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Predicting timothy mineral concentrations, dietary cation-anion difference, and grass tetany index by near-infrared reflectance spectroscopy

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ABSTRACT

The mineral concentration of forage grasses plays a significant role in 2 metabolic disorders in dairy cattle production, namely, hypocalcemia (milk fever) and hypomagnesemia (grass tetany). Risks of occurrence of these 2 metabolic disorders can be evaluated by determining the dietary cation-anion difference (DCAD) and the grass tetany (GT) index of forages and specific rations. The objective of this study was to evaluate the feasibility of predicting timothy (*Phleum pratense* L.) mineral concentrations of Na, K, Ca, Mg, Cl, S, and P, the DCAD, and the GT index by near-infrared reflectance spectroscopy (NIRS). Timothy samples (n = 1,108) were scanned using NIRS and analyzed for the concentration of 7 mineral elements. Calculations of the DCAD were made using 3 different formulas, and the GT index was also calculated. Samples were divided into calibration (n = 240) and validation (n = 868) sets. The calibration, cross-validation, and prediction for mineral concentrations, the DCAD, and the GT index were performed using modified partial least squares regression. Concentrations of K, Ca, Mg, Cl, and P were successfully predicted with coefficients of determination of prediction $\left(R_P^2\right)$ of 0.69 to 0.92 and coefficients of variation of prediction (CV_P) ranging from 6.6 to 11.4%. The prediction of Na and S concentrations failed, with respective R_P^2 of 0.58 and 0.53 and CV_P of 82.2 and 12.9%. The 3 calculated DCAD and the GT index were predicted successfully, with R_P^2 >0.90 and CV_P <20%. Our results confirm the feasibility of using NIRS to predict K, Ca, Mg, and Cl concentrations, as well as the DCAD and the GT index, in timothy.

Key words: *Phleum pratense*, milk fever, hypomagnesemia, near-infrared reflectance spectroscopy

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INTRODUCTION

Hypocalcemia (milk fever) and hypomagnesemia (grass tetany) are 2 important metabolic disorders in dairy cattle production. These disorders usually occur when the supplies of Ca and Mg are inadequate during early lactation (Jefferson et al., 2001). The risk of milk fever and grass tetany occurring can be evaluated by determining the DCAD and the grass tetany (**GT**) index of forages and specific rations. Three formulas are commonly used to calculate the DCAD in dairy cattle rations (Pelletier et al., 2008):

$$\text{DCAD1} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S}) \quad [1]$$

(Ender et al., 1971);

$$\text{DCAD2} = (\text{Na} + \text{K} + 0.15\ \text{Ca} + 0.15\ \text{Mg}) - (\text{Cl} + 0.6\ \text{S} + 0.5\ \text{P}) \quad [2]$$

(NRC, 2001); and

$$\text{DCAD3} = (\text{Na} + \text{K}) - (\text{Cl} + 0.6\ \text{S}) \quad [3]$$

(Goff et al., 2004), where DCAD is expressed in millimoles of charge (**$mmol_c$**)/kg of DM (g/kg of DM × 1,000 × valence/atomic weight). The GT index is calculated using the following formula:

$$\text{GT index} = \text{K}/(\text{Ca} + \text{Mg}) \quad [4]$$

(Kemp and ’t Hart, 1957).

The DCAD1 formula is widely used in applied dairy cattle nutrition because it is well correlated with urinary pH and is predictive of clinical milk fever. DeGaris and Lean (2008) suggested that DCAD1 was the most effective formula for predicting the risk of milk fever, based on the simplified strong ion model and the meta-analysis of Lean et al. (2006). However, Charbonneau et al. (2006) reported that DCAD3 was most highly associated with milk fever ($R^2 = 0.44$) and urinary pH

($R^2 = 0.85$) according to their meta-analysis of 22 published studies.

To prevent milk fever, dairy cows should be fed a ration with a DCAD1, DCAD2, and DCAD3 of approximately −50, 150, and −42 $mmol_c$/kg of DM, respectively, beginning from 3 to 4 wk before calving (Goff and Horst, 2003; Pelletier et al., 2008). The respective DCAD1 and DCAD3 values of forages should be no more than 250 and 290 $mmol_c$/kg of DM to avoid adding excessive amounts of anionic salts, such as those based on $CaCl_2$ or $MgCl_2$, which reduce DMI (Horst et al., 1997; Pelletier et al., 2008). The occurrence of grass tetany is greatly increased for cattle grazing forages with a GT index higher than 2.2 (Jefferson et al., 2001).

