Brix as an Indicator of Sugar Content and Nutritive Value in Alfalfa and Orchardgrass Herbage

Billman, E.*; Soder, K.*; Horst J.†; Balk, K†; Hafla, A.‡
*USDA-ARS; †Agri-King, Inc., Fulton, IL; ‡ Cargill Animal Nutrition, North America, Des Moines IA.

Keywords: Grazing; energy; forage; pasture; validation

Abstract

Brix, a measurement of total dissolved solids in solution, has been used by forage producers to provide real-time estimates of energy content of fresh herbage. However, its efficacy has never been validated in herbage through wet chemistry testing and comparisons with other nutritive value parameters. This study compared and correlated Brix measurements with sugar concentrations and common nutritive value parameters relating to protein, fiber, and energy to determine the viability of using Brix to predict when to graze or harvest fresh herbage. Brix measurements were collected monthly on fresh herbage samples of alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) from May to August in 2019 and 2021. Herbage was immediately flash frozen with liquid nitrogen and analyzed for sugar concentration and nutritive value. Brix did not differ among sampling dates for alfalfa and only differed at the May sampling for orchardgrass, indicating that Brix values were not affected by harvest date during late spring and summer months. When correlated across all sampling dates, Brix was positively correlated to sugar concentrations, reduced fiber, and greater net energy concentrations in alfalfa, but not positively correlated to any nutritive value parameters in orchardgrass. These results indicated that Brix should be used only in a limited fashion to predict energy content of fresh herbage and is more reliable when used with legumes than grasses.

Introduction

Forage nutritive value is a key component of ruminant livestock production. However, typical wet chemistry nutritive value analyses are time consuming and cost prohibitive for most producers. Hand-held refractometers offer a portable and low-cost method (Brix, a measure of total dissolved solids in solution) to estimate sugar concentration in real-time in the wine industry to rapidly make decisions about grape harvest (Harrill, 1998). Since the mid-2010's some grazing farmers have anecdotally used this method to estimate total sugar content of fresh herbage (Lemus and White, 2014). In turn, those results are assumed to be positively related to overall nutritive value and used to determine relative energy content of fresh herbage. However, little validation of Brix has been conducted with herbage samples to determine the efficacy of its application with grasses and legumes. This study compared Brix measurements with wet chemistry analyses of specific sugars and standard nutritive value parameters for orchardgrass (**OG**; *Dactylis glomerata* L.) and alfalfa (**ALF**; *Medicago sativa* L.). The objective was to determine if Brix is a viable method of estimating energy content of fresh herbage. We hypothesized that Brix would not be a reliable predictor of herbage energy content due to decreased concentrations of sugars in forage crops compared to horticultural crops.

Materials and Methods

Field Site and Herbage Material

Fresh herbage was collected at the Pennsylvania State University's Russell E. Larson Agricultural Research Center, Rock Springs, Pennsylvania (40° 43' 00" N, 77° 56' 24" W). Fresh herbage was sourced from two different species: OG (10-year stand last interseeded in 2014) and ALF (three-year stand, established in 2016). Soil type for both crops was a Hagerstown silt loam with slopes of 3-8% (fine, mixed, semiactive, mesic Typic Hapludalfs). Orchardgrass was fertilized with 34 kg N ha^{-1} as ammonium sulfate (34-0-0) between harvests, while ALF was never fertilized with N.

Biomass and Brix Sampling Procedure

Sampling occurred at monthly intervals (May – August) during 2019 and 2021. Sampling occurred between 1:00 – 3:00 pm, on sunny days, with no moisture on leaves, with the same person sampling Brix to minimize sampling error. At each monthly sampling, eight biomass samples were collected from each species at a cutting height of 10 cm, providing a total of 64 samples each of OG and ALF. Approximately 0.014 m^3 of material was collected for each whole-sample to facilitate freezing in liquid N. From these, three subsamples were used for Brix measurements on a digital MA871 Brix refractometer (Milwaukee Instruments, Inc., Rocky Mount, NC) within five minutes of harvest.

