



## Prediction of the Chemical Composition and Fermentation Parameters of Fresh Coarse Italian Ryegrass Haylage Using Near Infrared Spectroscopy

Hyung Soo Park

*National Institute of Animal Science, South Korea*

Sang Hoon Lee

*National Institute of Animal Science, South Korea*

Ki Choon Choi

*National Institute of Animal Science, South Korea*

Ji Hye Kim

*National Institute of Animal Science, South Korea*

Jong Geun Kim

*Seoul National University, South Korea*

*See next page for additional authors*

Follow this and additional works at: <https://uknowledge.uky.edu/igc>



Part of the [Plant Sciences Commons](#), and the [Soil Science Commons](#)

This document is available at <https://uknowledge.uky.edu/igc/23/2-1-2/10>

The 23rd International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

---

**Presenter Information**

Hyung Soo Park, Sang Hoon Lee, Ki Choon Choi, Ji Hye Kim, Jong Geun Kim, and Gi Jun Choi

## **Prediction of the chemical composition and fermentation parameters of fresh coarse Italian Ryegrass haylage using near infrared spectroscopy**

**Hyung Soo Park<sup>1\*</sup>, Sang Hoon Lee<sup>1</sup>, Ki Choon Choi<sup>1</sup>, Ji Hye Kim<sup>1</sup>, Jong Geun Kim<sup>2</sup> and Gi Jun Choi<sup>1</sup>**

<sup>1</sup>National Institute of Animal Science, RDA, Cheonan, South Korea

<sup>2</sup>Institute of Green Bio Science & Technology, Seoul National University, Pyeongchang, South Korea

Corresponding author e-mail: [anpark69@korea.kr](mailto:anpark69@korea.kr)

**Keywords:** Calibration model, Chemical composition, Fermentation parameters, Italian ryegrass haylage, Near infrared spectroscopy

### **Introduction**

Italian ryegrass (*Lolium multiflorum*) is an annual forage grass species which is widely cultivated in South Korea. It grows extensively in the southern part of the country and is an important component of winter forage for livestock. Each year, in Korea, over one million hectares of Italian ryegrass is converted to round bale haylage. Quality control is an important field in forage utilization research and marketing, and involves the estimation of forage nutrient content. Wet chemistry is the traditional method used to analyze the nutrient content of forage. However, this technique is often destructive, expensive, and time consuming, and it is not suitable for real-time feedstuff analysis. Near infrared spectroscopy (NIRS), on the other hand, is an alternative technique that has several major advantages over traditional methods. The analysis of silage nutrient content, using NIR, conventionally includes the drying and milling of samples. However, these processes can lead to reduction of volatile acids, which are important components of silage. NIRS can be affected by spectral regions, drying and grinding methods, particle size, packing density and the temperature of samples (Reeves and Blosser, 1991). In order to obtain accurate NIRS results, sample preparation, and the measurement conditions of the calibration set and predicted samples, need to match. The objectives of this study were to (1) assess the usefulness of NIRS in determining the nutritional composition and fermentative parameters of fresh coarse samples of Italian ryegrass haylage, (2) assess the predictive value of various NIRS calibration models, and (3) explore cost-effective and time saving methods for forage quality estimation, in field populations.

### **Materials and Methods**

**Sample and NIR spectra collection:** Whole crop Italian ryegrass haylage samples (n = 216) were collected from cattle farms and total mixed ration companies in Korea, between 2011 and 2014. Samples were stored at -20°C, immediately after collection. Prior to the NIRS scanning of fresh samples, haylages were thawed overnight at 4°C, and cut into 3 to 5 cm pieces, in order to facilitate packing into sample cells. Reflectance spectra were obtained using spectra star 2500 (Unity Scientific, Brookfield, CT) that can scan a wavelength range of 680 to 2500 nm; coarse fresh haylage samples (particle size 3 to 5 cm) packed in a circular rotating quartz sample cup of 150 mm diameter for spectral analyses. Data were recorded at 1 nm intervals, as log 1/R (where R is reflectance at the given wavelength), yielding 1820 data-points per sample analyzed.

**Reference analysis:** All reference analyses, except those of fermentation parameters, were performed on dried samples; a subsample of each haylage was dried at 68°C and milled in order to prepare the sample for the determination of crude ash (CA), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content, according to the methods of AOAC (1990). Crude protein (CP) was estimated from total nitrogen, determined by the Dumas combustion method using a Rapid N III (Elementar GmbH, Hanau, Germany). Aqueous extracts of the haylage samples were used to quantify volatile fatty acids, as described by Porter (1992).

