APPRAISAL BY NIRS OR BY LABORATORY ANALYSES OF HOUSING EFFECTS IN YOUNG BULLS

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Background

Near Infrared Reflectance Spectroscopy (NIRS) is a physical not destructive method appreciated for rapid chemical analyses, when operating in a quantitative way on raw (Naes et al., 1996) or freeze-dried meat (Masoero et al., 1994), as well as in a more synthetic qualitative approach (Fearn, 1997) and in a global discrimination (Downey, 1996; Masoero, 2000).

Objectives

This paper concerns the capabilities of discrimination of environmental factors which long time operated on the growing animal, by using only the NIR Spectroscopy of anhydrous muscle tissues, as compared to a wide set of conventional laboratory analyses.

Methods

Thirty young bulls of Piemontese breed were divided in two groups of 15 subjects, tie housed or held in boxes (5 m^2/head). The animals were fed hay and concentrate and were slaughtered at an average weight of 560 kg. At 1 h and 24 h *post mortem* pH was measured. Seven days *post mortem longissimus thoracis et lumborum* (9th *T.V.* - 1st *L.V.*) was taken to perform chemical (8 variables), physical (7 variables) and sensorial (7 variables) analyses. The analytical methods were reported by Destefanis et al. (2000).
Samples of raw or cooked muscle were freeze-dried, then finely ground and stored at –20°C till to NIRS analysis. The NIR spectra recorded by a NIRSystem 4500 Foss-electric monochromator device were mathematically treated as 1,4,4,1 and submitted to Modified Partial Least Squares (MPLS) regression to calibrate equations of binary discrimination, fitting 1 (tie) or 2 (free-stall) dummy values. Chemometrics performed by NIRS 2 v. 3.0 (ISI, 1993) standard software was also used to calibrate by MPLS 22 laboratory variables. Furthermore a stepwise regression allowed to ascertain the most important wavelengths as well as the most relevant laboratory variables ($P<0.01$). ANOVA results of laboratory variables was arranged in descending R^2 values in order to discriminate the most important ones.

Results and discussion

NIRS calibration of the experiment design for the variable housing (1 = tie or 2 = free-stall) was successful both on raw muscle and even more on cooked muscle; the calibration R-squares coefficients raised to 0.51 and 0.68 respectively (table 1). When all the 22 conventional laboratory variables were involved simultaneously in multivariate MPLS analysis the reached result was the same achieved by the raw NIRS examination ($R^2 = 0.54$).
This means by first that a significant ontogenetic factor affects the housing methods (Tab. 2), resulting the tie-stalled animals to be characterised by a muscle with lower content of hydroxyproline (-18%) and with a slightly lower water content (-0.5 % relative). In the frame of the laboratory variables the most significant were, in descending R^2 order as univariate: hydroxyproline, protein content, hue, water content, yellowness, with R^2 coefficients statistically significant, but halved than the previous ones. When a stepwise regression at probability level <0.01 was performed to discriminate the two groups (Tab. 1), the most distinctive variables became only water content and hydroxyproline, because, after linear combining, the other variables (protein and colour parameters hue and yellowness) were absorbed.
In cross-validation mode the relationships built by the MPLS method appeared strong in the freeze-dried raw muscle, while in the NIR spectra of cooked freeze-dried muscle and also in the laboratory whole analyses the relationships fallen of about 40%, thus reaching a R^2 near 0.3 values: the low number of samples in this study could be responsible of this instability. When a simple regression on the most significant wavelength was performed in a binary discrimination of groups, the R^2 coefficients were so high as 0.46 (at 2222 nm, a wavelength linked to protein compounds) for the raw and 0.22 (at 1364 nm) for the cooked muscle. These values fallen of only some 0.05, after cross-validation, reaching a value similar to the multivariate model in validation for raw muscle. The bivariate regression on water content and hydroxyproline ($R^2cv=0.41$) appeared more stable than MPLS equation based on 22 variables ($R^2cv = 0.33$) when the internal cross-validation was performed.
In this study NIRS capabilities (Tab. 2) appeared to be quite effective into discriminating muscles with an average difference of about 18% in hydroxyproline content, explained by the NIRS with $R^2c= 0.49$, at 1552 nm, and a very scarce difference amount of 0.5% in the original water, when, it must be outlined, the NIRS inspection was carried on in the dried (not fresh) muscle tissues, raw or cooked. In effect NIRS of dried muscles was linked to hue and yellowness, which were explained by the 2230 nm wavelength ($R^2c = 0.37$ and 0.29 respectively) and not far from the region responsible of the variation in the original water content ($R^2c= 0.49$, at 2326 nm). Nevertheless the differences of spectra reflected in the 2222 nm region must be representative of other undetermined substances, putative of the differentiation of muscles after prolonged exercise in free stalling conditions.
The emerging differences in cooked NIR Spectra were located around 1364 nm. In absence of laboratory analyses of the cooked samples, except for the shear force and cooking losses, a correlogram with the analyses carried on raw samples revealed in that spectral region there were some relationships (not tabulated) with hue (1324 nm, $R^2c= 0.29$), yellowness (1348 and 0.13), lightness (1327 and 0.47) and also with iron (1316 and 0.59).
The concordance of NIRS evaluation of the individuals, within the groups, on the raw and on the cooked tissues appeared to be quite high (Fig. 1) because overlapping was limited to two cases of the thirty samples compared. On the contrary, in spite of the equivalent R^2c, the discrimination by the 22 laboratory variables was wrong in five cases. Nevertheless when the results of classification criteria were elaborated averaging and pooling the three estimates by NIRS of raw, NIRS of cooked and MPLS of 22 variables a perfect individual discrimination of the animals in the two categories was possible in all cases (Fig. 1).
As a short conclusion, NIRS technique of dried muscles can anticipate in a few time and at a least-cost, that true experimental factors and strong effects are present inside the trial, thus further analyses can be worked out with a degree of certain success. On the contrary, if the NIRS binary contrasts of the mean estimated values were negative, the stop of pursuit in the analyses can be reasonably invoked.