For each reading, approximately 33 cm^3 of subsampled material was placed into the well of a hand-held garlic press. The press was then held over the refractometer lens and squeezed to extract fluid from the OG or ALF tissue. During collection of the Brix readings, the remaining biomass of the whole-sample was submerged in liquid nitrogen (-200°C) for 25 to 30 seconds to slow metabolic activity and preserve the samples as close to the timing of Brix sampling as possible for wet chemistry. Whole-samples were then transferred to a -80°C freezer, then freeze-dried.

Laboratory Analyses

Wet chemistry analyses of freeze-dried samples were conducted for total and individual sugars, energy, fiber, and protein concentrations (Analab, Agri-King, Inc., Fulton, IL). At the testing lab, samples were ground to pass through a 6-mm mesh screen in preparation for sugar extraction assays followed by a second grind step through a 0.8-mm mesh screen. All sugar profiles were obtained from samples using HPAEC-PAD (high-performance anion-exchange chromatography with pulsed amperometric detection) adapted from Ellingson et al. (2016). This process was slightly modified to use a 50% acetonitrile solution for extraction, rather than 50% ethanol solution, to improve mannitol recovery.
Other parameters tested via wet chemistry were dry matter (**DM**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and crude protein (**CP**; AOAC, 2006). Energy estimates were calculated, including total digestible nutrients (**TDN**), net energy of lactation (**NE_L**), net energy of maintenance (**NE_M**), and net energy of gain (**NE_G**).

Statistical Analyses

Data were pooled within each species (OG and ALF) across both years and analyzed as repeated measures using PROC GLIMMIX in SAS 9.4 (SAS Institute, Cary, NC). Correlation analyses were conducted using the PROC CORR command of SAS 9.4. Significance was determined at $P < 0.05$ for both the GLIMMIX and CORR procedures.

Results and Discussion

Changes in Sugars and Nutritive Value over Time

For both OG and ALF samples, there were significant ($P < 0.05$) effects of sampling date on most wet chemistry sugar and nutritive value parameters (Table 1). Brix, glucose, hemicellulose, and ash concentrations were not affected ($P > 0.10$) for ALF, while all parameters differed ($P < 0.05$) for OG. In general, sugar concentration increased throughout the growing season for ALF, while sugars were lower during the mid-summer sampling dates for OG. These results indicated that OG appears to produce less sugar during hot summer months, which aligns with its C3 photosynthetic pathway and reduced summer growth potential. This was attributed to photorespiration and a net carbon loss. However, Brix of OG was similar ($P > 0.10$) from among the June, July, and August samplings. This suggests that Brix may be affected by non-sugar compounds in solution in the OG samples. When examining fiber and protein, ALF increased in CP while decreasing NDF and ADF as the season progressed, while for OG the effects were reversed over the same time span. Similar effects were also noted in the energy categories (TDN, NE_L, NE_G, and NE_M), but this was likely related to changes in NDF, ADF, and hemicellulose for ALF and OG.

Correlation of Brix to Sugars and Nutritive Value

When examining the relationship among Brix and total and specific sugars, Brix had stronger correlations to sugar concentrations in ALF compared to OG (Figures 1a and 1b). Brix measurements in ALF were positively correlated to total sugars, fructose, glucose, and mannitol ($P < 0.01$). Conversely, Brix measurements in OG were not correlated ($P > 0.10$) to any sugar components. Brix measures total

Table 1. Nutritive value parameters for alfalfa (ALF) and orchardgrass (OG) pooled over two growing seasons (2019 and 2021), including sugars, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (Hemicell), total digestible nutrients (TDN), net energy of lactation (NE_L), net energy of maintenance (NE_M), and net energy of gain (NE_G).

