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NEAR INFRARED SPECTROSCOPY APPLIED TO LIVING RABBITS TO ESTIMATE BODY COMPOSITION AND CARCASS AND MEAT TRAITS: A CALIBRATION STUDY.

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I T A L Y

ABSTRACT. 39 NZW and "Carmagnola Grey" young rabbits from a 2^7 incompletely confounded experiment design (age, feeding level, sex, fasting 24h pre-slaughter, housing, diet , breed) were exposed to Near InfraRed Spectroscopy (NIRS) at 1200-2400 nm by a continuous analyzer. The rabbits were locally hair-cut and then read over the shoulder-blade in 25 seconds by a fibre-optic scanner device (diameter 8 mm). Body components as blood, gut, skin and warm carcass were recorded at slaughter. After cooling 24 h at 3°C the carcass components (liver , kidney fat , scapular fat) were dissected, the pH of Long. dorsi at 6LV was recorded and the drip loss calculated. One hindleg was analyzed for colour parameter at the medial face and a narrow sample from the semi tendinosus muscle was then submitted to INSTRON device.

Multiple correlation coefficients from calibration analysis

VARIABLE		(R)
BLOOD % LW	BP	0.84
SKIN % LW	SP	0.67
GASTR-INTEST. %	GP	0.72
DRESSING %	DP	0.79
PERIRENAL FAT %	PFP	0.80
SCAP.FAT %	SFP	0.76
LIVER %	LP	0.71
DRIP_LOSS %	DLP	0.88
COOLING LOSSES %	WCP	0.72
pH24_L6	pH24_L6	0.70
L.Dorsi_MOIST.%	LD_MOIS	0.76
COLOUR_L	L	0.70
COLOUR_b	b	0.81
INSTRON_PEAK 2	IN_P2	0.74
INSTRON_BREAK	IN_BR	0.69
PREFERENCE PANEL	PANEL	0.71
A.D.Gain	ADG	0.34
A.D.F.Intake	ADF	0.81
F.C.Index	FCI	0.63

Finally the carcasses were splitted, cut and reassembled for a family paired panel test with 409 valid trimodal preferences (+/-/=) collected and computed as marginal ratios. Multilinear regression analysis of the whole spectra, transformed as log(1/R), standardized and 1^{th} derivated, from calibration study (max 4 wavelenghts) showed that this living technique, immediate and not-destructive, seems to be a suitable tool for scientific purposes. Critical regions of spectra from live rabbits were identified and related to many quanti-qualitative traits. The domain of prediction must to be confirmed especially in condition of limited biological variation. Correlations of spectra with the studied design variables were high: from 0.95 for breed to 0.74 for fasting and sex. This fact introduce possibilities about discriminative purposes. Further studies need in this promising area.

Keywords: Rabbit, NIRS, Body Composition, Meat Quality, Consumer Preference, Prediction from Live animals.

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Introduction

Estimation of body composition of living animal is well assessed in pigs and also -to a less extent- in beef production (Lister, 1983; Hedrick, 1985). Prevision by ultrasound is direct to fat tissue and consequently indirect to water and protein (Simm, 1983). The power of prediction depends mainly from the amount of fat in the carcass as well as from its distribution: weak percentages and thin deposition in sites of fat tissue obstacle reliability of estimation from carcass and from living animal mainly because of lack of interfaces who interact with ultrasound waves. This kind of investigation was never applied to meat rabbits.

Accurate external measurements of young rabbits can be highly informative in term of body mass components, but less as percentages or ratios (Lukefahr and Ozimba, 1991).

The Spectroscopy in the Near Infra Red, 800-2500 nm (NIRS) is a non destructive method which can capitalize overtones of fundamental vibrations of the organic compounds. Technological advances in monochromators and in detectors allow to capture signals in reflectance or in transreflectance, by using an advanced optic fiber device. In man the method gave reliable estimation of body mass fat from skin spectra (Conway et al, 1984). In rabbit carcasses it was recently and successfully used from Dutch colleagues for prediction of chemical analyses (Steverink and Steunenberg, 1990).

The object of this investigation was to explore the limits of the NIRS calibration on living rabbits for a wide set of variables (body, meat, quality) in a wide range of biological variability.

Material and Methods.

