COMPARISON BETWEEN PROVISAL AND HYLA RABBIT STRAINS
2. NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) OF MUSCLES AND LIVER TISSUES

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Abstract - The data were collected on sixty fattening PROVISAL rabbits and sixty fattening HYLA rabbits of both sexes, slaughtered at 74, 84 and 94 days in two replicates, in order to investigate the presence and the bigness of direct and indirect relationships between genetic and ontogenetic (age-sex) effects with the Near Infrared Reflectance Spectroscopy (NIRS) of two muscles (Hindleg=HL and Longissimus dorsi=LD) and liver (=Lv) freeze dried tissues. The results confirmed previous findings about chemical variables : lipids (R^2 calibration = $R^2c>0.93$) ; protein ($R^2c>0.78$) ; collagen ($R^2c=0.72$), and also about some slaughtering weight-linked performances : perirenal fat ($R^2c>0.57$), weight traits of hindleg parts : meat/bone ratio ($R^2c>0.48$). The genetic type was fitted as binary dummy variable (1 or 2) by NIRS evaluation with $R^2c=0.33$ from HL, 0.24 from Lv, and only 0.06 from LD spectra : the unexplained NIRS response after the indirect contribution was 0.28 from HL and 0.20 from Lv suggesting substantial presence of other factors (fibre types in biceps femoris, chemicals in liver as indicated by $R^2c=0.55$ of liver percentage from liver spectra).
The age (discrete) effects were not transparent to NIRS evaluation and furthermore a consistent prevalence of direct NIRS contribution *vs* indirect (0.16 to 0.23 of R^2c unit) was identified from the three tissues. The sex effects, evaluated as binary, appearing in spectra were totally indirect from other variables (mainly hindleg and protein percentages).
It was hoped to realize a NIR spectra data-bank of experiments, with HL muscles being more informative than LD or liver.

INTRODUCTION

The spectrometry of organic tissues in the near infrared region (from 800 to 2400 nm) was largely investigated for rapid determination of water, lipids and protein contents. The cost of a modern instrument with monochromator may be around 25.000-40.000 ECU, while the cost of a conventional laboratory already powerful (min. 100 analyses/d) is tenfold: this is matter of development and production.
In experimental studies concerning the rabbit the NIRS of dried muscles gave good and useful calibrations for chemical analysis (MASOERO *et al.*, 1994a), for assessment of stress transportation effects (MASOERO *et al.*, 1994b), and for discrimination of muscles untreated or treated with beta adrenergic agonist and phytohormones administered to fattening rabbits (MASOERO *et al.*, 1994c).
The aim of this trial (second part by LAMBERTINI *et al.*, 1996a) was to evaluate by NIR spectroscopy the differences : between two commercial genetic lines very popular in Italy: the PROVISAL and the HYLA strains, between three slaughtering ages and the two sexes. Three sites were considered: the Longissimus dorsi (LD), the hindleg (HL) and the liver (Lv).

MATERIAL AND METHODS

The sixty animals described in the companion paper (LAMBERTINI *et al.*, 1996a) were analyzed according to the harmonized methodology. The Longissimus dorsi (LD) and the liver (Lv) were preserved in freezer as integral at -18 °C; after thawing the two minced tissues were freeze-dried for 3 days, then milled in a domestic short-bladed cutter device for 30" and stored in freezer till the NIRS analysis. The hindleg (HL) was also preserved till the boning operations, then one sample of the separated muscles was traited exactly as the LD sample.
The NIR spectrometry of sampled tissues was performed, after moisture stabilization at 40°C overnight, by a monochromator instrument NYR System 4500, which scanned from 1300 to 2400 nm, step 2. The spectra were mathematically traited as code 1,4,4,1 and submitted to chemometric analysis by software ISI 2 ver. 3.0 (ISI, 1991). The method of calibration was the Modified Partial Least Squares (=MPLS) without exclusion of the outliers, however detected at $H>3$ or $t>2.5$. The crossvalidation check was performed by 5 groups. Because of

