



Application Note AN-NIR-131

草近外光分析

Multiparameter determination in a few seconds

Forages are plants or parts of plants eaten by both livestock and wildlife. Forages provide bulk, help with weight maintenance, and combat several issues [1]. Alfalfa forage is high in protein—well-suited to promote muscle mass in beef cattle or to increase the production of dairy cows [2]. Alfalfa is also commonly used for horse feed. These animals require forages with good palatability along with high digestibility, intake potential, and protein levels, thus increasing the demand for alfalfa and other high-quality feeds. Farmers have responded by producing even

higher-quality alfalfa in recent years. Since forage quality depends on chemical, biological, and dynamic properties, both measured and calculated methods must be used. Standard alfalfa assays measure neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ash, moisture, and protein. For alfalfa pellets, the mean particle size is also important. Near-infrared spectroscopy (NIRS) offers **rapid and reliable prediction** of fat, moisture, crude protein, fiber, ash and starch in a few seconds and without sample preparation.

EXPERIMENTAL EQUIPMENT

Samples of alfalfa (including fresh alfalfa, alfalfa pellets, and alfalfa cubes) were analyzed on a Metrohm NIR Analyzer. All measurements were performed in reflection mode using the large cup. The samples were measured in rotation to collect spectral data from several areas. Spectral averaging of signals from several spots helped to

reduce sample inhomogeneity.

The reference values were measured according to ISO norms described at the end of this Application Note. Metrohm software was used for all data acquisition and prediction model development.

RESULT

The obtained NIR spectra (**Figure 2**) were used to create a prediction model for quantification of fat, moisture, crude protein, fiber, ash, and starch in alfalfa. The quality of the prediction models was evaluated using correlation diagrams (**Figures 2–5**) which display a very high

correlation between the NIR prediction and the reference values. The respective figures of merit (FOM) display the expected precision of a prediction during routine analysis of different variations of alfalfa feed (**Tables 1–3**).

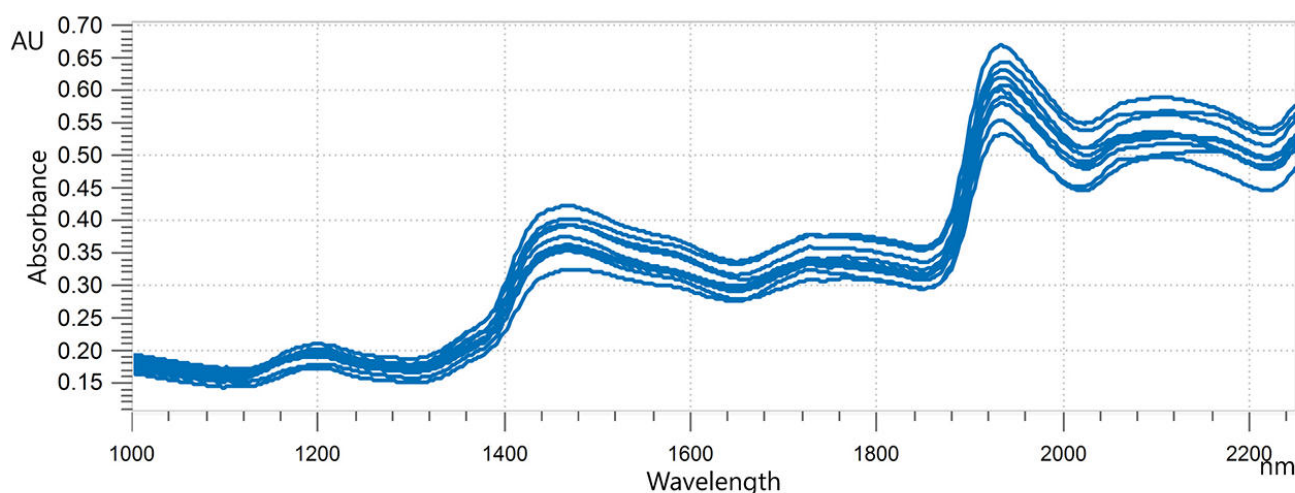


Figure 1. NIR spectra of alfalfa samples analyzed on a Metrohm NIR Analyzer.

Result protein content

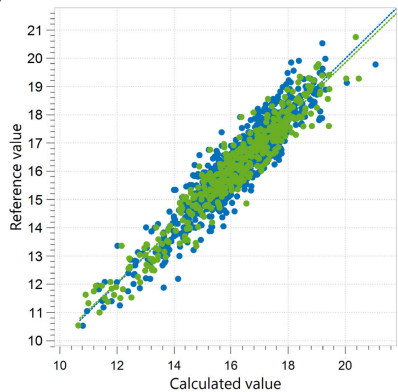


Figure 2. Correlation diagram and the respective figures of merit for the prediction of protein content in alfalfa pellets.

R2	SEC (%)	SECV (%)	SEP (%)
0.922	0.5	0.52	0.52

Result moisture content

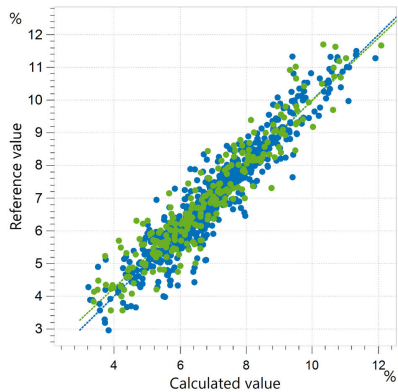


Figure 3. Correlation diagram and the respective figures of merit for the prediction of moisture content in alfalfa pellets.