Animals and design. The trial involved 39 NZW and "Carmagnola Grey" young rabbits from a 2^7 incompletely confounded experiment design:

$Y_{ijklmnop} = m + A_i + B_j + C_k + D_l + E_m + F_n + G_o + R_{ijklmnop}$ where:

- A = **Age** (0=100 d, 1=80 d); (**main factor**)
- B = **Feeding Level** (0=ad libitum; 1=restricted, at 110 g/d) (**main factor**);
- C = **Sex** (0=Male;1=Female) (confounding AxB);
- D = **Fasting** (0=No;1=Yes: 24_h solid fasting preslaughter); (**main factor**)
- E = **Cage** (0=Single cell;1=Feedlot on the ground); (conf. AxD interact.)
- F = **Commercial Pellets** (0=Growing formula;1=Lact.Does formula); (BxD);
- G = **Genetic type** (0=NZW; 1=Carmagnola Grey). (interaction AxBxD);

Confounding of factors was accorded to the following scheme:

	AGE	LEVEL	SEX (AB)	FASTING	CAGE (AD)	PELLET BD)	BREED (ABD)
Group #	A	B	C	D	E	F	G
1	0	0	0	0	0	0	0
2	0	0	0	1	1	1	1
3	0	1	1	0	0	1	1
4	0	1	1	1	1	0	0
5	1	1*	1	0	1	0	1
6	1	0	1	1	0	1	0
7	1	1	0	0	1	1	0
8	1	1	0	1	0	0	1

* Note: this level should have been set to 0 for perfect orthogonalisation.

The control group, 1, chosen to be the base-level (0 for each factor) included 1/2 of the effectives because of reference tools that needed in the panel test. Three were the replicates in the other cells.

Traits definition The stunned animals were slaughtered by bleeding. Body components (Blasco et al, 1990) were measured immediately and after a 24 h chilling at +3°C. Cold carcasses were halved and separated in six pieces (left regions identified by a series of threads) by a cut at the 7-8th TV and at 6-7th LV. From the six pieces a randomized carcass was then reassembled to organize a family paired panel test where one (unknown) half portion was a standard control rabbit. Thus 409 valid trimodal preferences (+/-/=) were collected, pooled within rabbit, and computed as marginal ratios of positives to negatives (minus 1), when positives exceeded, otherwise the contrary of the reciprocal (plus 1) was true.
The drip losses of Longissimus dorsi were measured as water lost in 24 h at +3°C of an intact sample of ~10 grams. Colour was measured on the medial side of the hindleg (rectus internus m.) by Hunterlab colourimeter . Physical traits of the meat were determined by Instron using a narrow sample of fascia lata tensor and vastus ext. muscles.
A total of 54 variables were then set in the individual vector.

Instruments and analyses A continuous Spectrometer (Quantum 1200, from LT Industries) was equipped by an optic fibre device (diameter 8 mm). The monochromator worked in the range from 1200nm to 2400nm being connected to internal polystyrene standard and a lead sulfide detector. The scan was made in transreflectance mode over the shoulder. The scapular region of the rabbits were previously haircut. NIR Spectra were obtained as an average of 30 scans. Crude signals were transformed in standardized spectra of log(1/R) and further first derivated.
Statistical analyses were performed in four sections.

step a.- Study of the 7 designed factors was firstly conducted by GLM procedure of the SAS System.

step b.- An equivalent approach was directly attempted from transformed spectra used as predictors of the experimental levels (0 or 1). For this discriminant purpose it was used the Partial Least Squares (PLS) method by the LightCal software (from LTI); the principal components number was fixed to 4 for all the design variables. No observation was removed. Correlation coefficients "true-fitted" and figures of the loadings for the wavelenghts were reported.

step c.- Multilinear regression was used to obtain the best model for fitting 19 selected traits,reported in the tables, with maximum 4 wavelengths. Some outliers for growth traits or for registered parameters were removed from the study of variables.

step d.- Finally, another PLS analysis was attempted for prediction of preference panel results (Y) from the 53 indipendent variables (X) conventionally registered. This method allows to use a number of variables larger than observations, who were 39. The software UNSCRAMBLER II performed calculations (CAMO, 1991).

Results and Discussion.