variations in the residual moisture contents, the 3 chief water bonds were removed from the analyzed spectra, which finally consisted of 113 points.
The 23 continuous variables reported in the first paper –with collagen and lipids and protein contents of the second half of the samples estimated by NIRS equations– were analyzed by chemometric method. Furthermore, the genetic type and the sex were each fitted as a binary (1, 2) and the three classes of age were fined as discrete (1=74 d, 2=84 d and 3=94 d), like dummy variables.
In order to explain the relative importance of the variables useful to discriminate the two genetic, and the sex, and the three age classes a conventional linear stepwise regression analysis was performed (SAS, 1987). The realized Rsquare was then compared to the Rsquares obtained by NIRS fitting of the classes of the main factors in experiment in direct calibration mode (R^2c). In this manner it was possible to ascertain the indirect contribution (via correlated variables) to the NIRS ability of direct calibration : if the difference is favourable to NIRS, its ability is demonstrated. The check was easily realized, weighing the partial rsquares (r^2p) by their NIRS calibration coefficient of the single trait (R^2c) and summing up (sum of r^2p*R^2c) for the significant traits, and this was repeated for the three investigated tissues.
The same steps of analysis were used to discriminate the NIRS direct and indirect contribution to the meat/bone ratio of hindleg, a synthetic and very interesting continuous trait.
Because the NIRS analysis can easily discriminate the muscle type, to verify this possibility, a similar analysis (as above) was performed with all the chemical variables (lipids and protein contents of dry matter of HL and LD) versus NIRS direct and indirect calibration of the type of muscles, coded as 1 and 2.

RESULTS AND DISCUSSION

NIRS fitting of the continuous variables (Table 1)

According to expectations the maximum of NIRS ability was detected in estimating the chemical composition of muscles from homologous site: the lipids (R^2c=0.96 and 0.94 for HL and LD respectively) ; the protein (0.86 and 0.79), while the heterologous prediction - for instance LD spectra *vs* HL composition- gave poor results. This depends on the low basic intercorrelation of the trait between muscle : r=0.55 for lipids and r=0.44 for protein.

The collagen contents of LD was predicted by NIRS analysis with useful accuracy: R^2c=0.72 but the relationship was not very strong, being the Rsquare in crossvalidation decreased to the value of 0.56 ; however the obtained result is interesting because of the complexity of the conventional analysis.
The NIR spectra of muscles were also related to the slaughtering performances with limited successes for the live-slaughter-weight and the perirenal fat percentage. The carcass composition traits were also slightly related to NIR spectra of muscles and particularly the hindleg weight and the femur weight.

The meat-bone ratio was related to NIRS of HL and LD muscles by a Rsquares of 0.49 and 0.50 in calibration mode respectively. These values are clearly unusable for predictive purposes of the individuals, but they are highly significant of a true relationship if we consider that the statistical linear model of fixed effects (mainly age, genetic type and sex) gave a R^2 of 0.52 (LAMBERTINI *et al.*, 1996a) : thus the variability of the meat/bone ratio accounted for by the whole experiment was explained at a similar level by the only spectra of the muscles or of the liver.

To clarify this unusual relationship the indirect pathway was explored by stepwise regression (Table 2). The total Rsquare, excluding the two variables of the ratio, approached to 0.99 with contribution of hindleg weight and of the three separated bone components.

The NIRS direct appraisal of meat/bone ratio was superior to indirect contribution of the four weighted significative correlated variables, and for all the tissues: liver (+0.11), hindleg muscles (+0.07) and, minimum, LD (+0.04). Obviously no rationale exists to support a functional relationship between NIRS of tissues and meat/bone ratio of the hindleg and consequently of the carcass; but these results, which were early enhanced (MASOERO *et al.*, 1994 c,d) and pointed out in Piemontese and Chianina beef cattle (MASOERO *et al.*, 1996), could open new suggestive hypothesis.

