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COMPARISON OF SIX BREEDS OF RABBITS BY NIRS EVALUATION OF THREE TISSUES

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ABSTRACT

Meat composition of rabbits from six breeds: *Vienna White* (VW), *Fauve de Bourgogne* (FB) *Argenté de Champagne* (AC), *Belgian Hare* (BH), *English butterfly* (EN) and *Control Strain INRA A9077* (C77) was examined by Near Infrared Reflectance Spectroscopy (NIRS). From February to May 1999, 150 rabbits between 77 and 84 days were slaughtered. Three samples were collected from *Longissimus Dorsi* (LD), hind leg (HL) and *Obliquus abdominis* (OA), frozen at –18°C 24 h after slaughtering and preserved till August 1999, when they were freeze-dried. The between-breed variability was maximum for live weight (R^2=0.60), high for lipids in LD and OA (R^2=0.35 and 0.33), while null for lipids in HL. The leanest were *Belgian Hare rabbits,* the fattest those from the Control strain. The NIRS fitting of design increased from sex, to age, to live weight (R^2c = 0.59, 0.31, 0.63 for LD, HL, OA) and was maximum for duration of storage (R^2c= 0.83, 0.44, 0.76). The genetic differences in NIRS properties were consistent (average R^2c: 0.47, 0.45, 0.40 by tissues) and constant, when cross-validated. The cluster analysis put in evidence a strong originality of *Belgian Hare*; similarities between VW and –EN and between AC and C77; intermediate position for FB. The LD muscle appeared as the favourite site for genetic and ontogenetic expressions, while OA picked up significant complementary knowledges.

INTRODUCTION

The Near Infrared Reflectance Spectroscopy (NIRS) technique is a powerful physical tool used in agricultural industry for rapid and accurate analyses. The meat of rabbit or beef was investigated for study of ontogenetic (age, weight, categories), environmental (caging system, transportation), or genetic factors (Masoero et *al*., 1996, 1998; Bergoglio et *al*., 1997, 1999). In the frame of a European research project on European rabbit genetic resources (Bolet et *al*., 1999), NIRS was used to investigate the between-breed variability of meat composition. We present here preliminary results based on a first set of samples from six breeds.

MATERIAL AND METHODS

Animals, slaughtering and sampling tissues.
In this part of the research project 150 pure-bred rabbits were raised in the INRA Centre of Auzeville (France): *Vienna White* (VW, n=15), *Fauve de Bourgogne* (FB, n=48), *Argenté de Champagne* (AC, n=11), *Belgian Hare* (BH, n=16), *English butterfly* (EN n=28) and the *Control strain A9077*, from INRA (France) (C77, n=32). After weaning at 35 days the young rabbits were raised in single cage. The slaughtering term was fixed between 77 and 84 d with contemporaneous groups of the Control strain. The animals were slaughtered between February and May 1999. Live weight was determined at slaughtering. From each carcass 3 samples of muscle or meat were taken out: 1- a square shape of 5*5*1.5 cm of ground muscle of *Longissimus dorsi* (LD); 2- a square shape of 5*5*1.5 cm of ground of dissected meat from hind leg (HL); 3- two little strips of muscles (10-15 g in all) cut around the *linea alba* of *Obliquus abdominis* muscles (OA), not clean and not ground (Masoero et *al.*, 1997). Each sample was enveloped into an aluminium sheet, marked and stored into a plastic bag under vacuum. All samples were frozen at -18°C. These frozen samples were shipped to National

Institute of Animal Production in Turin (Italy) to be freeze-dried (on August 1999), then ground by mill for 20" and submitted to NIRS analysis.

NIRS measures, chemometrics and binary contrasts.

Reflectance spectra of muscle samples were recorded using a NIRSystem 4500 (Silverspring, MD, USA) scanning spectrophotometer, which yielded reflectance values every 2 nm. Data used in statistical procedures were those obtained every 10 nm between the wavelengths of 1308 nm and 2388 nm, corresponding to 113 points of measurement for spectre, excluding two water regions (1386-1430 and 1844-1988 nm). Spectrophotometer control and preliminary spectral file handling was performed using NIRS2 software (ISI, 1993), after mathematical transformation by first derivative of raw spectra of absorbance (log 1/R).

