

NIRS potential use for the determination of natural resources quality from *dehesa* (acorn and grass) in Montanera system for Iberian pigs

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SUMMARY

ADDITIONAL KEYWORDS

Free-range systems.

Nutritive quality.

Feeding.

Predictive technologies.

NIRS technology has been used as an alternative to conventional methods to determinate the content of nutrients of acorns and grass from *dehesa* ecosystem. Dry matter (DM), crude fat (CF), crude protein (CP), starch, total phenolic compounds (TP), α -tocopherol, γ -tocopherol, fatty acids, neutral detergent fiber (NDF), total antioxidant activity (TAA) and total energy (TE) were determined by conventional methods for later development of NIRS predictive equations. The NIR spectrum of each sample was collected and for all studied parameters, a predictive model was obtained and external validated. Good prediction equations were obtained for moisture, crude fat, crude protein, total energy and γ -tocopherol in acorns samples, with high coefficients of correlation (1-VR) and low standard error of prediction (SEP) (1-VR=0.81, SEP=2.62; 1-VR=0.92, SEP=0.54; 1-VR=0.86, SEP=0.47; 1-VR=0.84, SEP=0.2; 1-VR=0.88, SEP=5.4, respectively) and crude protein, NDF, α -tocopherol and linolenic acid content in grass samples (1-VR=0.9, SEP=1.99; 1-VR=0.87, SEP=4.13; 1-VR=0.76, SEP=10.9; 1-VR=0.82, SEP=0.6, respectively). Therefore, these prediction models could be used to determinate the nutritional composition of *Montanera* natural resources.

Uso potencial de la tecnología NIRS en la determinación de la calidad de los recursos naturales (bellotas y pastos) de *dehesa* destinados a la alimentación del cerdo Ibérico

RESUMEN

PALABRAS CLAVE ADICIONALES

Montanera.

Calidad nutritiva.

Alimentación.

Tecnologías predictivas.

INFORMATION

Cronología del artículo.

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La tecnología NIRS se utilizó como alternativa a los métodos convencionales para determinar el contenido de nutrientes en bellotas y pastos de la *dehesa*. Materia seca (MS), contenido en grasa (G), proteína total (TP), almidón, compuestos fenólicos totales (FT), α -tocoferol, γ -tocoferol, ácidos grasos, fibra neutro detergente (FND), actividad antioxidante total (AAT) y energía total (ET) fueron determinadas por métodos convencionales para el posterior desarrollo de las ecuaciones de predicción del NIRS. Se han realizado todos los espectros NIR de cada una de las muestras para todos los parámetros estudiados obteniendo un modelo predictivo y una validación externa. Se han obtenido buenas ecuaciones de predicción para la humedad, contenido en grasa, proteína total, energía total y γ -tocoferol en muestras de bellotas obteniendo un alto coeficiente de correlación (1-VR) y un bajo error estándar de predicción (SEP) (1-VR=0.81, SEP=2.62; 1-VR=0.92, SEP=0.54; 1-VR=0.86, SEP=0.47; 1-VR=0.84, SEP=0.2; 1-VR=0.88, SEP=5.4, respectivamente), así como la proteína total, NDF, α -tocoferol y contenido de ácido linolénico en muestras de pasto (1-VR=0.9, SEP=1.99; 1-VR=0.87, SEP=4.13; 1-VR=0.76, SEP=10.9; 1-VR=0.82, SEP=0.6, respectivamente). Por lo tanto, estos modelos de predicción podrían ser utilizados para determinar la composición nutricional de los recursos naturales de la *Montanera*.

INTRODUCTION

In Southwest of Iberian Peninsula, the Iberian pigs are traditionally fattened during the final phase with the intake of acorn and grass from *dehesa-mon-tado* ecosystem (typical silvo-pastoral-system). Several authors have demonstrated the variability in the nutritional composition of acorns and grass between different years, date of sampling in the same season

or geographical areas and their effect on meat quality from Iberian pigs (Tejerina et al. 2010, 2011). Therefore, it is necessary to perform a large number of chemical analysis for knowing the quality in each one. The Near Infrared Reflectance Spectroscopy (NIRS) (Prevolnik et al. 2005) is commonly used to predict the parameters of chemical composition as a rapid and effective technique and it could be used to determinate the quality of these natural resources at real time.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

For the study, 8 plots of dehesa ecosystem distributed in the southwest of the Iberian Peninsula (Extremadura-Alentejo), with *Quercus rotundifolia*, *Quercus suber* and pasture grasses were selected. Sampling of acorns ($n=80$) and grass ($n=80$) took place during the period of use of Montanera in the plots selected two consecutive years. Acorn samples were taken in trees selected randomly (a minimum of 10 trees per sample) and both, acorns and grass, were collected directly from the soil. They were taken at different random points along a path that will encompass a complete diagonal of the plot.

