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ABSTRACT

The use of near-infrared spectrometers (NIRS) for predicting meat quality traits directly in the abattoir was tested with three trials. For the calibration trial, spectra were acquired from the cross-cut surface of the Longissimus thoracis muscle on 1166 carcasses of Piemontese young bulls with a portable visible-near-infrared spectrometer (Vis-NIRS) and with a small hand-held instrument (Micro-NIRS). A sample of the same muscle was analyzed to provide the reference. Validation statistics of the two instruments were similar. Predictabilities of meat color and purge loss were good, whereas for the other traits they were less promising. The repeatability trial showed that post-slaughter factors, not predictable by NIR spectra collected in the abattoir, affect reference meat quality values. A trial under operative conditions showed that both spectrometers were able to capture the major sources of variation in most of the meat quality traits. Overall, NIRS could be used to predict the animals' "native" characteristics exploitable for genetic improvement of meat quality traits.

INTRODUCTION

The quality of meat depends on many different properties that can be determined by meat sampling, instrumental laboratory analyses, and/or sensory description by a trained panel of experts (Przybylski & Hopkins, 2016). The analysis of meat quality traits is therefore almost always carried out for research purposes (Hocquette, Bauchart, Micol, Polkinghorne, & Picard, 2016).

Quality control in the beef industry, besides for sanitary reasons, is often limited to the evaluation of carcass quality on the basis of mus- cularity and fatness (Brad Kim, Channon, D'Souza, & Hopkins, 2016). When meat is to be evaluated on a freshly-cut muscle section, as in the case of a carcass side divided into two quarters, certain traits (muscle development, color, marbling) are subjectively scored or undergo computer-aided visual inspection (Jackman, Sun, & Allen, 2011). In practice, the beef industry lacks reliable methods that are also rapid, objective and cheap for predicting beef physical traits and that can be applied at-line in the abattoir. As a consequence, there is currently no affordable payment system based on meat quality at the commercial level, nor phenotyping procedure, which is needed to establish a se- lection program for genetically improving meat quality traits.

Some attempts have been made to use infrared spectroscopy for predicting the meat quality traits of different species, as reviewed by Karoui, Downey, and Blecker (2010) and Prieto, Pawluczyk, Dugan, and Aalhus (2017), and to use these predictions for genetic purposes. In the case of beef production, a previous study (Cecchinato, De Marchi, Penasa, Albera, & Bittante, 2011) on the use of near-infrared spectro- scopy (NIRS) in the laboratory on aged meat samples collected in the abattoir showed that this could be a valuable method for phenotyping beef carcasses, estimating genetic parameters, and predicting the breeding values of the sires of slaughtered animals for meat color traits, purge loss, and also some fatty acids (Cecchinato et al., 2012), but not for cooking loss and shear force.

Laboratory NIRS instruments can help reduce the cost of some meat analyses, but they cannot be the basis of a routine system of meat quality prediction for either commercial or genetic purposes. The col- lection of meat samples from every carcass, and the subsequent trans- portation, and processing in the laboratory depreciate the carcass and increase the labor requirement and cost of analysis.

Since portable NIR spectrometers have become available, there has been increasing interest in testing their use in the abattoir or in meat processing units, which would eliminate the need to take samples and transport them to the laboratory (De Marchi, 2013; Piao, Okura, & Irie, 2018; Wang, Peng, Sun, Zheng, & Wei, 2018). The suppliers of spec- trometers are now offering instruments with very different character- istics. Some of them also cover the visible part of the spectrum (Vis- NIRS), making them particularly suitable for predicting meat color traits (Qiao et al., 2015). Moreover, very small instruments (Micro- NIRS), previously used for at-line industrial applications, have been adapted for hand-held use (Wiedemair, De Biasio, Leitner, Balthasar, & Huck, 2018; Zamora-Rojas, Garrido-Varo, De Pedro-Sanz, Guerrero- Ginel, & Pérez-Marín, 2013; Zamora-Rojas, Pérez-Marín, De Pedro- Sanz, Guerrero-Ginel, & Garrido-Varo, 2012), although they have not been widely tested for predicting beef quality.