In order to generate predictive models, the calibration was made using the Stepwise Regression method, with internal cross-validation, selecting the optimal number of components in each case. Scatter correction by standard normal variates (SNV) and Detrend were also performed. Calculated calibration statistics included the coefficient of multi-determination in calibration (R^2c), the coefficient of determination in cross-validation (R^2cv) and the error estimation of cross-validation (SECV). The fitted factors were first the variables in the experimental design: age, live weight, sex and duration of storage between slaughter and analysis. Additionally, binary contrasts and distances matrices of breeds for the three tissues were calculated. The fitting degree was expressed by coefficient of determination (R^2c), which varies between 0 (it can not discriminate the two groups) and 1 (maximum discrimination). The R^2 values, as distance between the two groups, allow to perform a double triangular matrix of distances with the null values on the diagonal, reporting the binary contrasts values for the breeds. From these, six cluster analyses were built, using the average method (SAS, 1988). Finally, two pooled-cluster analyses were performed on the matrices of the average coefficients of calibration and validation from all muscles.

RESULTS AND DISCUSSION

The live weight (see Table 1) was responsible of a high amount of variation (ANOVA: R^2 =0.60), with a min-max interval of 1000 g, which is quite important, but in agreement with the controlled standard-weight of these breeds in the exhibitions. The experiment took place from February to May 1999. Because the six breeds were run together, the duration of storage between slaughter and analysis was balanced among breeds. As far as chemical composition is concerned, the estimate of lipids content was obtained using previous NIRS equations

Table 1. Characteristics of the 150 animals in the experiment.

Breeds	1 VW *Vienna White*	2 FB *Fauve de Bourgogne*	3 AC *Argenté de Champagne*	4 BH *Belgian Hare*	5 EN *English butterfly*	6 C77 *Control Strain*	ANOVA R^2	ANOVA P > F
N	15	48	11	16	28	32		
Age, d	82±4	81±4	81±4	81±4	81±4	82±4	0.01	0.86
Live weight, g	2467 ±264	2588 ±253	2843 ±368	2418 ±221	1802 ±233	2716 ±344	0.60	0.0001
Duration of storage, d	121±16	132±26	136±41	132±33	124±34	139±43	0.03	0.42
Estimated LD lipids,%	2.8±1.2	4.0±1.3	3.6±1.1	1.9±0.7	3.1±0.8	4.9±1.6	0.35	0.0001
Estimated HL lipids, %	11.5±2.6	11.9±3.3	10.7±4.4	10.2±3.1	11.3±3.6	11.1±4.0	0.02	0.63
Estimated OA lipids, %	24.1±10	29.2±9.3	24 ±9.80	11.9±6.2	17.6±7.3	26.4±6.8	0.33	0.0001