Every sample was freeze to -20°C and, for each one, a subsample (50 kernel in the case of acorns, and 200g for grass) was freeze drying until their analysis. Dry matter (DM), crude fat, crude protein, starch, total phenolic (TP), total antioxidant activity (TAA), α -tocopherol, γ -tocopherol, total energy (TE), neutral detergent fibre (NDF) and fatty acid profile were analysed by conventional methods and by NIRS predictive equations.

CHEMICAL DETERMINATIONS

The dry matter (g DM/100g), neutral detergent fiber (g NDF/100g DM), crude protein (g CP/100 g DM), total energy (MJ/kg DM) were assayed following the official AOAC method (AOAC, 2003). The crude fat (g CF/100g DM) was assayed following the method described by Folch et al. (1957). The α - and γ -tocopherol contents in acorns and grass samples were assayed by the method proposed by Liu et al. (1996) with some modifications proposed by Cayuela et al. (2003). Results were expressed as mg of α - and γ -tocopherol/kg DM. Total phenolic compounds were determined colorimetrically following the method described by Singleton and Rossi (1965). Total phenolic compounds

were quantified using a gallic acid standard curve ranging 2.5–50 μ g/mL by Waterman et al. (1994). Results are expressed as g of gallic acid (GAE)/kg DM. The total antioxidant activity (TAA) was determined according to Cano et al. (1998). TAA was determined as the sum of lipophilic antioxidant activity (LAA) and hydrophilic antioxidant activity (HAA). The difference between the initial and final absorbance gave the resulting LAA or HAA by comparison with the Trolox standard. Results are expressed as g Trolox/kg DM. The starch was determinate using assay Kit based in AOAC Official Method 996.11. Results are expressed as g of starch/100g DM.

NIRS ANALYSIS

Spectral data were taken with turn-table probe coupled to LabSpec 2500 ASD Inc.® instrument by continuous measures from 1000 to 2500 nm, which were processed with the software IndicoPro®.

The predictive equations were developed by software Unscrambler by CAMO® by multivariate regression method by partial least square (PLS) and the evaluation of the effects of different spectral pre-treatments (1st and 2nd derivate, EMSC (extended multiplicative scatter correction), SNV (standard normal variate) and their combinations).

RESULTS AND DISCUSSION

NIRS PREDICTION IN ACORN SAMPLES

Table I shows the statistics of NIR equations to predict different parameters of acorn composition according to the mathematical treatment in which better 1-VR and SEP values were obtained. The results showed a good predictive ability in dry mater, crude fat, crude protein, total energy and γ -tocopherol for acorns. For dry matter, crude fat, crude protein, total energy and γ -tocopherol all the equations had r^2 (1-VR)

Table I. NIRS predictive equations (calibration and validation) in acorn samples of dry matter, crude fat, crude protein, starch, total energy, γ -tocopherol, total phenolic, total antioxidant activity and oleic acid (Ecuaciones de predicción NIRS (calibración y validación) del contenido materia seca, grasa, proteína, almidón, energía total, γ -tocoferol, actividad antioxidante total y ácido oleico en muestras de bellotas)

