Comparison of two spectral acquisition conditions for the evaluation of genetic parameters of duck fatty liver quality

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Introduction

In France, over 95% of the duck fatty liver (Foie Gras) production comes from overfed male mule ducks, an intergeneric hybrid resulting from mating between male Muscovy ducks (*Cairina moschata*) and female common ducks (*Anas platyrhynchos*). In animal genetic studies, the need for a large number of samples requires simplified measurement procedures. Until now, as the composition of fatty liver is difficult to record in large numbers, no genetic parameters were available to describe such quality traits in mule duck. The present study (GENECAN) aims at studying the effect of two different NIR spectroscopic measurement approaches on the estimation of genetic parameters of fatty liver composition traits.

Materials and Methods

Animals

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 livers were taken and weighed. Two samples were deep frozen in liquid nitrogen for chemical analyses and laboratory NIR measurements.

Spectral acquisition

Direct / ASD

Spectra were recorded on the surface of fresh whole fatty liver just after slaughter. The spectrometer used was an ASD labspec Pro (ASD, Boulder (Co), USA) with a spectral range of 350-2500nm by 1nm. The spectrometer was equipped with a reflectance probe (spot size=10mm). Four points on each liver were recorded twice *i.e.* 8 spectra per sample and the final spectrum was the average of these 8 individual spectra. The work was performed at +4°C (carcass preparation room); fatty livers were in equilibrium with this temperature.

Laboratory / FOSS

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

Chemical analyses

A total of 200 samples were selected for chemical analysis on the basis that they were representative of the whole spectral population following a PCA performed on the FOSS spectra. These 200 samples were analysed with the reference laboratory methods:

DM	Dry matter: drying in oven at 104°C until constant sample weight
ASH	Total mineral content: ashing at 550°C
FAT	Cold lipid extraction with chloroform-methanol[1]
PROT	Protein content (N*6.25) by Dumas (combustion) method on LECO apparatus

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Analyses were done in duplicate which allowed calculation of the repeatability of the laboratory measurements (SEL, standard error of laboratory).

NIR calibrations

Data analysis was done using WINISI II software (Infrasoft international, Port Matilda (PA), USA). On FOSS spectra, the visible wavelengths of the spectra (400-800nm) were discarded to avoid models taking into account colour differences not linked with composition. So the wavelengths used in FOSS were 800-2500nm. On ASD spectra, the first segment (350-1000 nm) was discarded for the same reasons while wavelengths 2350-2500 nm were also removed because of the presence of some noise. So the wavelengths used in ASD were 1000-2350nm. Several statistical pre-treatments were tested and the best one on both spectral datasets was the first derivative (calculated on 10 datapoints) and smoothing (on 5 datapoints) on normalised spectra (standard normal variate).Calibration equations were developed by partial least squares (PLS) regression. The calibration performance was described by the coefficient of determination (R^2), residual standard error of calibration (SEC) and cross-validation (SECV). The ratio RPD = SD/SECV was calculated as a criterion of model quality.

Genetic analyses

Genetic parameters were estimated by combining pedigree information from both parental population (Common and Muscovy) and mule duck performances[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. Laboratory measurements (with FOSS spectrometer) were satisfactory for DM and FAT, with SECV values of 1.26 and 1.81 respectively. The corresponding RPD (3.9 and 3.6) indicated useful models. In the case of DM, SECV was much higher than measured SEL while SECV for FAT was only 36% higher than SEL. The prediction of ASH content was very poor, obviously due to the low variability in the population: standard deviation of the population was close to the repeatability of measurement. Crude protein (PROT) content was not very accurately predicted as evidenced by SECV=0.73 leading to a RPD value of 1.9 only. This was surprising because the laboratory precision was satisfactory (SEL=0.19) and this parameter is generally well-predicted by NIRS.

Measurements made on whole livers (ASD spectrometer) led to lower performance: on average SECV values were 25% higher, except for PROT for which SECV values were equivalent. This lower performance of direct measurements can be explained by the conditions of spectra collection (+4°C), the higher heterogeneity of sample and the "surface" effects (moisture on the surface of livers). However the magnitude of the degradation was not very important and the RPD values for the main quality parameters (3.0 and 2.9 for DM and FAT respectively) remained acceptable.

Table 1. Calibration equations.

	Population			Laboratory (FOSS)				Direct (ASD)			
	Mean	SD	SEL	SEC_f	R^2_f	$SECV_f$	RPD_f	SEC_a	R^2_a	$SECV_a$	RPD_a
DM	64.21	4.93	0.41	1.16	0.94	1.26	3.9	1.45	0.91	1.62	3.0
ASH	0.41	0.09	0.11	0.07	0.46	0.07	1.3	0.08	0.28	0.09	1.1
FAT	51.02	6.50	1.33	1.72	0.93	1.81	3.6	2.07	0.89	2.24	2.9
PROT	8.06	1.39	0.19	0.65	0.78	0.73	1.9	0.66	0.80	0.72	1.9

Genetic parameters

Note: only genetic parameters estimated on common duck side (not Muscovy ducks) are detailed below.

The lowest heritability estimates with laboratory equipment (FOSS) were obtained on liver ash contents, which is logical given the low precision measurement of this parameter. Heritability of PROT was the

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highest, despite the lower calibration performance, while DM and FAT had intermediate values. In direct measurement (with ASD spectrometer), the heritability estimates were of the same magnitude but somewhat different in their relative values. ASH heritability had a higher value while PROT heritability was lower than with laboratory measurement. It is important to note that standard deviations of heritability estimates were not increased in proportion to calibration errors. Genetic correlations between the same traits estimated from the two predictions ranged from 0.92 to 0.95, indicating that the genetic ranking was similar with both measurements. This shows that the relatively lower accuracy of calibration equations in the case of direct measurements did not create major changes in the animal classifications. As heritability values were globally similar with data originating from direct laboratory spectral measurements, we can assert that composition traits predicted by FOSS or ASD spectrometers were genetically the same.

Table 2. Heritabilities (± st. dev.) of fatty liver composition traits estimated with both spectrometers and genetic correlations between spectrometer estimates.

	DM	ASH	FAT	PROT
Heritabilities				
Laboratory (FOSS)	0.139±0.034	0.118±0.030	0.154±0.033	0.170±0.032
Direct (ASD)	0.151±0.032	0.144±0.032	0.148±0.031	0.126±0.028
Genetic correlations				
ASD vs FOSS	0.945±0.025	0.920±0.050	0.923±0.030	0.927±0.031

Conclusion

Direct spectral measurement on unprepared samples led to lower calibration performance than standardised laboratory spectral acquisition. However, the use of prediction data with both strategies did not have consequences on the average values nor the precision of genetic parameter estimates. The direct measurement, although less precise, can therefore have the same use in genetic studies.

Acknowledgements

This study was undertaken in the framework of the GENECAN project, funded by ANR.

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