Table 1 : NIRS calibrations and crossvalidation from three tissues

			Hindleg		Longissimus dorsi		Liver	
			R^2c	R^2cv	R^2c	R^2cv	R^2c	R^2cv
1	LSW	Live Slaughter Weight	0.516	0.384	0.625	0.398	0.382	0.308
2	DOP	Dressing Out%	0.239	0.115	0.415	0.295	0.169	0.055
3	DLP	Drip Loss %	0.218	0.157	0.344	0.267	0.125	0.062
4	SKP	Skin %	0.323	0.200	0.361	0.227	0.057	0.040
5	FGTP	Full Gastrointestinal Tract %	0.069	0.010	0.164	0.120	0.281	0.202
6	CCW	Chilled carcass weight	0.437	0.372	0.584	0.456	0.369	0.301
7	LvP	Liver%	0.322	0.092	0.396	0.231	0.554	0.444
8	KiP	Kidneys %	0.406	0.136	0.333	0.202	0.056	0.000
9	PFaP	Perirenal Fat %	0. 645	0.491	0.569	0.434	0.169	0.154
10	SFaP	Scapular Fat %	0.030	0.000	0.016	0.000	0.010	0.000
11	HLP	Hind Leg %	0.285	0.139	0.211	0.102	0.443	0.262
12	HLW	Hind Leg weight	0.537	0.397	0.591	0.476	0.354	0.263
13	FmW	Femur weight	0.499	0.311	0.474	0.372	0.427	0.278
14	TiW	Tibia Weight	0.230	0.146	0.176	0.072	0.262	0.098
15	CxW	Coxa weight	0.045	0.024	0.244	0.095	0.030	0.011
16	F&TW	Fat and Tendons Weight	0.328	0.274	0.330	0.144	0.338	0.105
17	MBRH	Meat Bone Ratio of Hindleg	0.486	0.357	0.499	0.410	0.433	0.335
18	MFRHL	Meat fat Ratio of Hindleg	0.082	0.057	0.068	0.019	0.046	0.025
19	IFaHLP	Intramuscular Fat %	0.960	0.948	0.291	0.181	0.115	0.099
20	PrHLP	Protein %-Hindleg	0.855	0.850	0.218	0.084	0.089	0.071
21	IFaLDP	Intramuscular Fat % -L. dorsi	0.140	0.119	0.935	0.899	0.031	0.000
22	PrLDP	Protein % -L. dorsi	0.117	0.108	0.788	0.733	0.055	0.000
23	Co11LDP	Collagen %-L. dorsi	0.395	0.159	0.719	0.555	0.361	0.206

Table 2 : Comparative stepwise regression and NIRS of meat/bone ratio

			Stepwise	NIRS coefficients R^2c		
#	item	Variable	regression r^2p	Hindleg	L.dorsi	Liver
13	FmW	Femur Weight	0.458	0.499	0.474	0.427
12	HLW	Hind Leg Weight	0.323	0.537	0.591	0.354
15	CxW	Coxa Weight	0.170	0.045	0.244	0.030
14	TiW	Tibia Weight	0.039	0.230	0.176	0.262
		Total R^2	0.989			
		Indirect contribution to NIRS		0.418	0.456	0.325
		NIRS ability direct calibration		0.486	0.499	0.433
		NIRS revalence		0.068	0.043	0.108

r^2p=partial r^2

The NIRS of the liver tissues was even somewhat interesting for the liver percentage (R^2c=0.55) supposing a quanti-qualitative relationship of the organ; the same comment as above has to be repeated being the R^2 of the linear model only 0.15.

NIRS fitting of genetic type main effect (Table 3)

The PROVISAL rabbits were separated from the HYLA by 5 variables: kidneys, gut tract percentage, intramuscular fat of LD and femur and tibia weights ; the R^2 of multiple regression was 0.22. When NIRS of HL muscles was calibrated to the genetic type (1 or 2) the R^2 value raised to 0.34 whose 0.05 was derived from indirect relationships ; thus the net NIRS prevalence amounted to 0.29, a very high value: this fact demonstrates the presence of other significant differences trapped by the NIRS. In effect the percentages of the red, intermediate and white fibres of Biceps femoris were significantly different in the two genetic types (LAMBERTINI *et al.*, 1996 b).

Nevertheless the LD muscle did not display any NIRS contribution over the indirect relationships, supposing that no other genetic differences could be discovered in this site.

Furthermore the liver spectra gave a net NIRS prevalence of 0.20, indicating that chemical differences of that organ could be investigated on a genetic basis.