				Harvest					
Category	Species	Parameter	Unit	May	June	July	August	SEM	Significance
Sugars	ALF	Brix	°Brix	4.3†	4.6	3.9	3.7	0.51	‡
		Fructose	%	1.1[a]	1.0[b]	0.7[c]	1.0[b]	0.05	***
		Glucose	%	5.1	4.6	4.5	5.2	0.03	‡
		Sucrose	%	2.7[b]	2.5[b]	2.7[b]	3.2[a]	0.13	***
		Mannitol	%	0.0[b]	0.0[b]	0.0[b]	0.3[a]	0.05	***
		Total Sugar	%	5.1[ab]	4.7[bc]	4.4[c]	5.3[a]	0.24	**
	OG	Brix	°Brix	1.9[b]	3.5[a]	3.4[a]	3.2[a]	0.45	**
		Fructose	%	1.6[a]	1.4[ab]	1.2[b]	1.6[a]	0.13	***
		Glucose	%	6.4[a]	5.8[a]	4.7[b]	6.5[a]	0.49	**
		Sucrose	%	3.6[a]	2.9[b]	2.4[c]	3.4[a]	0.18	***
		Mannitol	%	0.03[c]	0.08[b]	0.08[ab]	0.1[a]	0.01	***
		Total Sugar	%	6.7[a]	5.7[b]	4.7[c]	6.6[a]	0.32	***
Protein & Fiber	ALF	CP	%	19.3[c]	18.2[d]	20.7[b]	22.8[a]	0.46	***
		NDF	%	37.5[a]	34.9[b]	34.8[b]	29.6[c]	0.90	***
		ADF	%	28.9[a]	27.0[b]	27.4[b]	22.4[c]	0.71	***
		Hemicellulose	%	8.6	7.9	7.5	7.3	0.60	‡
	OG	CP	%	24.3[a]	20.9[b]	20.3[b]	21.4[b]	0.71	***
		NDF	%	47.0[b]	48.8[b]	54.9[a]	52.9[a]	1.11	***
		ADF	%	27.9[c]	30.4[b]	32.0[a]	30.2[b]	0.71	***
		Hemicellulose	%	19.1[b]	18.4[b]	22.9[a]	22.7[a]	0.90	***
Energy	ALF	TDN	%	63.1[c]	65.5[b]	64.7[b]	70.1[a]	0.80	***
		NE_L	Mcal kg^{-1}	1.54[c]	1.56[bc]	1.58[b]	1.72[a]	0.018	***
		NE_M	Mcal kg^{-1}	1.41[c]	1.43[bc]	1.45[b]	1.63[a]	0.022	***
		NE_G	Mcal kg^{-1}	0.81[c]	0.84[bc]	0.88[b]	1.03[a]	0.020	***
	OG	TDN	%	64.5[a]	61.5[bc]	60.1[c]	61.8[b]	0.82	***
		NE_L	Mcal kg^{-1}	1.58[a]	1.50[bc]	1.47[c]	1.52[b]	0.020	***
		NE_M	Mcal kg^{-1}	1.45[a]	1.36[bc]	1.30[c]	1.36[b]	0.026	***
		NE_G	Mcal kg^{-1}	0.88[a]	0.79[b]	0.73[c]	0.79[b]	0.024	***

** Significant at $P < 0.01$; *** Significant at $P < 0.001$; † Within rows, different letters indicate significant differences; ‡ Not significant.

dissolved solids in solution, not simply dissolved sugars. Fresh herbage is relatively low in sugar, comprising < 10% DM (Holzer et al., 2003) which makes Brix readings less accurate. Additionally, fluid extraction from OG samples was difficult due to greater amount of fiber and in the OG herbage material compared to ALF. Past work has also established that OG herbage has less nonstructural carbohydrates than ALF (Jonker, et al., 2001). It is likely that the greater amount of force exerted on the OG samples to extract fluid caused structural carbohydrates (fiber) to be extracted in the solution. Subsequently, Brix values would not directly correlate to sugar concentration.

Further correlations of Brix to parameters within the categories of protein, fiber, energy, and mineral components indicated that Brix accurately correlated to lower fiber and greater digestible energy concentrations in ALF (Figure 2a). Conversely, there were almost no correlations between Brix and other nutritive value parameters for OG (Figure 2b). This supports the findings from the correlations to sugar concentration, with Brix being reliable at predicting energy values of ALF, but not of OG herbage.

