Application Note: 50942

Prediction of Choline Chloride Concentration on Silage by FT-NIR Spectroscopy

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Key Words

- Antaris
- Animal Feed
- Choline Chloride
- Fodder
- FT-NIR

Abstract

Measuring the amount of choline chloride on animal feed is vital to the livestock industry. Choline chloride is sprayed on ground corn cob as an added vitamin that helps animal growth and promotes health. Current methods for analyzing the amount of choline chloride are tedious and time-consuming (up to 12 hours per sample). Analysis using the Antaris™ FT-NIR analyzer successfully measured choline chloride content on production samples directly through the plastic bag in less than 6 seconds without sacrificing accuracy or precision.

Introduction

Choline chloride (ChCl, chemical name trimethyl (2-hydroxyethyl) ammonium chloride) is an animal feed additive, classified as a water-soluble B-vitamin that increases animal growth. ChCl is added exogenously to feed stocks because it plays an essential role in fat transport, metabolism, and protects cell membrane structure. Corn cob (silage) is used for ease of ingestion.

Currently, the most common ways to measure choline chloride content on silage are the Volhardt Titration, perchloric acid titration, reaction with sodium tetra-phenylborate, and the Reinke Salt Colour comparison method. All of these methods require soaking the ground cob in solvent for 6-12 hours to extract the choline chloride, followed by filtration concentration, and finally titration. The measurement of choline chloride using the above techniques requires solvents, expensive reagents, and trained operators, in addition to a considerable amount of time to successfully identify the amount of ChCl. The accuracy of these methods is also in question as the Volhardt method contains contaminants like NaCl or $CaCl_2$ that interfere with the measurement. The Reinecke method is unsuitable for high concentrations of choline chloride (above 50%).

The current study shows the advantages of using Fourier transform near-infrared spectroscopy (FT-NIR) to quickly, accurately, and non-destructively analyze silage for choline chloride content. In addition, it can be shown that FT-NIR can be used to acquire the concentration of the choline chloride by scanning directly through the plastic bag that the ground cob arrives in, negating the need for unpacking, soaking, and titrating the samples.

Experimental

Twenty-five bags of silage were measured on the Antaris FT-NIR Solid Sampling analyzer at 4 cm^{-1} resolution using 8 co-averaged scans through the bags. The samples were then quantified using a silver chloride titration to assess the amount of choline chloride. These reference numbers were then combined with the spectral data in a calibration model using TQ Analyst™ software, our chemometric analysis package.

The algorithm for the analysis was Partial Least Squares and the raw data was pretreated as a first derivative. Other pretreatments in this calibration include a Multiplicative Scatter Correction and a Norris smooth with a segment length of 11 and a gap of 3.

Results and Discussion

Spectral data collected by diffuse reflectance on silage is shown in Figure 1. There is a substantial amount of spectral variance between samples which, in this case, were plastic bags of choline chloride-sprayed silage. Spectral variance is essential to establish a relationship between concentrations taken from reference values and FT-NIR data. Peaks in the Near-IR spectrum that are due to varying concentrations of choline chloride will show differences in spectral absorbance.

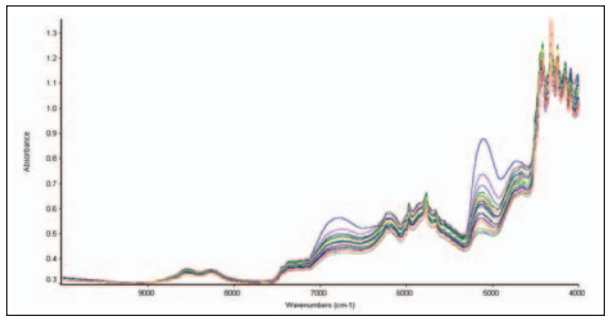

Figure 1: Spectra of choline chloride treated ground corn cob

Raw spectral data, however, is rarely used in FT-NIR spectroscopy. Mathematical processing of raw spectral data is common in Near-IR spectroscopy due to NIR's broad, overlapping peaks. Using a first or second derivative treatment of the raw spectral data enhances the ability of chemometric models to pick out the spectral variation that is relevant to the component of interest. An example of first-derivative processed spectra is shown in Figure 2.