We hypothesized that predictions of meat quality traits could be simply, rapidly and cheaply obtained directly in the abattoir using small infrared spectrometers with the aim of either genetically improving the population, or monitoring quality for commercial purposes. The specific objectives of this study were: a) to test the use of infrared spectrometers in the abattoir on a large number of carcasses to predict beef quality traits; b) to compare the predictions obtained from a top-of-the-range portable instrument using a wide spectrum (Vis-NIRS) with those obtained from a very small hand-held spectrometer (Micro-NIRS) through cross-validation and external validation; c) to analyze and compare the sources of variation in laboratory-measured beef quality traits, beef infrared absorbance spectra, and NIRS-predicted beef quality traits; and

d) to field test the ability of NIRS predictions to identify the main sources of variation (beef production system, farm within system, parity of dam, season of birth, batch of slaughter and carcass weight) in beef quality traits (pH, color traits, purge loss, cooking loss, meat shear force).

MATERIALS AND METHODS

Farms and animals

This study utilised a sub-set of 1166 animals from a previous re- search described by Savoia et al. (2019b). The larger study was carried out on 1327 young bulls from 115 farms operating six different beef farming systems. The clustering and characteristics of the beef systems, feeding practices, and animal slaughter, are described in detail by Savoia et al. (2019b).

Briefly, the young bulls selected for the study were all enrolled in the Herd Book of the Italian Piemontese breed, were sired by artificial insemination bulls, and were reared on commercial farms re- presentative of the farming systems in the Piemonte region (north- western Italy). The average age at slaughter was about 18 months (541 ± 63d), the average carcass weight was 438 ± 44 kg, (corre- sponding to an average carcass daily gain of 0.82 ± 0.11 kg/d), and 66.7% of the carcasses had a muscularity grading of E (excellent) de- termined according to the SEUROP classification system (Commission of the European Communities, 1982).

Experimental design

The study comprised three specific phases:

- Calibration/validation trial, to achieve objective a) to test portable NIRS instruments in the abattoir on a large number of carcasses, and objective b) to compare a top-of-the-range portable instrument with a very small one;

- Repeatability trial, to achieve objective c) to analyze the sources of variation in measured beef quality traits, beef infrared absorbance spectra, and NIRS-predicted beef quality traits;

- Testing under commercial conditions, to achieve objective d) to test at the population level the ability of NIRS predictions to identify variations in beef quality traits.

NIRS instruments and spectra collection

Two very different spectrometers were tested in this study and the most important characteristics differentiating them are summarized in Table 1. Vis-NIRS is a top-of-the-range instrument that collects an ex- tended spectrum spanning the visible and NIR sections of the electro- magnetic wave interval (wavelength: 350 to 1830 nm) measured every 1 nm (1481 data points per sample). Micro-NIRS is a very small in- strument (weighing 60 g vs the 5600 g of the Vis-NIRS) with a shorter spectrum spanning only the NIR section (905 to 1649 nm) measured every 6 nm (125 data points per sample).

Spectra were collected with both instruments in the abattoir after each carcass side had been divided into two quarters (the pistol cut) the day after slaughter (about 24 h). The instruments were first calibrated using a standard white barium sulfate surface, and the spectra were then collected on the surface of a cross-section of the Longissimus thoracis muscle between the 5th and 6th ribs. Five spectra were ob- tained with each instrument in reflectance (R) mode from different sites on the same cut muscle surface. Each spectrum represented the average of three replicates on the same position. The average and standard deviation intervals of the absorbances obtained as log(1/R) from the two spectrometers are shown in Fig. 1.

Spectral data editing and processing

In order to compare the two technologies/instruments without the confounding effects of differences in data processing, meat spectra were analyzed in same statistical environment (RStudio, version 3.4.1) rather than in the native software installed in the instruments. Each spectrum was centered and standardized, and the Mahalanobis distance (De Maesschalck, Jouan-Rimbaud, & Massart, 2000) was then calculated. Spectra with a Mahalanobis distance greater than the square root of the critical value of a chi-squared distribution with $\alpha = 0.001$ and degrees of freedom equal to the number of wavelengths were treated as outliers and discarded.

