Use of near infrared spectroscopy for the genetic study of duck fatty liver quality

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Introduction

Melting rate (MR) is an important quality parameter in fatty liver production. It has been shown that MR is a genetically heritable trait[1]. For genetic selection purposes, it is essential to have a reliable prediction of this trait. It is also interesting to explore the informative content of the spectrum itself, in terms of genetic correlations between the trait of interest (MR) and absorbance at each wavelength, and also whole heritability of each absorbance.

Materials and Methods

Origin of samples

Male mule ducks (n=1550) were hatched in 2 year and 2 annual pedigree batches at the INRA experimental farm of Artiguères (UEPFG, France). These mule ducks were hybrids between 2 experimental populations: the female ducks were 382 back-cross (BC) common ducks and the male ducks were 56 Muscovy drakes. At 12 weeks of age, ducks were bred for 12 days in collective cages of 4 or 5 individuals and were overfed (by 2 different crammers) twice a day. At the end of the overfeeding period, animals were slaughtered after electronarcosis and the liver were taken and weighed. A sample was taken and frozen in liquid nitrogen for NIR spectroscopic analysis while another sample was taken for immediate MR measurement.

Spectral acquisition

Spectra were recorded on a FOSS NIRSystems 6500 (Foss, Silver Spring (MD), USA) equipped with a Direct Food Contact Analysis (DCFA) module. Samples were thawed at room temperature (+20°C), then manually mixed and poured into small cups (40mm diameter) with quartz glass which were placed on the DCFA window for spectra acquisition. Three different readings, with separate cup filling, were taken and the final spectrum was the average of these three individual spectra.

Reference data

All 1550 samples were submitted to melting rate measurement immediately after liver collection. The measurement was done by recording the quantity of fat released after cooking a 60 g liver sample at 105°C during 50 min.

NIR calibrations

Data analysis was done on WINISI II software (Infrasoft International, Port Matilda, PA, USA). Visible wavelengths of the spectra (400-800 nm) were discarded to avoid models taking into account colour differences not linked to composition; wavelengths used were 800-2500 nm. Several statistical pre-treatments were tested and the best results were obtained with first derivative (calculated on 10 datapoints) combined with smoothing (on 5 datapoints) on normalised spectra (standard normal variate).Calibration equations were developed by partial least squares (PLS) regression. Calibration performances were described by their coefficient of determination (R^2), and their standard error of calibration (SEC) and cross-validation (SECV). The ratio RPD = SD/SECV was calculated as a criterion of model quality. Calibrations were done either on all (1550) samples or on a subset of 200 samples chosen after PCA analysis on spectra, with the objective of testing the loss of information which would occur in such experiments if total measurement of all samples was replaced by NIR prediction based on the reference analysis of a subset of samples. In this case, a SEP was calculated on the 1350 samples not used in the calibration.

Reference paper as:
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Genetic analyses

Genetic studies were first performed on the melting rate trait, either the measured MR or the predicted MR (with total or reduced calibration databases); heritabilities and genetic correlations between measured and NIR predicted values were estimated. Then each absorbance value of the spectra was considered as a variable: we estimated heritability of each absorbance and genetic correlations of each absorbance with measured MR. This leads to a "spectrum" of the heritability and genetic correlations values as presented in Figure 1.

For the genetic study on melting rates, livers with extreme weights (higher than 830g and lower than 300g) were removed because they are not representative of commercial products. Genetic parameters were estimated by combining pedigree information from both parental population (common and Muscovy) and mule duck performances (n=1422)[2]. The model included two random effects, corresponding to the additive genetic values of sires and dams in the 2 parental populations and a fixed effect corresponding to the combination of year, batch and crammer effects (12 levels). Pedigrees were traced back up to 5 generations of ancestors on both parental lines and consisted on 596 animals in the common line and 201 animals in the Muscovy line. Genetic parameter computations with a multitraits approach were performed by REML and confirmed by Gibbs sampling using "remlf90" and "gibbsf90" programmes respectively.[3] A total chain length of 100,000 iterations was run and 20,000 samples were discarded as burn-in.