**NIR calibration:** A spectral correction algorithm—standard normal variate (SNV) and detrend (SNV&D)—was used to decrease the effects of light scattering, due to particle size, on spectral readings. Mathematical transformations described by four digits (e.g., 1, 4, 4, 1) were tested. Calibration equations were obtained from a modified partial least squares (MPLS) regression. In order to select the optimal number of terms in the equation, and avoid over-fitting, cross-validation was conducted by determining the standard error of cross-validation (SECV) and the proportion of observed variance ( $R^2_{cv}$ ). The best spectral mathematical treatments were selected from a total of 20 calibrations, developed for each

chemical parameter, based on their  $R^2_{cv}$ , SECV, and RPD values. Spectral data processing and statistical analyses were performed using the U-Cal (V.2.04) software package.

## Results and Discussion

**Reference data:** The haylage samples varied widely in their chemical composition and fermentation parameters (Table 1). Variation in the values of all fermentation parameters, except for lactic and total acids, was small. The haylage samples contained non-detectable (or nearly non-detectable) propionic and butyric acids that were not found in good quality silage.

**Table 1:** Chemical composition and fermentation parameters of fresh coarse Italian ryegrass haylage.

Constituent	n	Mean	Min	Max	S.D.
<b>Chemical composition</b>					
Dry matter (%)	216	51.76	20.60	88.57	14.23
Acid detergent fiber (% DM)	216	39.16	33.12	47.40	2.67
Neutral detergent fiber (% DM)	216	61.41	52.37	69.95	3.57
Crude protein (% DM)	216	10.07	4.66	19.44	2.99
Crude ash (% DM)	171	8.48	4.62	17.17	2.04
<b>Fermentation parameters</b>					
pH (1:5)	216	4.99	3.81	6.72	0.65
Acetic acid (% DM)	216	0.38	0.01	2.21	0.42
Propionic acid (% DM)	216	0.68	0.00	3.21	0.58
Butyric acid (% DM)	216	0.69	0.00	3.82	0.94
Lactic acid (% DM)	216	3.96	0.04	18.85	2.83
Total acid (% DM)	216	4.66	0.07	20.97	3.21

**Table 2.** Mathematical treatments and statistical indicators of the calibrations developed for the prediction of fermentation parameters.

Constituents	Math treatment	N	Mean	SD1	$R^2$	SEC2	$R^2_{cv}$	SECV	RPD
pH	1,4,4	195	4.99	0.62	0.84	0.25	0.70	0.28	2.21
	2,8,8	197	4.98	0.60	0.86	0.23	0.68	0.28	2.14
	1,8,8	194	4.98	0.63	0.86	0.24	0.78	0.22	2.86
	2,16,16	190	4.98	0.59	0.86	0.23	0.77	0.26	2.27
Acetic acid (% DM)	1,4,4	130	0.23	0.38	0.76	0.14	0.29	0.16	2.38
	2,8,8	131	0.22	0.38	0.75	0.12	0.34	0.19	2.00
	1,8,8	134	0.23	0.39	0.73	0.15	0.31	0.17	2.29
	2,16,16	128	0.22	0.40	0.74	0.13	0.35	0.32	1.25
Propionic acid (% DM)	1,4,4	112	0.53	0.54	0.60	0.32	0.35	0.34	1.59
	2,8,8	108	0.50	0.56	0.61	0.32	0.34	0.33	1.70
	1,8,8	113	0.53	0.54	0.60	0.33	0.31	0.34	1.59
	2,16,16	107	0.51	0.50	0.50	0.32	0.28	0.33	1.52
Butyric acid (% DM)	1,4,4	57	0.30	0.59	0.90	0.11	0.55	0.26	2.26
	2,8,8	63	0.32	0.52	0.66	0.33	0.41	0.36	1.44
	1,8,8	53	0.24	0.59	0.66	0.14	0.62	0.24	2.46
	2,16,16	73	0.52	0.58	0.85	0.25	0.41	0.55	1.05
Lactic acid (% DM)	1,4,4	160	3.56	4.63	0.74	1.25	0.66	1.36	3.40
	2,8,8	164	3.47	4.83	0.70	1.31	0.65	1.66	2.91
	1,8,8	159	3.58	4.64	0.77	1.19	0.73	1.30	3.57
	2,16,16	165	3.52	4.88	0.71	1.32	0.63	1.41	3.46
Total acid (% DM)	1,4,4	170	4.26	4.91	0.72	1.43	0.67	1.54	3.19
	2,8,8	173	4.18	4.81	0.65	1.57	0.58	1.66	2.90
	1,8,8	166	4.27	4.92	0.76	1.35	0.72	1.46	3.37
	2,16,16	176	4.16	4.92	0.71	1.47	0.66	1.58	3.11