Variables and experimental factors The table 1 reports distributive properties of the main variables.
The coefficient of variation was quite high for a lot of variables, but

Table 1. - Statistics of the sample.

Predictand		N	mean	min	max	sd	cv%
SW	SLAUGHTER WEIGHT	39	2524	1610	3250	373	14.8
BP	BLOOD % LW	38	2.9%	2.1%	3.6%	0.4%	13.3
CSP	COMM. SKIN % LW	39	15.8%	12.8%	18.7%	1.5%	9.7
GP	GASTROINTESTINAL%LW	39	18.2%	13.7%	24.7%	2.8%	15.4
DP	DRESSING %LW	39	59.7%	52.4%	63.5%	2.5%	4.2
PFP	PERIRENAL FAT %	39	1.7%	0.0%	3.5%	1.0%	58.6
SFP	SCAPULAR FAT %	39	0.5%	0.1%	1.1%	0.3%	62.4
LP	LIVER %	39	5.5%	3.4%	9.1%	1.4%	24.6
DLP	DRIP_LOSS %	38	4.76	2.08	13.44	1.90	39.9
WCP	COOLING LOSSES %	38	1.99	0.4	3.25	0.7	35.2
pH24_L6	pH24_L6	39	5.94	4.98	6.31	0.21	3.6
LD_MOIS	Long.Dorsi_MOISTURE	38	74.94	72.98	78.16	1.03	1.4
L	COLOUR_L	38	48.02	43.70	54.22	2.39	5.0
b	COLOUR_b	38	8.25	5.8	10.8	1.17	14.2
IN_P2	INSTRON_PEAK 2	38	14.27	4.98	23.35	3.73	26.1
IN_BR	INSTRON_BREAK	38	7.5	5.04	12.9	1.96	26.1
PANEL	PREFERENCE PANEL TE	38	0.34	-5.00	10.00	2.97	864.3
ADG	AVERAGE DAILY GAIN	37	24.8	11.1	45.2	8.3	33.3
ADF	AVERAGE DAILY FEED	39	137	107	184	25	18.5
FCI	FEED CONVERSION EFF	36	5.88	3.33	9.16	1.67	28.4

very high for growth rate and for drip losses, for fatness traits and for Instron peak 2. The family panel test for preference showed the greatest coefficient of variation, due to its intrinsic variability and because the mean tended to zero.

In general the attempt of adding sources of biological variation was met in order to pool the most part of variability for a correct NIRS calibration.

The table 2 enhances the amount and the statistical significativity of the factors of variation involved in this model. Before all it is necessary to remark the small number of observations within each level of the 7 factors: obviously, inferences cannot be generalized, besides this was not our work purpose .

The severe feeding restriction adopted strongly reduced the performances as well as it affected body composition according to a well described general pattern (Ouhayoun et al., 1985) The restricted rabbits, obviously, grew slowly , preserved more viscera and cumulated less skin and carcass; further, they were strongly leaner (perirenal fat), and they had more moistured, light, tender, acid muscles. The preferability vs the control was decreased of 165%, but not statistically significant (P=0.24).

The younger rabbits confirmed the natural tendency of growth potential, they tended to reduce carcass -in opposition to visceral- while meat was more moistured, yellow, tender and water-lossing: the final pH resulted significantly increased of 3% .

The females (or interaction effects between the two previous factors) showed reduction of digestive tract and consequently the mass carcass increased. Cooling losses were increased and the meat was more yellow. Higher growth rate probably derived from small sampling.

Table 2.- Effect of the design factors, as a ratio of level 1 to zero level.

