolium perenne* L.) and meadow fescue ( *Festuca pratensis* Huds.) were studied. Six cultivars of each grass species were analysed. The samples were taken on May 15, 1999 (full vegetative stage).

Chlorophyll a + b content (SPAD readings, dimensionless value) was determined in 15 leaves at 20 measurements alongside the leaf blades ( due to chlorophyll concentration heterogeneity) by chlorophyllmeter SPAD – 502 (Minolta, Japan). The content of N in leaves was analysed by gas chromatography (CHN analyser 1016, Carlo Erba, Italy). Small amounts of leaf dry matter ( 1.0 mg) was burned at the temperature of 1020 °C. Crude protein (CP) concentration was calculated (  $CP = N \times 6.25$  ).

On the basis of regression between SPAD values and crude protein concentrations (CP) predicted crude protein concentrations were calculated (CP' ).

## Results and Discussion

Variation of SPAD values was found (41.5 - 35.8) and there was decreasing tendency for grass species: fescue > timothy > cocksfoot > ryegrass. Similar order was confirmed for crude protein concentration: fescue > cocksfoot > timothy > ryegrass. Variation between cultivars of both parameters was also observed (Table 1).

The results obtained demonstrated that there is a relationship between SPAD values of individual species and crude protein content. The correlation coefficient determined were: 0.906<sup>++</sup>; 0.706<sup>++</sup>; 0.653<sup>++</sup> and 0.541<sup>++</sup> for timothy, cocksfoot, fescue and ryegrass, respectively. Similar results ( unpublished data) were obtained in another experiment with red clover (*Trifolium pratense* L.). The relative differences ( mean of six cultivars ) between determined crude protein (CP) and predicted concentration calculated by linear regression (CP<sup>ˆ</sup>) were: - 0.3 %; -0.6 %; - 0.6 % and -0.9 % for timothy, cocksfoot, fescue and ryegrass, respectively. Results obtained in this study confirmed earlier assumption (Gáborèík, 1986, 1998a) that SPAD values can be used in grass selection for higher CP concentration in grasses due to close correlation between SPAD readings and CP concentration.

Usually cultivars of tall fescue (*Festuca arundinacea* Schreb.) of higher chlorophyll a + b content in leaves were characterized by higher root weight ( Gáborèík, 1998b ) which can better use water and mineral nitrogen from the soil.

A close relationship between chlorophyll a + b content determined by portable chlorophyllmeter SPAD-502 and crude protein content in leaves of four C<sub>3</sub> grass species was confirmed. Using this equipment it should be possible to detect grass genotypes of higher crude protein content *in situ* but also some morphological traits must be taken into account.

## References

- Arron, D.J., Allen M.B. and Whatley F.** (1956). Photosynthesis by isolated chloroplasts. *Biochim. Biophys Acta.* **20**: 449-461
- Gáborèik, N.** (1986). Relation between the concentrations of chlorophyll and crude protein in some fodder grass species. *Po³nohospodárstvo* **32**: 408-417 (in Slovak)
- Gáborèik, N.** (1998a). Content of chlorophyll – a trait applicable in grass breeding ? In: *Breeding for multifunctional agriculture* (Boller, B. and Stadelmann F. J., Eds.). Proceedings of 21<sup>st</sup> Eucarpia Meeting, Switzerland: 50-53
- Gáborèik, N.** (1998b). The root system and chlorophyll content in cultivar of tall fescue (*Festuca arundinacea* Schreb.). In: *Proceedings of 5<sup>th</sup> International Symposium on Structure and Function of Roots*, Stará Lesná, Slovakia, 64
- Hiscox, J.D. and Israelstam.** (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**: 1332-1334
- Kantety, R.V. van Santen E., Woods F.M. and Wood C.W.** (1996). Chlorophyll meter predicts nitrogen status of tall fescue. *Journal of plant nutrition*, **19**: 881-899
- Virtanen, A. and Peltonen J.** (1996). Field chlorophyll measurements for evaluation of timothy nitrogen status for optimized seed yield. *Acta Agriculturae Scandinavica (Section B, Soil and plant science)* **46**: 258-262
- Wood, C.W. , Reeves D.W. and Himelrick D.G.** (1994). Relationships between chlorophyll meter reading and crop chlorophyll concentration, N-status, and yield. A review. *Proceedings of Agronomy Society of New Zealand*, **23**: 1 – 9
- Yadava, U.L.** (1986). A rapid and non destructive method to determine chlorophyll in intact leaves *HortScience*, **21**: 1149 - 1450

**Table 1** - SPAD readings, determined (CP) and calculated (CP') crude protein content (mg g<sup>-1</sup>) of four grass species

Species	Parameters			Species	Parameters		
<b>Timothy cultivars</b>	SPAD	CP	CP'	<b>Ryegrass cultivars</b>	SPAD	CP	CP'
VV/II/85	46.2	189.4	193.4	Kentaur	35.2	165.2	143.2
Kaba	46.1	191.4	193.1	Kerem	30.2	121.2	128.4
Vitrov	40.3	188.2	176.3	Quickstar	41.8	157.0	162.7
Bartimo	38.2	169.7	165.4	Sakiki	33.2	143.6	137.3
G/H/	39.4	172.1	173.0	Gator	37.9	165.0	151.1
Feriol (WL)	35.0	154.1	159.8	Numan	36.2	116.2	145.5
<b>Cocksfoot cultivars</b>	SPAD	CP	CP'	<b>Fescue cultivars</b>	SPAD	CP	CP'
DP-65 05	36.3	172.3	161.8	FP-4	37.8	154.4	171.2
Rela	44.1	186.3	188.3	Premil	38.6	186.7	173.5
Lada	46.9	186.8	198.7	Roznovska	38.8	174.0	174.0
Vega	46.0	210.3	195.5	Bundy	40.5	184.0	178.9
Lemba	41.1	189.7	178.5	Szarvasi	45.6	210.0	193.5
Baraula	38.6	147.9	169.8	Swift	47.7	185.0	195.5