Table 3 : Comparative stepwise regression and NIRS of the Genetic type as binary

#	item	Variable	Stepwise regression r^2p	NIRS coefficients R^2c Hindleg	L.dorsi	Liver
8	KiP	Kidneys %	0.076	0.406	0.333	0.056
5	FGTP	Full Gastrointestinal Tract %	0.094	0.069	0.164	0.281
21	IFaLD	Intramuscular Fat % L. dorsi	0.017	0.140	0.935	0.031
13	FmW	Femur Weight	0.015	0.499	0.474	0.427
14	TiW	Tibia Weight	0.017	0.230	0.176	0.262
		Total R^2	0.219			
		Indirect contribution to NIRS		0.051	0.066	0.042
		NIRS ability direct calibration		0.336	0.064	0.242

r^2p=partial r^2

NIRS fitting of age main effect (Table 4)

In all the previous NIRS experiments with rabbits the age factor was calibrated with medium-high Rsquares and with predicted error around 7 days. Also in this experiment the age was calibrated with medium Rsquares from both the muscle tissues (>0.52) and low Rsquare from liver tissue (0.41). However it must be pointed out that, detracting the indirect contribution from the most explicative variables, a remarkable residual of unexplained NIRS response remained, maximum for HL (0.23).

Table 4 : Comparative stepwise regression and NIRS of the Age clas as discrete

#	item	Variable	Stepwise regression r^2p	NIRS coefficients R^2c Hindleg	L.dorsi	Liver
6	CCW	Chilled Carcass Weight	0.567	0.437	0.584	0.369
17	MBRH	Meat Bone Ratio of Hindleg	0.031	0.486	0.499	0.433
5	FGTP	Full Gastrointestinal Tract %	0.022	0.069	0.164	0.281
4	SkP	Skin %	0.030	0.323	0.361	0.057
3	DLP	Drip Loss %	0.030	0.218	0.344	0.125
2	DOP	Dressing Out %	0.028	0.239	0.415	0.169
7	LvP	Liver %	0.012	0.322	0.396	0.554
18	MFRHL	Meat Fat Ratio of Hindleg	0.007	0.082	0.068	0.046
		Total R^2	0.727			
		Indirect contribution to NIRS		0.292	0.388	0.246
		NIRS ability direct calibration		0.526	0.554	0.409
		NIRS prevalence		0.234	0.166	0.163

NIRS fitting of sex main effect (Table 5)

The sex differences, as appreciated by NIRS appeared only in the muscles from the hindleg region. This was consistent with the first part of paper where sex differences were linked to hindleg proportions and anatomical composition. In effect no unexplained NIRS contribution was detected out of the indirect contribution and all the three tissues gave negative NIRS prevalence. This finding agrees with the more usual lack of sex-dimorphism in fattened rabbit.

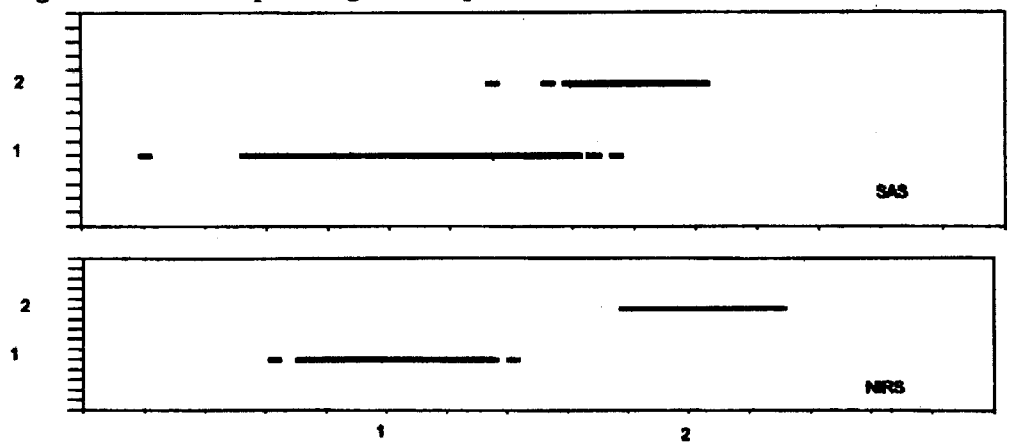
Table 5 : Comparative stepwise regression and NIRS of the sex as binary