Timothy (*Phleum pratense* L.) is the dominant perennial forage grass species grown in eastern Canada (Bélanger et al., 2001). Several studies have reported lower values of K concentration, the DCAD, or the GT index for timothy compared with other forage grasses such as orchardgrass (*Dactylis glomerata* L.) or phalaris (*Phalaris aquatica* L.; Thomas et al., 1998; Tremblay et al., 2006; Pelletier et al., 2008). Timothy is therefore a forage species well suited to decrease risks of milk fever and grass tetany. Data on the DCAD and the GT index of timothy and other forage species are commonly obtained after chemical determinations of several minerals, rendering the acquisition of these indices time-consuming and expensive.

Near-infrared reflectance spectroscopy (**NIRS**) has been widely used as a fast and cost-effective method for determining forage nutritive value (Shenk and Westerhaus, 1994). The ability of NIRS to predict forage nutritive value and many other organic substances is based on the rotational or vibrational energies of hydrogen bonds. Although minerals theoretically do not absorb energy in the near-infrared spectrum, some of the inorganic minerals in forages can be predicted by NIRS (Clark et al., 1987, 1989; Halgerson et al., 2004) through their association with organic molecules. The NIRS prediction of minerals has produced many inconsistent results in forage analyses. Clark et al. (1987) reported that NIRS calibrations for the macrominerals Ca, P, Mg, and K were useful in crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] and alfalfa (*Medicago sativa* L.). Halgerson et al. (2004) obtained similar results, in which concentrations of Ca, P, and K were accurately predicted in leaves and stems of alfalfa hay, whereas predictions of Mg and S were less consistent and prediction of Na failed. In contrast, Stoltz (1990) reported that calibrations for Ca, K, P, and Mg in alfalfa and white clover (*Trifolium angustifolium* L.) were unsuccessful, and in other studies, difficulties were also found in obtaining accurate NIRS predictions for minerals (Redshaw et al., 1986; Saiga et al., 1989). Based on reported successful NIRS predictions for some minerals needed in calculation of the DCAD and the GT index, we hypothesized that predicting the DCAD and the GT index is possible. To our knowledge, information on the possibility of predicting the DCAD and the GT index of timothy with NIRS is limited. The objective of this study was to evaluate the feasibility of using NIRS to predict Na, K, Ca, Mg, Cl, S, and P concentrations, as well as the DCAD and the GT index in timothy.

MATERIALS AND METHODS

Samples

This study was part of a larger experiment described in detail by Pelletier et al. (2007) in which we determined the influence of Cl and N fertilizer applications on DCAD. Timothy (*P. pratense* L., cv. Champ) samples (n = 1,108) were collected in 2003 and 2004 at 4 locations in the province of Québec, Canada, as described by Pelletier et al. (2007). Timothy was sown in 2002 at Sainte-Anne-de-Bellevue (45°24′N, 73°57′W), Normandin (48°51′N, 72°32′W), and Saint-Augustin-de-Desmaures (46°44′N, 71°27′W), and in 1998 at Sainte-Perpétue (46°05′N, 72°28′W). Ten fertilizer treatments (seasonal applications of 0, 80, 160, and 240 kg of Cl/ha as $CaCl_2$, 160 kg of Cl/ha as NH_4Cl, all combined with N fertilization that provided 70 or 140 kg of N/ha as NH_4NO_3 for $CaCl_2$ treatments or a mix of NH_4NO_3 and NH_4Cl for NH_4Cl treatments) were applied in a split application: 60% before the start of spring growth and 40% after the first harvest. Individual plot size was 6 × 1.5 m. In Sainte-Anne-de-Bellevue, Normandin, and Saint-Augustin-de-Desmaures, half of the timothy plots were harvested to a 5-cm height when timothy reached the late-heading stage, and the other half were harvested 7 d later in both spring growth and summer regrowth. In Sainte-Perpétue, timothy was harvested at the late-heading stage only. Plots were harvested with a self-propelled flail forage harvester (Carter MGF Co. Inc., Brookston, IN) at Normandin and Sainte-Perpétue and with a REM flail forage harvester (Swift Machine and Welding, Swift Current, Saskatchewan, Canada) at Sainte-Anne-de-Bellevue and Saint-Augustin-de-Desmaures.

A sample of approximately 500 g was taken from each plot, weighed, dried at 55°C in a forced-draft oven for 3 d, and then ground using a Wiley mill (Standard model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. All dried and ground timothy samples (n = 1,108) were stored in plastic containers at room temperature in a dark room. Samples were scanned between July 7 and 19, 2006, at 2-nm intervals