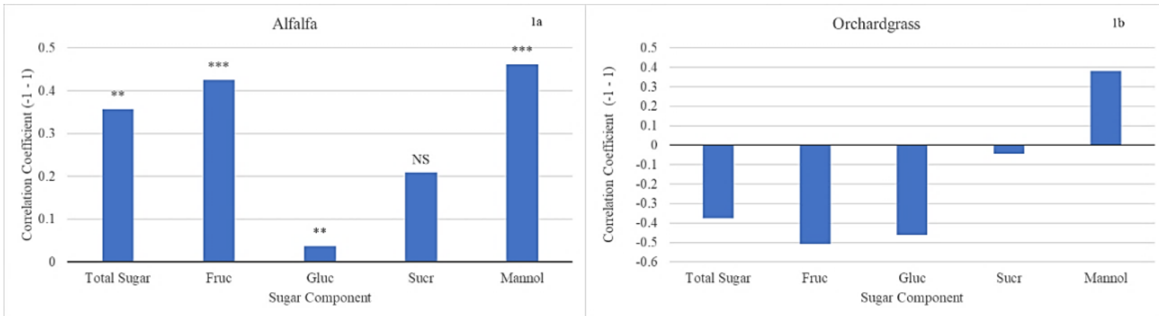

Figure 1. Pearson correlations between Brix and total sugars, fructose (Fruc), glucose (Gluc), sucrose (Sucr), and mannitol (Mannol) for alfalfa (1a) and orchardgrass (1b) over two growing seasons. **Significant at $P < .01$; ***Significant at $P < .001$; NS = not significant $P > .05$

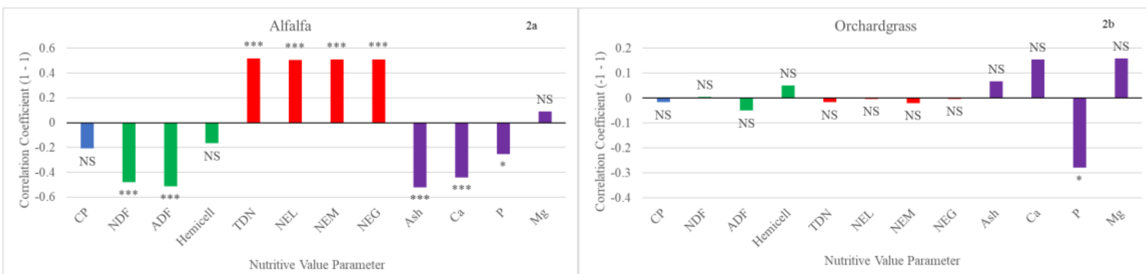

Figure 2. Pearson correlations between Brix and crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (Hemicell), total digestible nutrients (TDN), net energy of lactation (NE_L), net energy of maintenance (NE_M), net energy of gain (NE_G), ash, calcium, phosphorus, and magnesium for alfalfa (2a) and orchardgrass (2b) over two growing seasons. *Significant at $P < 0.05$; ***Significant at $P < 0.001$; NS = not significant $P > 0.05$.

Conclusions

Brix measurements collected over two growing seasons on ALF and OG had differing efficacy at predicting energy content of fresh herbage. Based on correlations to sugars and to nutritive value parameters, high Brix values in ALF were indicative of lower fiber, higher energy herbage, while Brix values for OG were not useful at predicting sugar or nutritive value components. This was possibly due to less moisture content in grasses compared to legumes, and the greater difficulty of fluid extraction from OG compared to ALF. Brix could prove useful in predicting energy content of other forage legumes, but it should not be the sole method of determining herbage nutritive value.

References

AOAC International. 2006. *Official methods of analysis.* 18th ed. AOAC Int. Gaithersburg, MD.

Ellingson, D. J., Anderson, P., and Berg, D. P. 2016. Analytical method for sugar profile in pet food and animal feeds by high-performance anion-exchange chromatography with pulsed amperometric detection. *J. AOAC Internat.* 99(2): 342-352.

Harrill, R. 1998. Using Refractometer to Test the Quality of Fruits and Vegetables. http://www.living-foods.com/articles/fruitquality.html (accessed 11/02/2022).

Holzer, M., Mayrhuber, E., Danner, H., and Braun, R. 2003. The role of *Lactobacillus buchneri* in forage preservation. *Trends Biotech.* 21(6): 282-287.

Jonker, J. S., Cherney, D. J. R., Fox, D. G., Chase, L. E., and Cherney, J. H. 2001. Orchardgrass versus alfalfa for lactating dairy cattle: Production, digestibility, and nitrogen balance. *J. Appl. Anim. Res.* 21: 81-92.

Lemus, R. W., & White, J. A. (2014). Brix level in your forage: What does it mean? Mississippi State University Extension Service. 7(2).