Beef quality reference analyses

The collection and processing of the meat samples, and the analyses of meat quality traits are described in detail by Savoia et al. (2019b).

Briefly, the meat quality traits of all the carcasses were analyzed on a section of the same muscle as that used for spectral acquisition (Longissimus thoracis at the level of the 6th thoracic vertebra) after one week of ageing under vacuum at 4 °C. The meat quality traits were analyzed according to the methods recommended by the Commission of the European Communities (Boccard et al., 1981):

- pH, measured 3 times using a portable Crison pH-meter equipped with a glass electrode;

- color traits, averages of 3 measures taken on the freshly-cut surface after 1 h of blooming at 4 °C using a Minolta CR-331C colorimeter and expressed in CIELAB coordinates: Lightness (L*), redness (a*) and yellowness (b*); Hue angle (H*) and Chroma (C*) were calcu- lated as H* = tan-1 (b*/a*) and C* = (a*2 + b*2)0.5;

- purge loss (PL, %), was determined as the difference between sample weight at packaging and sample weight after ageing, ex- pressed as a percentage of original sample weight

- cooking loss (CL, %), computed as the difference between the weight of the sample cut before and after cooking in a sealed bag immersed in a water-bath until it reached an internal temperature of 70 °C (Honikel, 1998), expressed as a percentage of the raw meat sample;

- shear force (SF, N), determined on six 1.27 cm-diameter cylindrical cores of cooked meat with a V-shaped Warner-Bratzler blade fitted to an Instron Universal Machine model 5543 (AMSA, 2015).

Calibration/validation trial

The data for this study were obtained from 1166 Piedmontese young bulls from 95 farms, and consisted in the meat quality traits of each animal measured on a meat sample and two average NIR spectra (one per instrument).

A Bayesian model (Bayes B) implemented in the BGLR library of the R software (Pérez & de los Campos, 2014) was used to develop cali- bration equations for each meat quality trait, as described by Ferragina, De Los Campos, Vazquez, Cecchinato, and Bittante (2015). The data from each instrument were partitioned into a calibration sub-set con- taining 80% of the observations randomly selected, and a cross-vali- dation sub-set containing the remaining 20% of the observations. This procedure was repeated 15 times for each trait. Determination coeffi- cients, calculated as the square of the correlation between the observed and predicted values in the calibration set (R2) and in the cross-validation set (R2), were used to evaluate the accuracy of the predictions. As the most important source of variation in meat quality traits was shown to be the slaughter batch formed by animals slaughtered on the same date, with their meats aged together and analyzed on the same day (Savoia et al., 2019b), external validation was also carried out. This was done by predicting the observations for all the animals slaughtered in a batch from the regression equations developed from the data from all the other batches, and repeating this procedure for every slaughter batch (leave-one-batch-out procedure). The determination coefficients (R2Ext) were then calculated only once on the final dataset, containing all the predictions from the “leave-one-batch-out” procedure.

Repeatability trial

Analysis of the most important sources of variation, and evaluation of the repeatability of the reference beef quality analyses, the absor- bance of meat spectra at the level of each individual wavelength, and the meat quality traits predicted with both spectrometers were per- formed on 30 young bulls' carcasses. A double thickness meat sample was collected from each side of each carcass (60 sides) so that two replicated meat quality trait analyses were performed per side (four data point for animal, 120 in total). In addition, 5 spectra were taken from different sites on the cross-sectional area of the muscle of each side (300 spectra in total), each being the average of three replicates from the same site. The predicted beef quality traits were obtained by ap- plying the equations developed in the calibration trial to the individual spectra of each cross-sectional muscle position (300 predictions per trait).

The sources of variation in the data obtained were quantified using the Mixed Procedure in SAS (2013) and the following statistical model:

y = slaughter batch + animal + carcass side (animal) + Ɛ

where y is the vector of the traits being considered (analytical va- lues for each of the meat quality traits; absorbances at every wave- length of the spectrum on each muscle site by the two NIR spectro meters; predicted values for each of the meat quality traits by the two NIR spectrometers). The terms slaughter batch, animal, and carcass side (nested within animal) are random variables assumed to have σ2 , σ2 , and σ2 variances, respectively; ε ~ N(0, σ2) is the random residual term. Parameters from the mixed model were estimated using the re- stricted maximum likelihood method (REML).