	AGE A	LEVEL B	SEX C AxB	FASTING D	CAGE E AxD	PELLET F BxD	BREED G AxBxD
BP	0.95	1.15	0.85 +	1.18 *	0.88	1.21 *	1.00
CSP	0.96	0.92 *	1.05	1.00	0.94 +	1.11 ***	0.99
GP	1.07 +	1.29 ***	0.90 **	0.98	1.05	0.95	0.99
DP	0.98 +	0.93 ***	1.03 *	0.99	1.01	0.99	1.01
PFP	0.91	0.54 *	1.42	0.68 +	0.52 **	1.04	0.86
SFP	0.66 +	0.68	0.85	0.52 **	0.76	0.64 *	1.10
LP	1.07	1.17	0.96	0.78 **	0.82 *	0.86 +	1.20 *
DLP	1.51 **	1.20	1.03	1.13	1.30 +	1.31 *	1.03
WCP	1.02	0.77	1.39 *	0.80	1.53 **	1.04	0.74 *
pH24_L6	1.03 *	0.95 **	1.02	0.97 +	1.01	1.01	1.01
LD_MOIS	1.02 **	1.02 *	1.01	1.01 **	1.01	1.00	1.02 +
L	1.01	1.05 +	1.06	1.01	1.01	0.99 *	1.13 ***
b	1.11 *	1.00	1.08 +	1.02	0.96	0.96	0.95
INST_P2	0.83 **	1.00	1.00	1.15 +	1.09	1.09	0.96
INST_BR	0.71 **	0.80 *	0.82	1.05	1.08 **	1.00	1.02 +
PANEL(ab.dif)	-0.53	-1.65	0.08	0.08	-1.13	0.53	-1.19
ADG	1.19	0.76 +	1.30 *	0.87	0.74 *	1.36 *	0.99
ADF	1.03	0.79 ***	0.91 +	1.07 +	0.92 +	0.92 +	0.89 *
FCI	0.87	1.14	0.91	1.13	1.24 +	0.70 **	0.83

+ P(0.10; * P(0.05; ** P(0.01; *** P(0.001 (belowed).

Preslaughter fasting affected above all the liver mass, which was reduced, and in a less measure, the blood percentage, which was increased: this last finding could be an apparent effect due to a better bleeding of fasted animals. The level in liver reduction (20% observed) in our recent experiments was also confirmed, while blood increasing was not.

Rabbits housed in feedlots on the ground strongly reduced the growth rate and consequentely their liver mass and perirenal fatness. Properties of muscles were deeply changed from exercise, as it appeared from higher

