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Visible spectroscopy on carcass fat combined with chemometrics to distinguish pasture-fed, concentrate-fed and concentrate-finished pasture-fed lambs

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ABSTRACT

We used visible spectroscopy of fat to discriminate lambs that were pasture-fed ($n = 76$), concentrate-fed ($n = 79$) or concentrate-finished after pasture-feeding ($n = 69$). The reflectance spectrum of perirenal and subcutaneous caudal fat was measured at slaughter and 24 h post mortem. In Method 1 (W450–510), the optical data were used at wavelengths in the range of 450–510 nm to calculate an index quantifying light absorption by carotenoids. In Method 2 (W400–700), the full set of data at wavelengths in the range of 400–700 nm was used to differentiate carcasses using PLS-DA as a classification method. W400–700 proved more reliable than W450–510 ($P < 0.0001$). The proportion of correctly classified lambs using W400–700 was 95.6% and 95.9% for measurements made on perirenal fat at slaughter and 24 h post mortem. The intensity of light absorption by carotenoids decreased exponentially with live weight gain during the finishing period.

1. Introduction

It has been demonstrated that meat from pasture-fed ruminants has healthier fatty acid composition than meat from animals fed concentrate diets (Aurousseau, Bauchart, Calichon, Micol, & Priolo, 2004). Also, consumers are showing increased interest in the origin and method of food production by demanding clear information in this regard (Prache, Cornu, Berdagué, & Priolo, 2005). It is therefore important to be able to identify products obtained through different production systems.

Visible reflectance measurements of fat in the region of light absorption by carotenoid pigments have been shown to be of interest for authenticating pasture-feeding in sheep and cattle (Prache & Thériez, 1999; Serrano, Prache, Chauveau-Duriot, Agabriel, & Micol, 2006). Prache and Thériez (1999) have proposed a mathematical analysis of the reflectance spectrum of the fat at wavelengths in the range of 450–510 nm (which corresponds to the zone of light absorption by carotenoids) to quantify the signature of these pigments and discriminate pasture-fed from stall-fed lamb carcasses. Dian et al. (2007a) further demonstrated that using the overall optical data of the visible reflectance spectrum at wavelengths in the range of 400–700 nm increased the reliability of visible reflectance of fat for discriminating between pasture-fed and stall concentrate-fed lambs.

However, forage shortage may lead to an abrupt change in the feeding regime of initially pasture-fed lambs, which are often stall-finished with low-carotenoid diets. This may make diet authentication more difficult. The two objectives of this study were therefore (i) to test the reliability of visible spectroscopy for discriminating among carcasses of lambs from three feeding regimes (pasture-feeding, stall-feeding, and 28 days stall-finishing after pasture-feeding), and (ii) to investigate the variation in the spectral features of the fat in the region of light absorption by carotenoid pigments after a switch from pasture to a concentrate-based diet. The interactions with the measurement site and the time elapsed between slaughter and visible reflectance measurements were also investigated, since these factors may affect carotenoid concentration (Kirton, Grane, Paterson, & Clare, 1975), the corresponding spectral characteristics of fat tissues (Priolo, Prache, Micol, & Agabriel, 2002), and therefore the reliability of discrimination among production systems.

2. Materials and methods

This study was carried out over 5 years (2008–2012) at two experimental farms, Unité Expérimentale des Ruminants de Theix (UERT) and Unité Expérimentale des Monts d'Auvergne (UEMA), and both run by the Clermont-Ferrand/Theix INRA Centre in France, but geographically separate. Working in these two farms enabled to enlarge the pasture-

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feeding period and to avoid potential complications linked to the onset of drought conditions at pasture, since one farm was situated in semi-mountainous conditions (UERT, 850 m a.s.l) and the other farm was located higher in the mountains (UEMA, 1000 m a.s.l). The UERT farm, where the lambings took place, enabled an early turn-out to pasture of P and PS lambs in spring, whereas the UEMA farm, which was less prone to drought conditions in summer, made pasture-feeding during summer more secure. The animals were handled by specialized personnel who ensured their welfare in accordance with European Union Directive No. 609/1986.

2.1. Animals, diets and slaughter procedures

A total of 224 Romane male lambs were used: 76 were fed on pasture for at least 60 days (P), 79 were stall-fed concentrate and straw indoors (S), and 69 were fed on pasture for at least 60 days followed by an abrupt switch to stall-feeding with concentrate and straw inside the stall (PS).

P lambs were born in spring 2008, 2009, 2010 and 2011, and PS lambs were born in spring 2010 and 2011. They were turned out on pasture (from early April to late June) and were offered ad libitum permanent pastures maintained in a leafy, green vegetative state. The pasture-feeding period of P and PS lambs was chosen to last for at least 60 days according to the results of Oliveira, Carvalho, and Prache (2012a), reporting the time of appearance of carotenoid pigments in the fat tissues relative to a change from a low to a high dietary carotenoid level. P lambs were fed pasture until slaughter, which occurred when a satisfactory degree of fatness was reached. PS lambs were fed pasture until the appropriate live weight (LW) for switching to the stall diet was reached. We chose 28-day duration for the stall finishing period of PS lambs to match common on-farm management practices. PS lambs were expected to show on average 7.0 kg LW gain (LWG) during this finishing period. The pasture-feeding period lasted 87 days on average (range 61–147 days) for P lambs and 86 days on average (range 61–128 days) for PS lambs.