<sup>1</sup>Standard Deviation, <sup>2</sup>Standard Error of Calibration, <sup>3</sup>Standard Error of Cross Validation, <sup>4</sup>Ratio of Performance Deviation (SD/SECV)

**NIRS calibration of chemical components of haylage:** A comparison of mathematical treatments for raw spectra showed that second-order derivatives consistently yielded the best results. The best mathematical treatment for DM, ADF, and NDF was 2, 16, 16, whereas the best mathematical treatment for CP and CA was 2, 8, 8. A comparison of  $R^2_{cv}$  values between models showed that all the selected calibrations were robust except for the total crude ash calibration ( $R^2_{cv} = 0.47$ ). The low predictive value of the CA calibration was expected because crude ash consists mainly of inorganic and mineral substances that do not have absorption in the near infrared region. Nevertheless, these compounds are associated

with organic structures, through oxides, chelates, and other compounds, which can allow their indirect estimation using NIRS (Shenk and Westerhaus, 1994). RPD values for our predictive calibration models ranged from 1.88 for CA to 6.84 for DM content, indicating that whereas the model used to predict total ash was not robust (supported by a low  $R^2_{cv}$  of 0.49), the predictive model for DM was. Previous studies, on coarsely ground hay (Reddersen *et al.*, 2014) and undried ground pasture silage (Park *et al.*, 1998) report robust predictions of total ash using calibration models with RPD values of 4.8 and 3.07, respectively.

**NIRS calibration of fermentation parameters of haylage:** The calibration models developed in our study were robust for all fermentation parameters except acetic, propionic, and butyric acids; the models for these acids had RPD values between 1.70 and 2.46 (Table 2). Further, first order derivative treatments provided better results than second order derivatives. The best mathematical treatment for pH, lactic acid, and total acid was found to be as 1, 8, 8. Although our calibration result for butyric acid was accurate ( $R^2 = 0.90$ ), the cross-validation result for butyric acid had low accuracy (mean of 0.68%, and range of 0.00 to 3.82% for fresh haylage). This is likely because either the range of butyric acid content in the population of haylages was too narrow for robust calibration or the butyric acid concentrations in our samples were too low to accurately represent the population of haylages from which our samples were drawn.

## Conclusion

Our study showed that the analysis of fresh coarse Italian ryegrass haylages, using NIRS, can provide accurate predictions for a wide range of chemical components, and fermentation parameters. These predictions may be applied to forage quality assessment in ruminant feeding systems. Furthermore, in comparison with scatter correction, the mathematical treatment approach for the analysis of spectral data could produce better predictions. Overall, our study findings highlight the considerable potential of NIRS for haylage analysis in routine adversary systems.

## References

- Association of Official Analytical Chemists (AOAC). 1990. *Official methods of analysis*. 15<sup>th</sup> Edition. Washington, DC.
- Park, R. S., R. E. Agnew, F. J. Gordon, and R. W. J. Steen. 1998. The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. *Anim. Feed Sci. Technol.* 72:155-167.
- Porter, M. G. 1992. Comparison of five methods for the determination of lactic acid in silage. In: Br. Grass. Soc., 3<sup>rd</sup> Res. Conf. pp.123-124.
- Reeves, J. B. and T. H. Blosser. 1991. Near infrared spectroscopic analysis of undried silages as influenced by sample grind, presentation method, and spectral region. *J. Dairy Sci.* 74(3): 882-895.
- Reddersen, B., T. Fricke, and W. Michael. 2014. Effects of sample preparation and measurement standardization on the NIRS calibration quality of nitrogen, ash and NDFom content in extensive experimental grassland biomass. *Anim. Feed Sci. Tech.* 183:77-85.
- Shenk, J. S. and M. O. Westerhaus. 1994. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In Forage Quality Evaluation and Utilization. p 406. *Am. Soc. Agron.*, Madison, WI.

## Acknowledgement

This work was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ009396012015) of the Rural Development Administration, Republic of Korea.