



Technical note: Rapid mineral determination in forages by X-ray fluorescence

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ABSTRACT

A large portion of the cow's ration is composed of forages that can vary greatly in mineral concentrations, which may affect animal performance and health. Current methods for mineral analysis require sample destruction either through wet or dry ashing and complex analytical techniques for individual minerals. Energy dispersive X-ray fluorescence (EDXRF) is a nondestructive, multi-mineral, spectroscopy technique, which makes mineral quantification simple, direct, and affordable. The study objective was to evaluate the prediction performance of EDXRF of Na, Mg, P, S, Cl, Ca, K, Mn, Fe, Cu, and Zn concentrations in forages. Twelve certified plant samples with a wide range in mineral concentrations were used to develop calibrations, and 35 forages (18 alfalfa hays, 10 grass hays, 7 corn silages) with measured mineral concentrations, which were collected over several years from 3 proficiency programs, were used as an independent validation set. All the samples were previously dried and finely ground and were prepared by compressing them into a round dense pellet, 40 mm in diameter, using a 40-ton pneumatic laboratory press. Samples were scanned using an EDXRF instrument enhanced for lighter minerals like Na and Mg. Samples were scanned at 20 keV and at 40 KeV associated with an Al filter, for a total analysis time of approximately 6 min. Calibrations were developed with Bruker SpectraEDX (Bruker, Hamburg, Germany) software and optimized to minimize the standard error of calibration. All of the minerals had acceptable calibration performance with coefficient of determination ranging from 0.93 (P) and 0.99 (Cl, Ca, and Mn) and coefficients of variation within 5 to 14%, which are similar to the coefficients of variation of the reference analysis. The coefficients of variation for Na was an exception, with a coefficients of variation of 29%. The validation set obtained similar statistical results as that observed in calibration. The

root mean square error of prediction corrected for bias was similar to the standard error of calibration, indicating that it is possible to build a robust calibrations that performed well across different type of forages by using 12 reference samples with a sufficient range in mineral concentrations that were determined accurately. A bias correction was necessary to improve prediction accuracy only for K (−0.23% dry matter) and Ca (−0.16% dry matter). Energy dispersive X-ray fluorescence demonstrated the ability to be an accurate, direct, and simple technique for forage mineral analysis.

Key words: mineral, forage, X-ray fluorescence

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Feeding a balanced ration for all nutrients, including minerals, is crucial for maintaining high levels of milk production and animal health (Goff, 2006). Forages usually represent a large portion of the ration, but also have a large variability in nutritional quality (Dairy One, 2018) that should be monitored regularly (St-Pierre and Cobanov, 2007). The current and most used analytical techniques such as atomic absorption and inductively coupled plasma mass spectrometry require extensive sample preparation, like acid hydrolysis (Tagami, 2006). This sample preparation, besides being time consuming and producing reagents waste, is not suitable for Cl because a significant portion of this halogen mineral is lost during the acid hydrolysis (Tagami, 2006). In comparison, energy dispersive X-ray fluorescence (**EDXRF**) is a nondestructive spectroscopy technique, which requires minimum sample preparation, that include drying, grinding, and pressing into a pellet. The EDXRF can measure many minerals simultaneously, which may have large ranges in mineral concentrations, making it attractive for plant and forage analysis (Djingova, 2013). The study objective was to evaluate the accuracy of EDXRF as a method to determine the concentration of macro- and some micro-minerals in forage samples.

The study was performed using an energy dispersive X-ray spectrometer (S2 Ranger LE, Bruker, Hamburg, Germany) with a Pd anode X-ray tube, Peltier cooled

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Table 1. List of certified reference standards used for calibration

Type of material	Reference code
Fescue plant	IPE965
Corn plant	IPE723
Wheat straw	IPE962
Wheat straw	IPE881
Alfalfa plant	IPE966
Grass mixture	IPE952
Corn stalk	IPE968
Mixed Polish herbs	INCT-MPH-2
<i>Pinus taeda</i> leaves	1575A
<i>Fagus sylvatica</i> leaves	BCR100
Olive leaves	BCR62
Hay powder	BCR129

detector, and maximum power of 50 W, 2 mA, or 50 KeV. About 5 g of finely ground sample was pressed into a pellet of 40 mm in diameter using a 40-ton pneumatic laboratory press (International Crystal Laboratories, Garfield, NJ). The pellet was placed within a round holder, inserted in a shielded chamber that was evacuated before it was irradiated with X-ray at different energy levels to excite different minerals until 100,000 cps was reached. First, samples were irradiated at 20 KeV without any filter for the determination of lighter minerals (Na, Mg, P, S, Cl) and then at 40 KeV with an Al filter to optimize fluorescence for the heavier (K, Ca, Fe, Mn, Cu, Zn) minerals. The total scan time per sample was approximately 6 min. Calibrations for the different minerals were developed using a set of 12 certified plant samples (Table 1) selected either from the one available from the European Commission Joint Research Center (JRC, Geel, Belgium), and the Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL, Wageningen, the Netherlands) catalogs. Calibrations were developed using Bruker SpectraEDX software by regressing the fluorescence emission at either 20 or 40 KeV scan against the known

