DEVELOPMENT OF A NIRS CALIBRATION EQUATION FOR IN SITU ANALYSIS OF GREEN PASTURES

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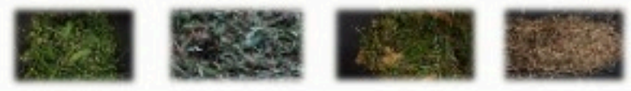
INTRODUCTION

The experimental phase was developed between May 2018 and November 2021, in six suckler cow farms representing different ecosystems in and around Castilla y León. In each farm, a pasture sample was taken every 45 days for chemical and NIRS analysis. The grass sampling was carried out using two different techniques: random sampling following the classical methodology and targeted sampling using GPS collars, placed on animals from three of the herds, in order to obtain their most frequent locations, as sampling points.

OBJECTIVES

To develop a rapid method of analysing the quality of grass consumed by grazing beef cattle in order to optimise the administration of concentrate feed according to the characteristics of the grass at each time of the year, using NIRS equipment in the laboratory and in situ, using portable NIRS equipment.



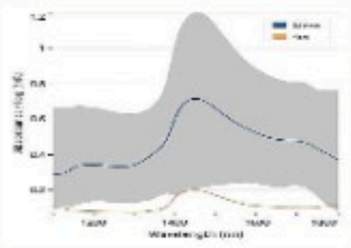
MATERIALS AND METHODS

OPTICAL DATA

Location by GPS Collars (Digitanimal)

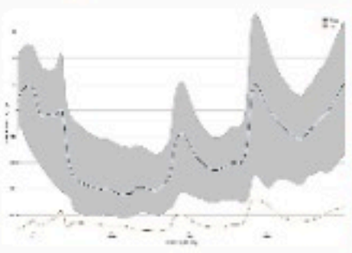
X-NIR (DINAMICA GENERALE)

NIR DS2500 (FOSS)



Sensor: (1100-1800,10) nm

- *Spectra on fresh substance*
- *Four sub-samples were taken and scanned separately.*
- *Sample based average*

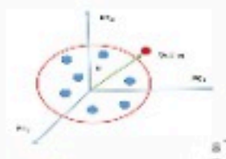


Sensor: (400-2500,0.5) nm

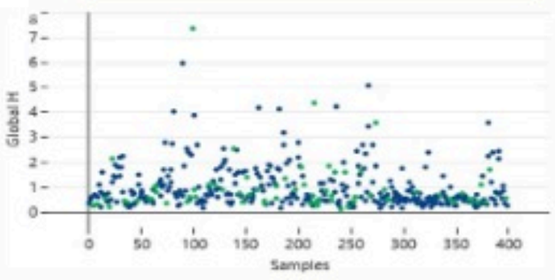
DATA ANALYSES

Qualitative analysis

- *Application of different pretreatments.*
- *Principal Component Analysis (PCA), create score from spectra file.*
- *Distance calculation: Detection of spectrally different individuals. Select samples from spectra file .*



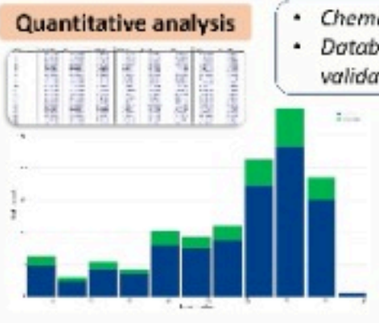
The score plot of the three PCs explained about 95% of the variance



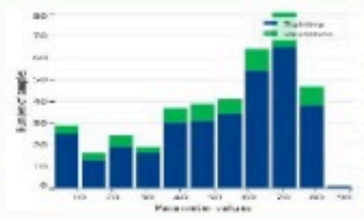
Determination of the Reference Analysis

Quantitative analysis

- *Chemical analysis of the selected samples.*
- *Database creation, calibration and validation group.*