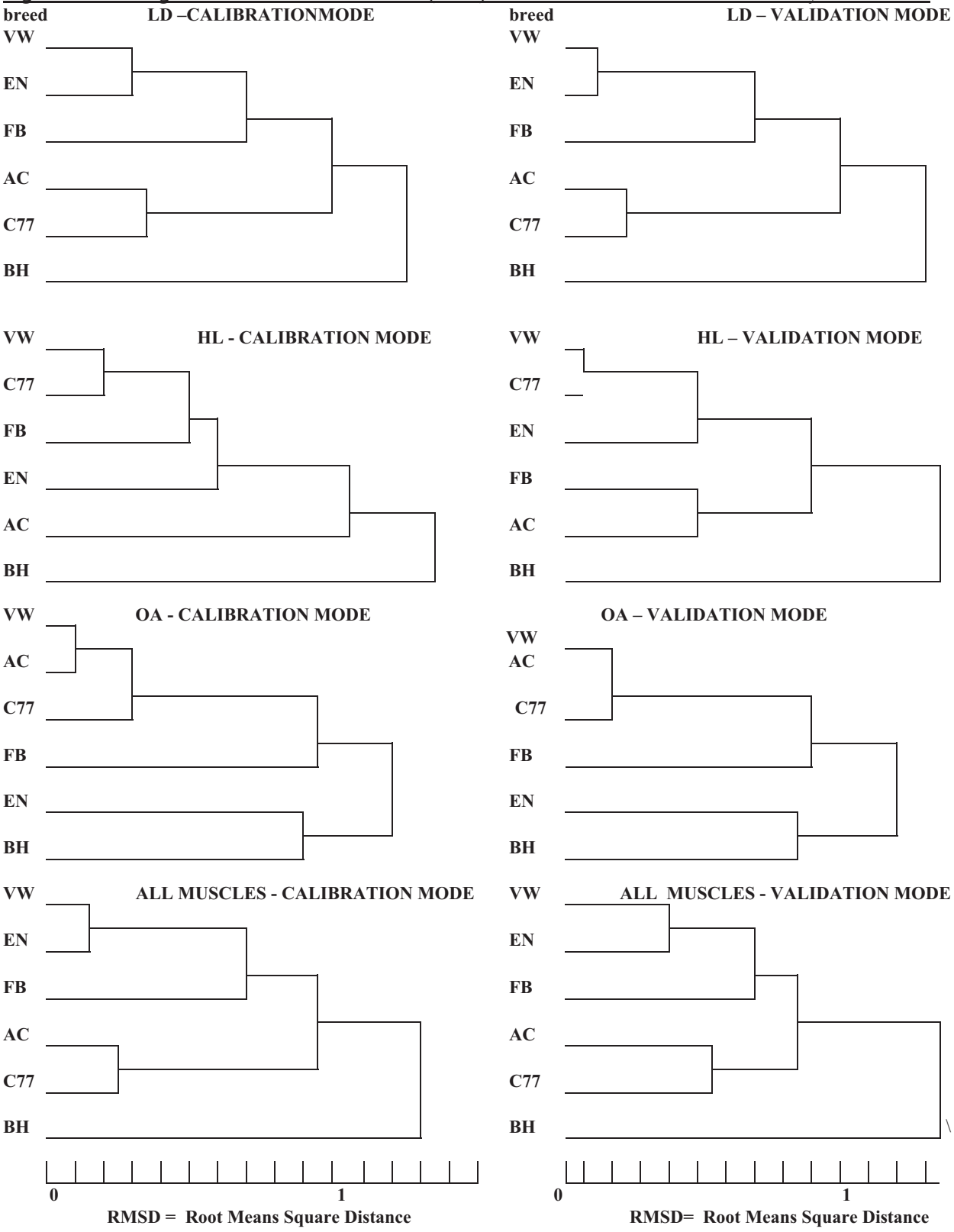
(Masoero et *al*. 1996). These estimated values showed consistent variation in *Longissimus dorsi* and *Obliquus abdominis* muscles (R^2 =0.35 and 0.33), with a minimum in *Belgian Hare* and maxima values in Control strain and in *Fauve de Bourgogne* breeds. Nevertheless, this finding was not replicated in the hind leg meat samples, where no difference was apparent in lipids content (R^2 =0.02).
The NIRS spectra were representative of the experimental design variables (see Table 2) in various degrees. Generally, *Longissimus dorsi* and OA were the most sensitive sites. In the ontogenetic traits, live weight fitted better than age. The R^2c values in calibration for the live weight were moderately high and stable in cross-validation (R^2cv between 0.59-0.29).

Table 2. Fitting of NIRS to the experimental design.

	LD muscle			HL meat			OA muscle		
Variables	R^2c	R^2cv	SECV	R^2c	R^2cv	SECV	R^2c	R^2cv	SECV
Age	0.15	0.13	3.5	0.16	0.14	3.5	0.08	0.05	3.7
Live weight	0.59	0.57	283	0.31	0.29	364	0.63	0.59	276
Duration of storage	**0.83**	0.82	14	**0.44**	0.41	25	**0.76**	0.74	17
Sex	0.07	0.04	0.49	0.07	0.05	0.49	0.07	0.05	0.49