Results and Discussion

Calibration equations

The statistics of calibration equations are reported in Table 1. On the whole database, the melting rate was predicted with SECV=5.76. No repeatability estimate is available for MR (which is a destructive measurement) but the precision obtained is known to be low, given the "industrial" type of reference measurement. The SECV obtained with the reduced database was equivalent to the one obtained on the whole database, but the corresponding R^2 and RPD were higher thanks to a higher SD of the population selected. The SEP calculated on the 1350 samples not used in the reduced calibration database was SEP=6.8, *i.e.* 18% higher than SECV.

Table 1. Statistics of calibration equations.

	Population		Calibration			
	Mean	SD	SEC	R^2	SECV	RPD
MR total database	39.02	12.19	5.65	0.79	5.76	2.12
MR 200 samples	35.69	14.82	5.17	0.88	5.75	2.58

Genetic parameters

Only genetic parameters estimated on common duck side are detailed below. Heritability of MR measured by reference method was 0.201 which is higher than heritabilities of liver biochemical traits (dry matter, fat and protein contents) obtained in the same study, despite the low precision of the MR reference measurements. Heritability of MR was not significantly different when estimated from measured or NIR predicted values and the NIR calibration used (whole database or reduced reference database) had no impact. Moreover precision (SD) of the three MR heritability estimates was similar.

Genetic correlations between MR measured or estimated by NIR spectroscopy was about 0.90 which suggests that there is a small part of the genetic information on MR that is not captured by NIR. Indeed it is understandable that the determinants of melting linked with chemical composition of liver (fat content, …) are well captured by NIR while some structural aspects of liver tissue, which also have an effect of melting, are less well accounted for, particularly in mixed samples as it was the case in this study.

Table 2. Heritabilities (mean and standard deviation) of melting rate estimated with data from reference measurement or NIR prediction (total database or reduced database)

	Heritability	SD
MR reference measurement	0.201	0.035
MR prediction (total database)	0.188	0.034
MR prediction (reduced database)	0.199	0.035

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Table 3. Genetic correlations between MR reference measurement and NIR prediction based on total or reduced database

	Genetic correlation with reference measurement	SD
MR prediction (total database)	0.912	0.028
MR prediction (reduced database)	0.894	0.034