Histogram of moisture values in percent for X-NIR



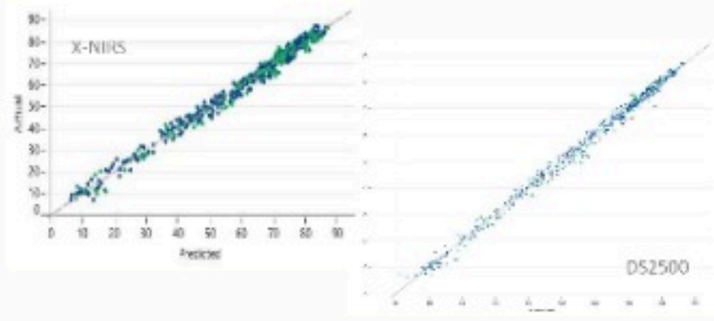
Histogram of moisture values in percent for DS2500

Component	Reference Analysis
Moisture	Moisture in two phases: Gravimetric method by previous and soft drying (65ºC) Method in stove at 105ºC
Crude Protein	Kjeldahl method
Crude Fiber	Weende method
FND	Van Soest method
FAD	Van Soest method
Lignin	Method of determination of fat-free organic substances insoluble in detergent acid and sulphuric acid solution (72%)
Crude Ashes	Incineration of a sample of muffle furnace at 550ºC

DEVELOPMENT AND EVALUATION OF QUANTITATIVE CHEMOMETRIC MODELS

ISI Nova and Mosaic software (from FOSS) were used to collect the spectral data. For the development of the equations, different mathematical treatments and cross-validation were tested with the WINISI IV software (Infrasoft International), regression models were generated using MPLS (Modified Partial Least Squares), combining different mathematical treatments and light scattering correction using SNV (Standard Normal Variate) and Detrend mathematical techniques.

The statistics used for the selection of the best calibration equations were SEC, SEVC, R^2, r^2,SEP, RSQ, RPD and RER.



Example: Relationship between the percentage of moisture predicted by NIR DS2500 and XNIR reference analysis

PREDICTION AND RESULTS

- Routine in the instrument software.
- Following the equation in routine.
- During routine work we will get:
  - Predicted value of the analysis.
  - Predicted value of "GH"
  - Predicted value of "NH"
  - T-statistic

- *GH<3*
- *GH >3<4 technical decision*
- *GH>4 wet analysis*

More robust equation Recalibration

GH: samples distant from center of the calibration group. NH: samples with distant neighborhood. T: differences between the residual and the estimated standard error.

RESULTS AND DISCUSSION

Range and statistics of the calibration and validation group obtained by MPLS regression for the estimation of each constituent in grass samples on fresh subtance

DS2500

Constituent (on fresh substance)	Calibration Statistics										Validation Statistics													
	N	Average	SD	Min	Max	SEC	R^2	SEVC	r^2	r^2Max	N	Average	SD	Min	Max	Slope	Bias	SEP©	RSQ	Predicted Average	Actual Average	Predicted SD	Actual SD	RPD	RER
Humidity	326	55,73	21,90	6,60	86,90	1,80	0,99	**2,25**	**0,99**	0,99	72	56,19	21,69	7,40	84,70	1,01	-0,01	**2,25**	**0,99**	56,20	56,19	21,44	21,69	9,65	34,39
Crude Protein	278	4,43	1,65	1,23	10,09	0,37	0,95	**0,46**	**0,92**	0,90	63	4,35	1,47	1,66	7,67	1,03	0,04	**0,45**	**0,91**	4,43	4,43	1,61	1,62	3,29	13,45
Crude Fiber	269	16,32	10,32	2,65	43,93	1,45	0,98	**1,66**	**0,97**	0,98	60	15,58	10,07	3,17	39,60	1,00	0,15	**1,46**	**0,98**	15,43	15,58	9,96	10,07	6,91	25,00
FND	258	30,90	18,37	5,90	80,46	1,37	0,99	**2,33**	**0,98**	0,99	63	30,63	18,74	8,22	75,15	0,98	0,03	**2,15**	**0,99**	30,59	30,63	19,00	18,74	8,72	31,16
FAD	241	19,89	11,87	3,83	51,23	1,34	0,99	**1,74**	**0,98**	0,97	66	18,32	12,12	4,13	47,87	1,01	-0,04	**1,72**	**0,98**	18,35	18,32	11,84	12,12	7,05	25,42
Lignin	229	4,29	2,36	0,72	11,02	0,72	0,91	**0,96**	**0,84**	0,67	57	4,14	2,18	0,95	9,14	0,92	0,03	**0,90**	**0,83**	4,11	4,14	2,16	2,18	2,43	9,12
Crude Ashes	206	3,54	1,58	1,09	9,01	0,43	0,93	**0,63**	**0,84**	0,91	44	3,23	1,26	1,38	6,12	0,86	-0,10	**0,52**	**0,85**	3,33	3,23	1,35	1,26	2,42	9,10