R2	SEC (%)	SECV (%)	SEP (%)
0.893	0.50	0.50	0.60

Result ADF content

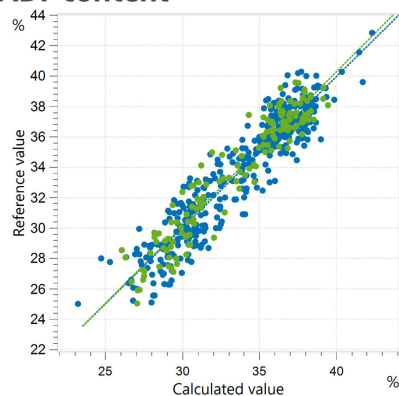


Figure 4. Correlation diagram and the respective figures of merit for the prediction of acid detergent fiber (ADF) content in alfalfa pellets.

R ²	SEC (%)	SECV (%)	SEP (%)
0.906	1.27	1.32	1.22

Result NDF content

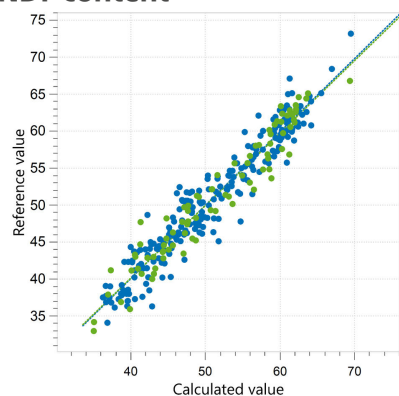


Figure 5. Correlation diagram and the respective figures of merit for the prediction of neutral detergent fiber (NDF) content in alfalfa pellets

R ²	SEC (%)	SECV (%)	SEP (%)
0.935	2.02	2.24	2.16

Figures of merit

The following tables display the figures of merit for the prediction models of alfalfa pellets (Table

1), alfalfa cubes (Table 2), and fresh alfalfa (Table 3).

Table 1. Figures of merit for the prediction of fiber, moisture, crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, and mean particle size (MPS) in alfalfa pellets.

Parameter (Range)	No. Spectra	SEC (%)	SECV (%)	SEP (%)	R2
Fiber (18–35%)	385	1.24	1.35	1.34	0.714
Moisture (16–34%)	976	0.50	0.50	0.60	0.893
Crude protein (10–21%)	1577	0.51	0.52	0.52	0.922
ADF (23–43%)	633	1.27	1.32	1.22	0.906
NDF (33–73%)	336	2.02	2.24	2.16	0.935
Ash (7–17%)	216	0.78	0.86	0.81	0.723
MPS (14–23 mm)	43	0.40	0.47	N/A	0.888

Table 2. Figures of merit for the prediction of ash, fiber, protein, moisture, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in alfalfa cubes.

Parameter (Range)	No. Spectra	SEC (%)	SECV (%)	SEP (%)	R2
Ash (8–14%)	72/23	0.33	0.36	0.30	0.887
Fiber (20–37%)	86/27	1.38	1.63	1.48	0.758
Protein (10–21%)	101/34	0.58	0.63	0.65	0.857
Moisture (10–20%)	87/28	0.23	0.30	0.29	0.974
NDF (34–56%)	96/22	1.73	2.11	1.44	0.918
ADF (25–43%)	102/35	1.38	1.65	1.44	0.837

Table 3. Figures of merit for the prediction of fiber, moisture, and crude protein in fresh alfalfa.

Parameter (Range)	No. Spectra	SEC (%)	SECV (%)	SEP (%)	R2
Fiber (18–35%)	385	1.24	1.35	1.34	0.714
Moisture (16–34%)	976	0.50	0.50	0.60	0.893
Crude protein (10–21%)	1577	0.51	0.52	0.52	0.922

CONCLUSION

This Application Note demonstrates the feasibility to determine multiple key parameters of alfalfa forage with NIR spectroscopy. Several analytical methods are usually required to

measure key quality parameters for forage (**Table 4**). NIRS forage analysis enables a highly accurate, cost-effective, and fast alternative.

Table 4. Overview of ISO norms used for determining the reference values for the different parameters of the alfalfa samples.

Parameter	Method
Starch	ISO 6493:2000 Animal feeding stuffs — Determination of starch content — Polarimetric method
Crude ash	ISO 5984:2022 Animal feeding stuffs — Determination of crude ash
Crude fiber	ISO 6865:2000 Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration
Crude protein	ISO 5983-1:2005 Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 1: Kjeldahl method
Moisture	ISO 6496:1999 Animal Feeding Stuffs — Determination of moisture and other volatile matter content
Fat	ISO 6492:1999 Animal feeding stuffs — Determination of fat content

REFERENCES

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2. Douliere Hay France. *Alfalfa Hay - Lucerne hay for beef and dairy cow, sheep, goat, broadmare and chicken*.
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