Pertinent literature

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Table 1. Calibration and internal cross-validation performances of binary discrimination of tied *vs* free stalled Piemontese young bulls

		Modified PLS		Stepwise regression			
	# PC	R^2c	R^2cv	R^2c	R^2cv	Sel Wavelenghts / Variables	
NIRS of LT_raw	1	0.508	0.470	0.456	0.416	2222 nm	
NIRS of LT_cooked	5	0.676	0.353	0.217	0.166	1364 nm	
22 Laboratory analyses	1	0.543	0.331	0.485	0.408	Water	Hydroxyproline

R^2c= R-square coefficient of determination in calibration mode; R^2cv= R-square coefficient of determination in cross-validation mode; # PC = number of terms in the MPLS equation

Table 2. Result of univariate analysis and of NIRS calibration of the raw muscles for the most discriminant variables

Variable	R^2	Root MSE	Tie-stall Housing	Free-stall housing	Prob	Tie/Free	NIRS R^2c	Wavelength nm
Hydroxyproline	0.288	0.74	4.19	5.10	0.0022	-17.9%	0.49	1552
Protein	0.240	0.57	22.12	21.49	0.0059	2.9%	0.20	1552
Hue	0.218	0.85	19.67	18.81	0.0094	4.6%	0.37	2230
Water	0.187	0.40	75.30	75.66	0.0171	-0.5%	0.49	2326
Yellowness	0.154	0.61	7.70	7.20	0.0321	6.9%	0.29	2230

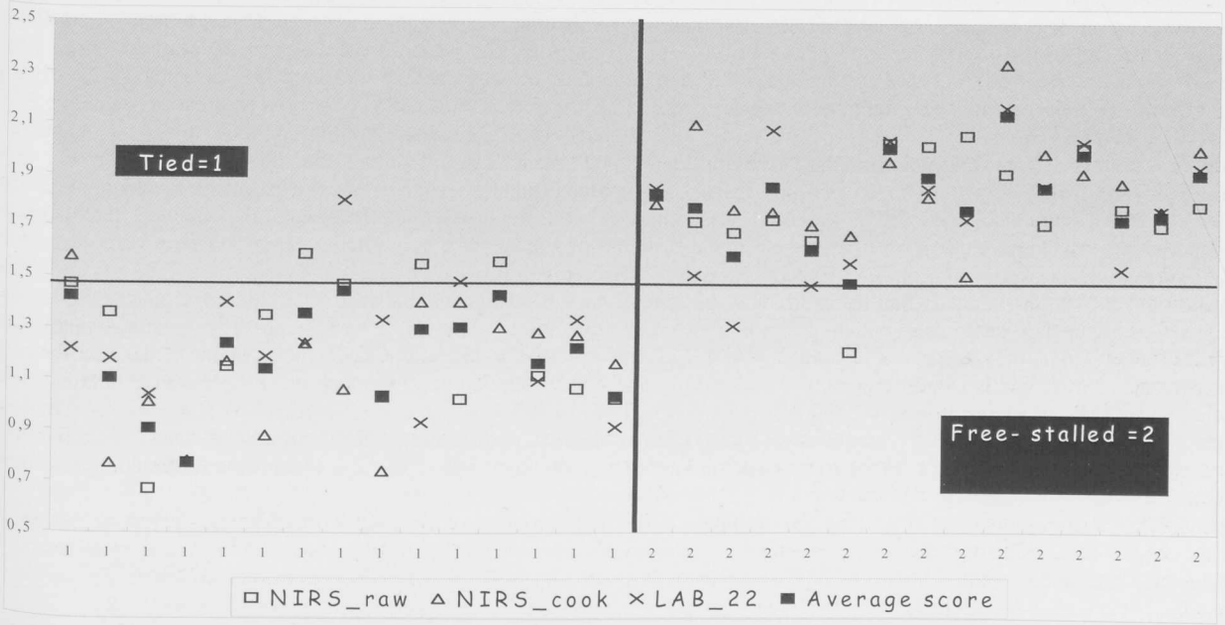

Figure 1. Plot of Modified Partial Least Squares estimated binary function of tied vs free-stalled young bulls discriminated by NIRS of freeze-dried raw or cooked *longissimus thoracis et lumborum* muscle or by Modified Partial Least Squares regressions of 22 Lab variables. X = assigned values ; Y = predicted values