#	item	Variable	Stepwise regression R^2	NIRS coefficients R^2c Hindleg	L.dorsi	Liver
11	HLP	Hind Leg %	0.192	0.285	0.211	0.443
20	PrHLP	Protein %-Hindleg	0.109	0.855	0.218	0.089
17	MBRH	Meat Bone Ratio of Hindleg	0.024	0.486	0.499	0.433
13	FmW	Femur Weight	0.017	0.499	0.474	0.427
14	TiW	Tibia Weight	0.054	0.230	0.176	0.262
		Total R^2	0.397			
		Indirect contribution to NIRS		0.181	0.094	0.127
		NIRS ability direct calibration		0.179	0.030	0.071
		NIRS prevalence		-0.002	-0.064	-0.056

Muscle characterisation

N.1-SAS figure enhances the separation of muscle type by using the lipids and protein analysis with a Rsquare 0.66. N.1-NIRS figure pictures the evident better answer obtained by the NIRS (R^2c=0.92 ; R^2cv=0.90).

Figure 1 : Fitting results of SAS stepwise regression equation and NIRS calibration for muscle type (1=HL, 2=LD)



This finding confirms similar previous results which relevance is not of practical use, but from scientific outstanding it is very promising. In effect the NIRS unexplained prevalence raised to plus 0.26 in the Rsquare, so fibre differentiation could be involved as a further planned examination has clarified (LAMBERTINI *et al.*, 1996b).

CONCLUSION

In the first place, each conclusion about body characteristics of different genetic lines must be set within the different studied sites: the conclusions about spectroscopic differences from LD are weaker than those from the liver and from the hindleg, thus the hindleg region should be recommended for comparative genetic studies. Some genetic differences in liver composition can be substantially hypothesized and it seemed to be of half the bigness *vs* age (ontogenetic) effects.

By second it was confirmed that a number of somatic traits can be statistically related to NIRS spectra, via the age-weight link. This fact does not have predictive purposes for individuals, but it could be explored as indicator of group (genetic, experiment, etc.) differences.

By third this method was confirmed as a quite perfect laboratory substitute for lipids and protein analysis in muscles as being indicative for the (difficult) collagen analyses.

Finally this tool should be accepted by the scientific community to realize a spectra data bank of experiments.

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Confronto fra conigli Provisal e Hyla. 2- spettroscopia NIR di muscoli e del fegato - Sessanta conigli all'ingrasso PROVISAL e sessanta HYLA ambosessi furono macellati a 74, 84 e 94 giorni di età, in due repliche, per studiare la presenza e la importanza di relazioni genetiche e ontogenetiche (età-sesso) dirette e indirette con lo spettro NIR di muscoli dell'arto posteriore (HL) del Longissimus dorsi pars lumbalis (LD) e del fegato (Lv) liofilizzati. I risultati confermano i precedenti circa le variabili chimiche: lipidi (R^2calibrazione=R^2c>0.93) ; proteina (R^2c>0.78) ; collageno di LD ($R^{-}c$=0.72), ed anche circa alcune caratteristiche di macellazione legate al peso vivo : grasso perirenale (R^2c>0.57), caratteri ponderali e componenti dell'arto posteriore ; rapporto carne/osso di HL (R^2c=>0.48). Il tipo genetico fu elaborate come variabile binaria (1 o 2) nella valutazione NIRS, con R^2c=0.33 dagli spettri HL, 0.24 da Lv, e solo 0.06 da LD : la parte di risposta NIRS che non fu spiegata dalla risposta indiretta fu 0.28 da HL e 0.20 da Lv suggerendo una sostanziale presenza di altri fattori (tipo di fibre nel Biceps femoris, sostanze chimiche net fegato, come indicato da una buona apparenza NIRS dell'organo : R^2c=0.55). Gli effetti dell'età (variabile discreta) non furono trasparenti alla valutazione NIRS; inoltre, una consistente prevalenza di effetti diretti NIRS rispetto a quelli indiretti (da 0.16 a 0.23 unità di $R^{-}c$) venne rivelata dai tre siti esplorati. Gli effetti del sesso, valutati in modo binario ed apparenti nello spettro NIR furono totalmente indiretti, dovuti ad altre variabili (principalmente proporzione della coscia e tenore proteico). Fu auspicato di realizzare una banca-dati degli spettri NIR degli esperimenti cunicoli considerando che i muscoli essiccati di HL paiono maggiormente informativi rispetto al LD o al fegato.