Different repeatability indices were then computed for the mea- sured beef quality traits:

- Sample repeatability = (σ2 2 + σ2) /

Animal repeatability = σ2/ (σ2 + σ2)

The same repeatability indices were also computed for the predicted beef quality traits obtained from both NIR spectrometers using all in- dividual spectra (5 per muscle section).

As it is common to use the average of 5 individual spectra to develop calibration equations, the repeatability indices of the average spectra were also computed:

- Sample repeatability = (σ2 + σ2)/ (σ2 + σ2 + σ2)

- Animal repeatability = σ2 / (σ2 + σ2)

The repeatability of the absorbances at every individual wavelength was computed as for the sample repeatability. Only one value of measured meat pH and purge loss per side was taken, so the sample repeatability could not be estimated.

Testing under commercial conditions

To evaluate the ability of the NIRS predictions, based on the meat absorbance spectra as the only source of information, in capturing the effects of the major sources of variation affecting the measured traits, the data were analyzed using the Mixed Procedure in SAS (2013). The laboratory measured traits were compared with the Vis-NIRS and Micro-NIRS predictions (those used for the external validation ac- cording the previously described "leave-one-batch-out" procedure) using the following model:

y = birth season + parity of dam + production system + carcass weight + farm(production system) + slaughter batch + Ɛ.

where y is the observation in each of the measured or predicted meat quality traits; birth season, parity of dam and production system are the fixed effects of the season of birth of the young bulls in 4 classes (January–March, April–June, July–September, October–December), the parity of the dam in 4 classes (1st, 2nd, 3–8, > 8), and the production system classified in 6 classes according to Savoia et al. (2019b); carcass weight is a fixed effect in 5 classes (< 350 kg, 350–400 kg, 401–450 kg, 451–500 kg, > 500 kg); farm(production system) is the random effect of the fattening farm nested within production system (98 levels); slaughter batch is the random effect of the day of slaughter (117 levels); and ε is the random residual term. Farms, slaughter batch and ε were assumed to be normally and independently distributed ~N(0, σ2). A minimum cell size of 3 observations was required for both the slaughter batch and farm effects. The least square means of fixed effects were compared with a Tukey-Kramer test (P < 0.05).

RESULTS

Calibration trial

Descriptive statistics of the meat quality traits of the Piemontese young bulls analyzed with the reference laboratory methods are shown in Table 2. These meat quality traits were reported and discussed in a previous survey (Savoia et al., 2019b).

Table 2 also reports the accuracy of the predictions of beef quality traits obtained from the spectra taken from the cross-sectional area of the Longissimus thoracis exposed in the abattoir when the carcass sides were divided to obtain the pistol cut the day after slaughter. The R2 varied from 0.51 (a* by Micro-NIRS) to 0.88 (L* by Vis-NIRS) for color traits, but was much lower for purge losses (0.29 by Vis-NIRS), cooking losses (0.26 by Vis-NIRS), and shear force (0.34 by Vis-NIRS). The only exception was pH predicted by Vis-NIRS with R2of 0.57). The R2 values were always greater for the predictions from the Vis-NIRS than for those from the Micro-NIRS. At cross-validation, the R2 values were always smaller than the R2 , especially for the equations based on Vis-NIRS. The external va- lidation, based on the prediction of individual batches of carcasses from the calibration equations developed using all the other batches yielded similar values, with no notable differences between the two spectrometers. The R2

values ranged from 0.52 to 0.80 for color traits, and were lower than 0.32 for the other meat quality traits (Table 2). As expected, the differences between the SD of the measured traits and the RMSEEXT of the corresponding predictions were related to the R2.

Repeatability trial

To better understand the differences in the degrees of accuracy of the predictions of meat quality traits through NIRS calibration equations, the sources of variation in the reference and predicted traits were quantified and are summarized in Table 3.