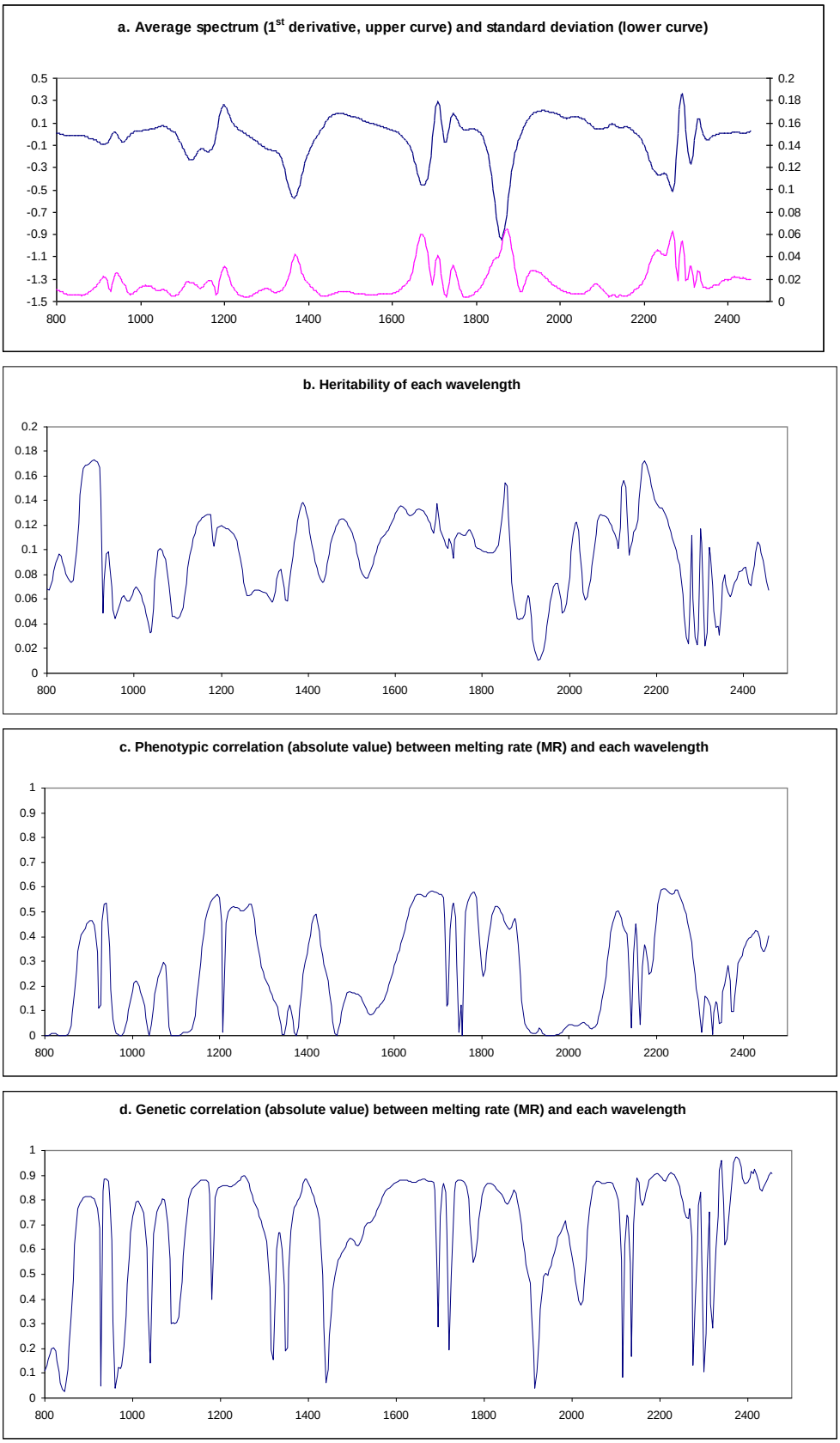

Figure 1. Spectral values, heritabilities of each wavelength and correlations with melting rate

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Heritabilities of absorbancies at all wavelengths can be compared with the spectrum values (and SD of spectra) and with the genetic correlations between absorbancies and measured MR (Figure 1). This facilitates examining the spectra from a genetic point of view. In some zones of the spectrum, a relatively high variability of absorbances is related to a high heritability and associated with a genetic correlation with MR. It is particularly the case in zones related to fat content (1720 nm, 2310 nm, etc.) which is logical because liver fat content is linked with MR (R^2>0.50). Some zones are particularly interesting because the link between the genetic parameters is broken; for example, around 2020nm, heritability is quite high (0.12) and the genetic correlation with MR is low. This means that there is a heritable trait at this wavelength but that this trait is not related to our parameter of interest (MR). Similarly, the observation of the phenotypic *vs* genetic correlation shows relatively parallel curves – although phenotypic variation is generally lower – but in some zones the phenotypic correlation between absorbances and MR is low while genetic correlation is high. This phenomenon is particularly visible in the 1940-2050nm zone or in isolated peaks like 1336nm. It shows that the single observation of the spectrum (absorbance, SD) or even its phenotypic correlation with a variable of interest cannot bring all the information on the spectral zones, especially when dealing with genetic prediction of a trait from the spectral information.

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Conclusion

Melting rate of fatty liver can be predicted by NIR spectroscopy. The prediction is not very precise but it allows genetic studies with the same results as if reference measurements were used. The study of the spectrum from a genetic point of view can be useful to identify spectral zones of particular interest for genetic studies, which can be distinct from the sole observation of spectra, spectra SD or phenotypic correlation with a parameter of interest.

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References

1. C. Marie-Etancelin, B. Basso, S. Davail, K. Gontier, X. Fernandez, Z.G. Vitezica, D. Bastianelli, E. Baéza, M.-D. Bernadet, G. Guy, J.-M. Brun and A. Legarra, *J. Anim. Sci.* **89**, 669-679 (2011).

2. L.L. Lo, R.L. Fernando and R. Grossman, *J. Anim. Sci.* **75**, 2877-2884 (1997).

3. I. Misztal, Proc. Inter. Workshop Comput. Cattle Breed. *Interbull Bul.* **20,** 33-42 (1999).

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