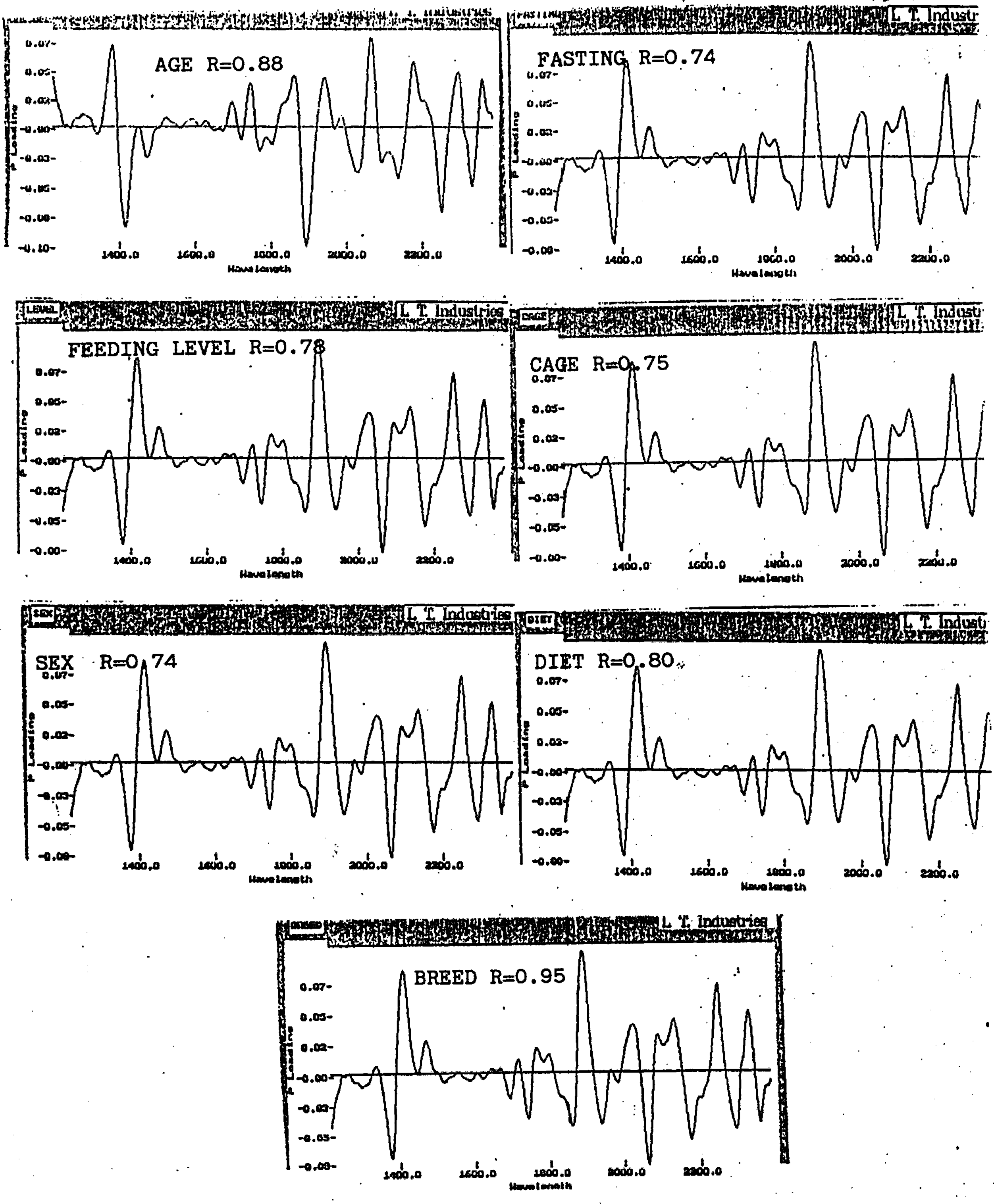

FIGURE 1.- Spectral loadings of the seven design variables by PLS method with 4 factors and correlation coefficients (no outlier removed: N=39).

cooling and drip losses, and they were also harder (higher shear force). Panel test disagreed the meat from that animals (-113% N.S.).
The availability of a diet for lactating does bettered rabbits growth and also it modified several somatic traits (more skin and blood, less scapular fat) as well as meat traits (higher drip losses, lower lightness).
The Carmagnola Grey rabbits showed higher liver percentage and as regard to meat traits, they reduced cooling losses in spite of an higher degree of moisture in muscle, and increased value for lighteness. The panel examination adversed this rabbits of some 119% (N.S.).
Interpretation of reports should be prudent mainly because of confounded effects A,B and D (see table of experiment design).
In general however it ressorted a wide range of possible modifications to our control "standard" condition.

Power of NIRS spectra on living to discriminate between classes. The figure 1 shows spectral loadings, that is the coefficient of correlation of single wavelengths with the predictable variable, for the seven designed factors. Correlation of the fitted with the true assigned value (0 or 1) of the levels is reported in the top. The number of principal components entered in the partial least square regression was fixed to 4 without discarding none of the realized spectra. This seemed a good conservative compromise, but the optimum number to include in calibration should be carefully investigated on an appropriate sample, having firstly to remove objects warned as outliers or for leverage. Nevertheless results were clearly promising about real capacity of NIRS to attribute individual spectra to the experimental group. Correlations were maximum for breed (0.95: see figure 2), high for age (0.88) and discrete for diet (0.80), feeding level (0.78), and fasting, cage and sex (0.74;0.75). Coat color may be responsible of the excellent characterisation of breed spectra. However it may be remembered that in the first experience Conway et al. (1984) did not account the color of the skin of human included in the experiment to predict very accurately the amount of fat in the body from epidermical NIR transreflectance.