minerals concentration of the 12 certified standards. Regressions were optimized by selecting either the peak height or peak area to minimize the standard error of regression also known as standard error of calibration (**SEC**). Validation was performed by comparing EDXRF mineral predictions to the reference mineral analysis of 35 forage samples (18 alfalfa hays, 10 grass hays, and 7 corn silages). Validation samples and their mineral analyses were obtained over years from 3 interlaboratory proficiency programs that provided reference mineral analysis to evaluate laboratory accuracy. Twenty samples were from the National Forage Testing Association (United States), 7 from the University of Piacenza (Italy), and 8 from Bipea (France). Not all the minerals predicted by EDXRF were available for the validation samples, such that Na, Mn, and Fe were available only for 7 samples and Cl was not reported for any of the samples from these proficiency programs (Table 2). All of the other minerals had at least 15 validation samples. Because of the lack of validation samples for Cl, 6 forage (2 alfalfa hays, 2 grass hays, 2 corn silages) samples were analyzed for Cl using the Volhard method (Eur-Lex, 2009).

Calibration performance was evaluated based on the coefficient of determination (R^2), SEC, and the coefficient of variation of the calibration error (**CVc**), calculated as $SEC \times 100/\text{mean concentration}$. Validation statistics were computed by using regression in Microsoft Excel (Microsoft Corp., Redmond, WA) to compare reference values of validation samples and EDXRF predicted values and determining the R^2 , the bias (difference between average reference values minus average EDXRF predictions), also known as systematic error, the prediction error expressed as root mean square error of prediction (**RMSEP**), and the standard error of prediction (**SEP**), which is the RMSEP after correction of the systematic error (Dardenne, 2009).

Table 2. Evaluation of energy dispersive X-ray fluorescence prediction compared with validation standards

Mineral (DM basis)	Calibration statistics ¹								
	n	Mean	SD	Minimum	Maximum	R^2	RMSEP	Bias	SEP
Na, mg/kg	7	929	1,053	200	3,300	0.984	153	-45	158
Mg, %	33	0.23	0.09	0.05	0.50	0.935	0.02	0.01	0.02
P, %	35	0.27	0.07	0.05	0.39	0.957	0.02	0.00	0.02
S, %	20	0.21	0.09	0.08	0.38	0.985	0.02	-0.02	0.01
Cl, %	6	0.35	0.04	0.05	0.70	0.987	0.05	-0.04	0.03
K, %	26	1.91	0.71	0.90	3.33	0.984	0.26	-0.23	0.13
Ca, %	35	0.99	0.77	0.18	3.97	0.998	0.21	-0.16	0.13
Mn, mg/kg	7	51	29	21	86	0.963	6	-3	6
Fe, mg/kg	7	506	406	192	1,361	0.995	160	-126	107
Cu, mg/kg	15	8	4	2	18	0.809	3	-2	2
Zn, mg/kg	35	40	60	9	360	0.996	7	-4	6

¹n = number of validation standards; RMSEP = root mean squared error of prediction; bias = average difference between predicted and reference values; SEP = root mean squared error of prediction corrected for bias.

Table 3. Calibration performance for energy dispersive X-ray fluorescence using certified reference standards

Mineral (DM basis)	Calibration statistics ¹							
	n	Mean	SD	CVc	Minimum	Maximum	R ²	RMSEC
Na, mg/kg	6	340	488	29	10	1,300	0.95	100
Mg, %	11	0.23	0.21	13	0.05	0.65	0.97	0.03
P, %	10	0.27	0.12	11	0.05	0.43	0.93	0.03
S, %	11	0.23	0.12	4	0.08	0.43	0.98	0.01
Cl, %	12	0.64	0.61	6	0.02	2.01	0.99	0.04
K, %	11	2.44	1.56	11	0.44	5.18	0.97	0.27
Ca, %	11	0.98	1.28	10	0.04	4.46	0.99	0.10
Mn, mg/kg	12	201	330	5	7	1,162	0.99	10
Fe, mg/kg	10	194	178	14	46	538	0.97	27
Cu, mg/kg	12	6	4	33	2	15	0.97	2
Zn, mg/kg	11	36	20	8	8	82	0.98	3

¹n = number of calibration standards; RMSEC = root mean squared error of calibration; CVc = coefficient of variation in calibration (RMSEC × 100/mean).