X-NIR PORTABLE

Constituent (on fresh substance)	Calibration Statistics										Validation Statistics													
	N	Average	SD	Min	Max	SEC	R^2	SEVC	r^2	r^2Max	N	Average	SD	Min	Max	Slope	Bias	SEP©	RSQ	Predicted Average	Actual Average	Predicted SD	Actual SD	RPD	RER
Humidity	380	60,70	20,29	7,40	86,90	2,18	0,99	**2,56**	**0,98**	0,99	96	58,91	21,33	7,60	86,70	1,00	-0,40	**2,81**	**0,98**	59,31	58,91	21,13	21,33	7,59	28,13
Crude Protein	311	4,32	1,57	1,23	10,09	0,44	0,92	**0,60**	**0,86**	0,90	73	4,35	1,49	1,38	8,11	1,01	0,07	**0,49**	**0,89**	4,29	4,35	1,39	1,49	3,06	13,82
Crude Fiber	263	15,40	9,87	2,65	43,93	1,55	0,98	**1,92**	**0,96**	0,97	67	15,77	10,51	3,82	41,11	1,06	0,30	**1,94**	**0,97**	15,48	15,77	9,74	10,51	5,41	19,20
FND	244	29,03	17,81	5,90	78,52	2,04	0,99	**2,49**	**0,98**	0,99	49	26,65	17,22	7,49	71,57	1,03	-0,23	**2,18**	**0,98**	29,69	29,91	17,06	17,93	7,89	29,37
FAD	357	18,24	11,65	3,10	51,23	1,94	0,97	**2,20**	**0,96**	0,96	67	19,25	12,04	4,60	47,87	1,03	0,04	**2,10**	**0,97**	19,20	19,24	11,52	12,04	5,75	20,65
Lignin	327	3,93	2,59	0,49	15,68	1,05	0,84	**1,26**	**0,76**	0,71	83	3,98	2,48	0,49	11,02	0,90	-0,01	**1,17**	**0,79**	3,99	3,98	2,44	2,48	2,12	9,01

NIR: Near Infrared Spectroscopy N: sample number; Min: Minimum; Max: Maximum; R^2(RSQ): coefficient of determination; SEC(SEP): calibration/validation standard error; SECV: cross-validation standard error; r^2: determination coefficient, RPD: Ratio between the standard deviation of the reference data for the validation group and the SEP. RER: Relationship between the range in the reference data for the validation group and the SEP.r^2max:theoretical maximum

The evaluation of the accuracy and reliability of the obtained equations would need further discussion, but in general terms the results obtained indicate calibrations with r^2 values higher than 0,9 which are perfect for quantitative predictions. The lower r^2 values remain high (0,8-0,9), except for lignin (0,79) in the portable X-NIR. These models also presented RPD greater than 3, a good indicator of the predictive capacity and robustness of the model, except for lignin and crude ash in the DS2500 NIRS (2,43 and 2,42 respectively) and for lignin in the Portable X-NIR (2,12). And although there is much work to be done on these types of instruments , the portable NIRS adds to the global control together with the laboratory NIRS equipment.

DISCUSSION

Therefore, the calibration models developed on both equipments give reliable and fast predictions in the laboratory and on the farm, so they can be used to evaluate the nutritional quality of a pasture area and adapt the complementary feeding in a short period of time (hours).

As a result of this work, a nutritional advice tool has been developed for extensive cattle farmers.

BIBLIOGRAPHY, ACKNOWLEDGMENTS AND COLLABORATIONS

(1) CENTER FOR AGRI-FOOD RESEARCH AND TECHNOLOGY OF ARAGON.

Albina Sanz, Isabel Casasús, Margalida Joy, Sandra Lobón, Mireia Blanco

❖ **Participating farmers.**

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- *Mark y Workman, 1991. r^2theoretical maximum.*