The table 2 confirm our previous knowledges concerning the absence of appreciable sex differences in NIRS properties, which were already confirmed by conventional methods.
The first newness in this study was the strong relationship of NIRS results with the duration of storage of the samples, because NIRS was apparently able to take very well into account in a quantitative mode this denaturative evolution. So it is important to replicate correctly the treatments for not having unbalanced sampling of breeds. In the literature concerning NIRS, the aspect of meat denaturation as time of storage goes on was not attended. The application of NIRS discrimination to fresh *vs* frozen and thawed heifer meat was recent and successful (Downey and Beauchene, 1997). In a hypothetical way, the breeds could be different in response to the processes, occurring in the freezer and this could be appreciated by NIRS.
The second newness of this work was the existence of high breed effects on the spectroscopic properties of the investigated tissues. The comprehension of the phenomenon arises from the contrasts reported in Table 3. On the average, the R^2c values raised to 0.47, 0.45 and 0.40 for LD, HL and OA respectively, with good and reliable values after getting through the cross-validation tests. If we compare these values to the more classical genetic distances indices, the NIRS values appeared very big and able often to class the individuals.
The cluster analysis of binary contrasts (figure 1) of average distances, obtained from the three tissues, put in evidence two strong and stable similarities between VW and EN on one hand and between AC and C77 on the other hand. The *Fauve de Bourgogne* breed was intermediate, but nearer to VW-EN cluster. The *Belgian Hare* appeared as well distinguished breed, confirming its originality. This seemed to be related with its extra-lean meat, although the same findings were confirmed also in hind leg where no differences in lipid content could be invoked (see below). The relationships among breeds were confirmed in validation mode.
The LD dendrograms grouped the breeds as those concerning all muscles together. Therefore, this muscle could be considered, in this case, the best site for thethe breeds classification by NIRS.
Concernig the hind leg meat, the relationships between calibration and validation mode were less stable, so this tissue appeared less reliable to detect differences among these genotypes. The result could be related to the reduced variability of hind leg lipid content. Nevertheless, BH was well distinguished from all the other breeds.

Figure 1 - Dendrograms of distances normalized (RMS) in calibration and validation mode by three tissues



In the *Obliquus abdominis* muscle there was a quite different clustering for *Belgian Hare*, that was paired with *English*, though at big distance. Thus, OA picked up significant

complementary knowledges, useful when the research will aim to classify the samples in the breeds by combination of the whole available sources of informations.
These results confirmed the ability of NIRS to establish genetic relationships in experiments with rabbits of different origin: affirmative of differences in the case of ten French sire strains (Bergoglio et al, 1997) and also confirmative of genetic proximity in the case of Provisal vs Hyla (Masoero et al, 1996). A confirmation of the methods could be obtained when the whole project will be analysed.

Table 3. Matrices of NIRS binary distances between breeds: breed contrasts.

Muscle	Breeds	1 VW	2 FB	3 AC	4 BH	5 EN	6 C77
Longissimus dorsi Muscle	1 VW	**0**	0.29	0.63	0.77	0.14	0.42
	2 FB	0.26	**0**	0.46	0.64	0.44	0.45
	3 AC	0.57	0.41	**0**	0.74	0.69	0.18
	4 BH	0.75	0.60	0.70	**0**	0.54	0.53
	5 EN	0.07	0.40	0.63	0.48	**0**	0.31
	6 C77	0.37	0.42	0.12	0.51	0.29	**0**
Hind leg Meat	1 VW	**0**	0.13	0.55	0.79	0.21	0.10
	2 FB	0.09	**0**	0.29	0.47	0.35	0.33
	3 AC	0.50	0.22	**0**	0.72	0.57	0.56
	4 BH	0.75	0.44	0.70	**0**	0.64	0.56
	5 EN	0.15	0.32	0.50	0.59	**0**	0.32
	6 C77	0.06	0.30	0.50	0.52	0.27	**0**
Obliquus abdominis Muscle	1 VW	**0**	0.38	0.05	0.44	0.29	0.12
	2 FB	0.32	**0**	0.39	0.52	0.37	0.39
	3 AC	0.00	0.34	**0**	0.52	0.53	0.12
	4 BH	0.41	0.50	0.44	**0**	0.35	0.60
	5 EN	0.26	0.35	0.48	0.31	**0**	0.46
	6 C77	0.07	0.35	0.06	0.57	0.44	**0**

R^2 c: calibration over the diagonal; R^2cv: cross validation below the diagonal.

CONCLUSION

NIRS of dried tissues was a reliable and powerful tool to investigate relationships among rabbit breeds, based on chemical composition of muscles. The capability should be enhanced when data from different muscles or tissues are available and combined together.
NIRS properties of such complex matrices, however, depend on the experiment conditions and how are chained the operative sequences in order to minimize denaturative effects on the meat samples.
The next challenge is to test equations in other animals from the same breeds, which are still on the experiment, the second –and more ambitious one - is to face ethiology.

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