Calibration statistics (Table 3) indicated R² greater than 0.93 for all minerals. Because lighter minerals (Na, Mg, and P) have lower X-ray fluorescence, their slightly lower R² compared with the other minerals was not unexpected, as they are more difficult to quantify by EDXRF. Nonetheless, even Na, the lightest mineral, demonstrated good calibration statistics, likely because the instrument was an enhanced version for detecting lighter minerals. The largest SEC was K at 0.27% DM, which was expected because it had the largest range in mineral concentration. Calcium, which is important in a balanced dairy ration (NRC, 2001), had an R² of 0.99 and SEC of 0.10% DM, indicating accurate quantification. The heavier minerals such Mn, Fe, Cu, and Zn demonstrated excellent performances with SEC for the heaviest Cu and Zn close to the lower limit of detection of 1 to 3 mg/kg (Ivanova, 1999).

Evaluating the relative errors as indicated by CVc, the best calibrations were obtained for S, Cl, Mn, and Zn with a CVc of <10% (Table 3). Then a second group of minerals (Mg, P, K, Ca, and Fe) yielded CVc between 10 and 14%. The poorest mineral calibrations were Na and Cu. Sodium had a CVc of 29%, which can be explained by the limitations of EDXRF detecting the lighter minerals (Jenkins, 1999), whereas Cu performance is related to the low concentration (less than 10 mg/kg), which is close to the EDXRF lower limit of detection. A SEC of 2 mg/kg is about the lowest possible error for Cu using this analytical technique. The coefficients of variation of reference analyses for the certified materials typically varied between 5 and 15%, which is similar to the range of CVc for most minerals. However, Cu, having a low average concentration, had a coefficient of variation analysis of 25%, not far from calibration results. Although the CVc for the different minerals may be large, the calibration performances

were comparable to the precision of analysis reported in the certificates of the certified materials. The only exception is Na that had the largest CVc, which was greater than reported in the reference standards.

Even with some limitation with reference analysis for some minerals (Na, Cl, Mn, and Fe), the validation data set provided a solid confirmation of the results obtained through calibration. With the only exception of Cu (R² = 0.81), all of the other minerals had an R² of 0.93 or greater. The validation error (RMSEP) for most of the minerals confirmed the SEC with the exception of Ca, Fe, and Zn. Calcium had a SEC of 0.10%, but in validation the RMSEP was nearly double at 0.21% even with a R² of 0.998. The bias for Ca was on average -0.16%, which can happen when comparing results coming from different laboratories and if we apply this correction systematically to all EDXRF Ca results, the corrected prediction error (SEP) would be 0.13% DM, which is very similar to the calibration performance. Iron had also a larger RMSEP, and even with a bias adjustment, the prediction error remained much larger (SEP = 107 mg/kg) than what could be expected from the magnitude of the calibration error (SEC = 27 mg/kg). The validation data set had a wider range in Fe concentration than the calibration database, and the 2 validation samples that had Fe concentration exceeding the upper calibration range had the largest errors. In this case it would be advisable to update the calibration using a wider range in Fe concentration. Similarly, the validation database for Zn had 2 samples that exceeded the calibration range and the validation sample with the highest concentration (360 mg/kg) had the largest prediction error. Removing that sample, the RMSEP would decrease to 5 and the SEP to 4, not too far from the calibration error. Like Ca, K had a fairly large bias value (-0.23%), which was similar in magnitude to the

RMSEP (0.26%). Adjusting the XRF predictions for systematic bias would lower SEP at 0.13%, even better than the calibration results.

Limited literature is available about the prediction performance of XRF for forage, but there are reports of using EDXRF for mineral analysis of plant material. Ivanova (1999) reported reliable prediction for Ca, Fe, K, Mn, and Zn for different plant sources. A few samples of hay and straw along cereals and mixed feeds were analyzed by Pukhovski (2002), with good predicting performances for P, K, Ca, Mn, Fe, and Zn. The prediction errors observed in this study indicate that using EDXRF to analyze forages would improve the accuracy of minerals for ration formulation compared with using mineral analysis from near-infrared spectroscopy (Clark et al., 1987), as it is often used. From a practical point of view, improving forage mineral analysis would allow more accurate supplementation of only the mineral needed, with the reduction of feeding costs, and of unnecessary excretions, which may affect the environment.

Mineral analysis by EDXRF using modern instruments can provide accurate quantification of the major macro- and some microminerals in forage samples. Simple sample preparation and the lack of using reagents make this technique easy to implement even in small forage laboratories, providing the opportunity to accurately measure forage mineral concentrations using a cost-effective method. Having a sample preparation similar to what is necessary for near infrared analysis (Abrams, 1989), EDXRF could be easily integrated into the operations of forage laboratories that use near infrared technology and enhance their ability to measure minerals.

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