Stall-fed lambs were born in 2008, 2010 and 2011, the last slaughtering occurring in March 2012. They were fed ad libitum a commercial concentrate containing no green vegetative matter and corn straw until slaughter, which occurred when they had reached an adequate fatness. These feeds were given to PS lambs from the time of switching to the stall diet until slaughter.

In the first 3 years of the study (2008, 2009 and 2010), P and PS lambs were born and raised at pasture in UERT farm from birth weaning, then they were transferred to UEMA farm to ensure sufficient pasture availability. In 2011, climatic conditions enabled raising all P and PS lambs at UERT farm from birth to slaughter. All S lambs were raised from birth to slaughter at UERT farm.

Water and salt blocks were made constantly available in all the feeding treatments. Animals were transported by truck to the abattoir located 500 m from the UERT farm and 25 km from the UEMA farm. Immediately after their arrival, the animals were electrically stunned and slaughtered by throat cut. The carcasses were placed in a chiller set at 4 °C until 24 h post mortem, and were stored in the dark.

2.2. Measurements

2.2.1. Animal and carcass characteristics

All lambs were weighed at birth and just before slaughter. The P and PS lambs were also weighed at turning out to pasture. For the PS lambs, we performed a double weighing at one-day intervals at the time of the switch to the stall diet and at slaughter to increase the precision of the estimation of LWG during the finishing period. Carcass and perirenal fat weight and subcutaneous fat thickness were measured 24 h post mortem.

2.2.2. Plasma carotenoid concentration

Blood samples were taken from the jugular vein of each lamb at 8 a.m. on the day of slaughter for all lambs, and on the day of switch to the stall diet for PS lambs, in order to measure plasma carotenoid concentration (PCC). Plasma was stored at −20 °C until assay. Carotenoids were extracted from the plasma within 3 months post-collection. Estimation of PCC was carried out according to the method described by Dian, Andueza, et al. (2007a).

2.2.3. Reflectance spectrum of perirenal and subcutaneous caudal fat

The reflectance spectrum (RS) of perirenal and subcutaneous caudal fat was measured on all lambs at wavelengths in the range of 400–700 nm, using a MINOLTA CM-2002 spectrophotometer (D65 illuminant, observer angle 10°). For each tissue, five measurements were made at five randomly selected locations at slaughter and at 24 h post mortem.

2.2.4. Methods used to discriminate pasture-fed from stall-fed and stall-finished grazing lamb carcasses

In the first method ($W_{450–510}$), the fat RS data were used at wavelengths between 450 and 510 nm to calculate an index quantifying light absorption by carotenoid pigments in the fat. The RS was translated (TRS) to give a reflectance value at 510 nm of zero. On the TRS, the integral value ($I_{450–510}$) was calculated as follows:

$$I_{450\text{-}510} = (TRS_{450}/2 + TRS_{460} + TRS_{470} + TRS_{480} + TRS_{490} + TRS_{500} + TRS_{510}/2) \times 10,$$

where TRS_i is the reflectance value of the translated reflectance spectrum at i nm.

$I_{450–510}$ was averaged over the five measurements, and linear discriminant analysis was then performed, followed by a cross-validation procedure to classify the fat samples according to feeding treatment, using Minitab software v. 13 (Minitab Inc., Paris). For measurements made at 24 h post mortem, the mean $I_{450–510}$ values were all negative, so the absolute value of the mean integral ($AVMI_{450–510}$) was used.

In the second method ($W_{400–700}$), the full RS data set at wavelengths in the range of 400–700 nm was used to discriminate P, S and PS lambs. The reflectance data (R_i, i ranging from 400 to 700 nm) were averaged over the five replicates, transformed (log $(1/R_i)$) and exported into Win ISI II version 1.5 software (Infrasoft International, Port Matilda, PA, USA) for multivariate analysis. The raw reflectance spectra of each tissue representing the three feeding treatments underwent discriminant analysis using a partial least squares discriminant analysis (PLS-DA) approach. PLS-DA is a classification method using dummy *Y*-variable values of 2 for the target category and 1 for the other categories being discriminated. This procedure was applied separately to each of the three categories (P, S and PS lambs), so producing three discriminant models. The models were tested via a cross-validation procedure, in which a quarter of all data samples (i.e. 56 randomly chosen samples) were temporally removed from the initial data set to be used for validation. The PLS-DA model was developed based on the calibration samples and used to classify the validation samples. This procedure was repeated four times, i.e. until all data set samples had been used through the validation procedure. Before chemometric analysis, the data were normalized. Sample assignment to a specific category was made on the basis of the predicted *Y*-value. Three predicted dummy values (corresponding to the three models developed using the P, S and PS calibration samples) were attributed to each sample, the sample then being assigned to the category for which the predicted dummy value was closest to 2. A principal component analysis (PCA) performed beforehand was used to rank the reflectance spectra from each feeding treatment according to the Mahalanobis distance (H) from the average reflectance spectrum, in order to detect sample outliers ($H > 3$). No outliers were found.