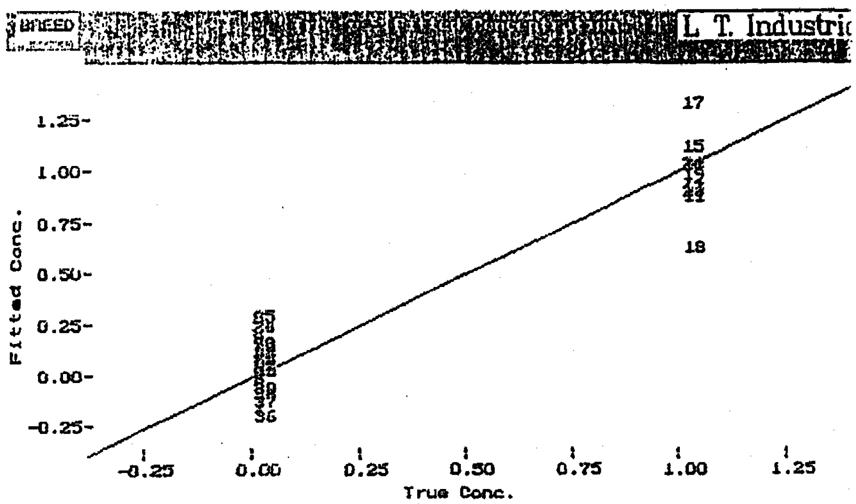

FIGURE 2.- Breed discrimination: 0=NZW 1=Carmagnola grey.
R=0.95

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Calibration of NIRS on living for observed characteristics and performances. The multiple linear regression (MLR) method was preferred to PLS for calibration of "ingredients" variables. This was a conservative purpose, because our sample was limited and unbalanced in extremities, thus very critical for cross-validation purposes. The multiple correlation coefficients (table 3) ranged from 0.88 for the drip loss percentage to 0.67 for skin incidence, with a minimum of 0.34 for ADG.

Table 3. - Multiple correlation coefficient and the most significant wavelenghts.

	(R)	(SE)	F-value:	nm1	nm2	nm3	nm4
BP	0.84	0.23	19.3	1717	2102	2263	2319
CSP	0.67	1.20	9.7	2076	2195	2364	
GP	0.72	2.04	12.8	1664	2231	2298	
DP	0.79	1.64	14.3	1251	1563	1602	1961
PFP	0.80	0.64	15.1	1249	1660	2074	2322
SFP	0.76	0.19	15.8	1664	1974	2326	
LP	0.71	1.03	8.5	1259	1609	1979	2136
DLP	0.88	0.37	30.6	1351	1651	1961	2303
WCP	0.72	0.73	11.4	2102	2124	2368	
pH24_L6	0.70	0.11	8.1	1614	1658	1549	2362
LD_MOIS	0.76	0.63	11.0	1657	1725	2041	2374
L	0.70	1.68	7.8	1721	1754	2270	2375
b	0.81	0.73	15.3	1580	1248	1953	2264
INST_P2	0.74	2.53	9.5	1248	1276	1754	2154
INST_BR	0.69	1.49	7.66	1344	1982	1630	1457
PANEL	0.71	2.14	8.4	1811	2299	2313	2343
ADG	0.34	8	4.53	2264			
ADF	0.81	15.8	16.6	1329	1918	2097	2160
FCI	0.63	1.38	6.9	1439	2264	2374	

Results of calibration could be improved by PLS1 alghoritm. Multilinear regression, however, is informative about possibilities of future true validation in new external datasets. In fact the highest multiple correlation coefficients were found for drip losses, blood percentage, yellow colour and perirenal fat and hot dressing percentage. It would be very useful by a simple spectrametric lecture to predict with sufficient precision also some qualitative meat characters till to the affective test as consumer preferences. Otherwise the statistically perfect response is reached by expensive, lating and difficult trials. Thus the problem is to fixe acceptable confidence level for fitted values: it depends both upon the purpose of investigation and upon the "noise" of the instrumentation for the investigated variable. In this prealable study standard errors were relatively high for individual predictions of traits, but experiment with rabbits can be easily implemented in numbers.

Panel prediction. The results of a PLS calibration (leverage correction) for overall preferences expressed in the panel test, where this Y variable was predicted from 53 X variables showed that after 6 factors the explained variance was 54.5% (R=0.73) and the SEP was 1.87. It was remarkable that the prediction from NIRS spectrum of the shoulder would have been so informative as a number of conventional and destructive analyses (R=0.71)

Conclusions

Critical regions of NIR Spectra from live rabbits were identified and related significantly to many quanti-qualitative traits.

The domain of prediction demandss to be confirmed by:

i- further PLS internal models also including lectures of loin not reported here;

ii- working in external samples similar to the original;

iii- working in others samples characterized by limited biological variation.

It is however clear that many variables can be the objective for this kind of studies, but they have to be intended in a more general biological and zootechnical sense and not only as an "ingredient variable", in the narrow chemical sense.

Further investigations need in the area of this living technique, immediate and not-destructive, applied to scientific, analytical and also classificative, purposes.

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