ACORNS	PRE-TREATMENT	CALIBRATION					VALIDATION			
		N	MEAN	SD	p	R ²	SEC	1-VR	SEP	RPD
Dry matter	1,11,15+SNV	66	54,36	5,61	4	0,89	1,96	0,81	2,62	2,14
Crude fat	1,11,15	74	6,55	1,83	4	0,94	0,48	0,92	0,54	3,37
Crude protein	2,10,15+SNV	74	5,74	1,14	5	0,91	0,36	0,86	0,47	2,44
Starch	2,10,15+SNV	76	49,06	3,78	7	0,83	1,69	0,67	2,4	1,57
Total energy	1,11,15+EMSC	80	18,98	0,48	3	0,88	0,18	0,84	0,2	2,4
γ -Tocopherol	1,11,15	65	36,84	15,1	7	0,92	4,33	0,88	5,4	2,78
Total phenolic	Absorbance	71	14,37	4,82	7	0,81	2,3	0,71	2,89	1,67
AAT	1,11,15	72	48,39	17,1	6	0,85	7,27	0,69	10,64	1,6
Oleic acid	2,10,15+SNV	59	61,49	2,9	8	0,89	1,05	0,71	1,77	1,69

EMSC: extended multiplicative scatter correction; SNV: standard normal variate; (1,10,5) and (2,10,5): the first term is the order of the implemented derivative, the second one refers to the interval of subtraction or GAP and the third one to the length of smoothing segment; N: quantity of samples; p: quantity of equation terms; R²: coefficient of determination in calibration; SEC: standard error of calibration; 1-VR: Coefficient of determination in cross-validation; SEP: standard error of prediction; SD: standard deviation; RPD: Ratio performance deviation.

Table II. NIRS predictive equations (calibration and validation) in grass samples of dry matter, crude fat, crude protein, NDF, α -tocopherol, total phenolic, total antioxidant activity and linolenic acid (Ecuaciones de predicción NIRS (calibración y validación) para el contenido en materia seca, grasa, proteínas, fibra neutro detergente, α -tocoferol, compuestos fenólicos, actividad antioxidante total y ácido linolénico en muestras de pastos).

GRASS	Pre-treatment	CALIBRATION					VALIDATION			
		N	Mean	SD	p	R ²	SEC	1-VR	SEP	RPD
Dry matter	SNV	77	80,38	5,85	3	0,77	3,23	0,74	3,4	1,72
Crude fat	1,11,15+SNV	65	4,66	0,57	1	0,65	0,42	0,63	0,44	1,29
Crude protein	1,11,15+EMSC	80	19,88	6,12	4	0,93	1,74	0,9	1,99	3,07
NDF	1,11,15	79	49,26	10,9	7	0,9	3,63	0,87	4,13	2,63
α -Tocopherol	2,10,15+SNV	76	32,29	20,9	9	0,88	7,57	0,76	10,9	1,92
Total phenolic	1,11,15+EMSC	71	11,90	4,2	7	0,78	2,25	0,64	2,9	1,45
TAA	SNV	68	33,47	10,6	9	0,82	4,98	0,67	6,9	1,54
Linolenic acid	2,10,15	70	38,85	13,5	3	0,86	5,5	0,82	6,2	2,17

EMSC: extended multiplicative scatter correction; SNV: standard normal variate; (1,10,5) and (2,10,5): the first term is the order of the implemented derivative, the second one refers to the interval of subtraction or GAP and the third one to the length of smoothing segment; N: quantity of samples; p: quantity of equation terms; R²: coefficient of determination in calibration; SEC: standard error of calibration; 1-VR: Coefficient of determination in cross-validation; SEP: standard error of prediction; SD: standard deviation; RPD: Ratio performance deviation.

values higher than 0.8, while in the determination of others parameters, they varied between 0.67 and 0.71. These results are similar to those observed by De Pedro et al. (2004) in previous studies on acorn samples in different species.

NIRS PREDICTION IN GRASS SAMPLES

Table II shows the statistics of NIR equations to predict different parameters of composition of grass, according to the mathematical treatment in which better 1-VR and SEP values were obtained. The results showed a good predictive ability in crude protein, neutral detergent fiber, α -tocopherol and linolenic acid for grass. For crude protein, NFD and linolenic acid, all the equations had r^2 (1-VR) values higher than 0.8, while in the determination of crude fat and α -tocopherol oscillated between 0.63 and 0.76.

CONCLUSIONS

NIR technology allows estimating accurately dry matter, crude fat, crude protein, total energy and γ -tocopherol in acorn samples and crude protein, neutral detergent fiber, α -tocopherol and linolenic acid in grass samples. Therefore, these prediction models could be used to determinate the nutritional composition of natural resources at real time, making it easier the livestock food management of Iberian pigs to generate a differentiated quality of the products.

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