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The calibration shows a strong correlation between the spectral information for choline chloride and the reference data from the Volhardt titration. This is displayed as a calibration curve showing the reference value for choline chloride on the x-axis and the value predicted by the Near-IR calibration on the y-axis. If a calibration were theoretically perfect, then the concentrations predicted using the NIR data would match each reference value exactly. This would give a calibration curve with a perfectly straight line that had a slope of 1.0 and an intercept of 0.0. In reality, no calibration is quite this good, although the closer you can get to these parameters, the better the calibration. The calibration curve predicting choline chloride on silage is shown in Figure 3. The correlation coefficient was excellent with a value of 0.98354. In addition, the calibration residual (Figure 4), data that shows the ± error of any calibration, describes prediction accuracy of 1.6%. The regions that were used for analysis are shown in Figure 5.

Conclusion

In this case study, we determined that ground corn cob could be successfully quantified for choline chloride concentration. The residual error in this calibration was ±1.6%. These analyses were performed in less than 6 seconds while the reference data for each sample was generated over the course of 6-12 hours due to the long extraction and titration times. This represents an enormous time savings over the traditional methods of analysis. In addition, the sample is analyzed non-destructively so any items that have been analyzed can be used for feed, whereas destructive methods like titration destroy the entire sample. Analysis with the Antaris FT-NIR analyzer also allows the user to analyze for more than one component using the same spectrum. For example, if there was interest in determining particle size of the cob granules or looking for the concentration of other exogenous vitamins or chemicals in addition to choline chloride, the analysis time would be the same. Multivariate analysis techniques allow a single spectrum to be used for predicting the concentrations of multiple components in complex systems.

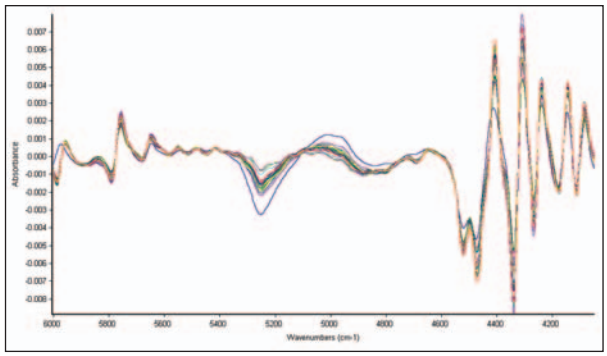

Figure 2: First derivative processed spectra

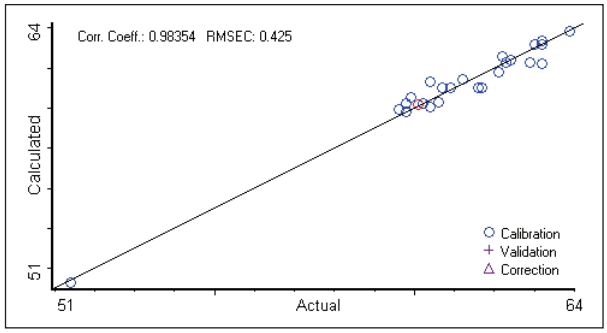

Figure 3: Calibration curve for choline chloride

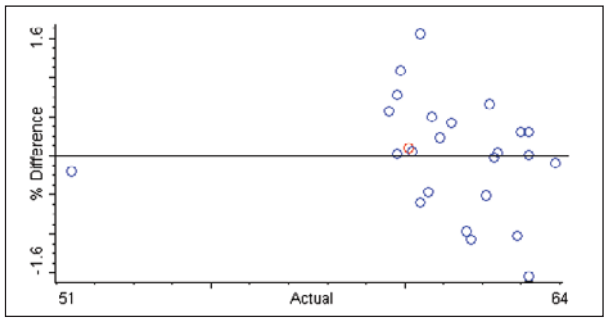

Figure 4: Residual for choline chloride

Index	Measurement Location / Range	CC
1	6134.45 - 6090.10	+
2	5974.39 - 5937.75	+
3	5708.26 - 5660.05	+
4	8531.54 - 8221.05	+

Figure 5: Region selection window in TQ Analyst software

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