The effect of the slaughter batch on sample variability of beef quality traits measured in the laboratory was moderate, ranging from 5% (for a*) to 28% (for pH), with the exception of shear force where it accounted for 55% of total variation. The effect of carcass side was always very small (≤ 14%), and the residual variation among replicates was also small (≤ 25%), with the notable exception of purge loss where it represented more than half the total variance. As expected, animal was the major source of variation for pH and color traits (≥ 58%), but represented a much smaller proportion of the total variance for purge loss (25%), cooking loss (44%) and shear force (23%).

The overall results gave sample repeatability for the physical analyses of meat ranging from 75% for cooking losses to 93% for L*. As expected, animal repeatability was lower than sample repeatability: −5 to −10 percentage points for color traits, and − 22 and − 40 per- centage points for cooking loss and shear force.

Moving on to the NIR spectra, the proportions of different sources of variation out of the total variance in the absorbance at each individual wavelength was highly dependent on the wavelength, as clearly shown in Fig. 2. Examination of the variability in the spectra yielded by Vis- NIRS shows that the visible light section of the electromagnetic spec- trum (350 to 750 nm) is characterized by largely heterogeneous waves (especially for violet radiations). The first section of the near-infrared spectrum (750 to 1300 nm) is much more homogeneous than the rest of the spectrum, and is characterized by high variability attributed to in- dividual animals (about 50% of total variance), whereas the remaining variability is explained by the other three sources of variation examined here (slaughter batch, carcass side, and muscle sampling/residual variability) at similar levels. This pattern explains the sample repeat- ability (75–80%) shown by the visible red and the first portion of the infrared radiations.

In the fraction of the electromagnetic spectrum with wavelengths ranging from 1300 to 1400 nm there was a dramatic change in the proportions of the different sources of variation: over 1400 nm the absorbance of the meat samples was strongly affected by the specific site within the cross-sectional area of the muscle (position dependent), and the effect of animal and the repeatability of the measurement fell to very low values.

It is worth noting that in the section of the spectrum they have in common, Micro-NIRS and Vis-NIRS exhibited very similar patterns.

The strong dependence of the meat spectrum on position within muscle section area explains the large proportion out of the total var- iance represented by the variation in residual/muscle site in the NIRS predictions of meat quality traits, which accounted for 18 to 78% of total variance in the case of Vis-NIRS, and 17 to 58% of total variance in the case of Micro-NIRS (Table 3). The corresponding values for the reference analyses were 7 to 56% of the total variance. The animal effect was the second largest source of variation for all meat traits in the case of Micro-NIRS, and for the majority of the traits obtained from Vis- NIRS. Slaughter batch was a greater source of variation than carcass side for most of the traits with both instruments (Table 3).

The high incidence of residual/muscle site variance in the absor- bances of many NIR wavelengths explained the lower sample and animal repeatabilities of predicted traits obtained with both instru- ments compared with those obtained with the reference analyses.

The above-mentioned repeatabilities refer to predictions obtained from a spectrum representing the average of three replicates taken at a single position on the muscle. To overcome this variability, in practice the average of several spectra taken at different positions on the muscle cross-sectional area are often used, as in the calibration trial reported in this study. In this case, the sample and animal repeatabilities calculated after excluding the residual/muscle site component are more in- formative. These were based on the average of 5 spectra taken from different positions on the muscle section area with both infrared spec- trometers, and were, with few exceptions, similar to or greater than those measured by the reference analyses (Table 3).

Testing under commercial conditions

Tables 4 and 5 report the descriptive statistics, the proportion of total variance captured by random effects (slaughter batch and farm within beef production system), and the F-value and significance of the fixed effects (birth season, parity of dam, beef production system and carcass weight classes) for each beef quality trait and each analytical method (laboratory reference, Vis-NIRS and Micro-NIRS predictions). As carcass weight was by far the greatest source of variability, the least squares means of this trait and their comparisons are also included.

The general means of color traits were almost identical across the analytical methods, whereas the standard deviation of the predicted traits tended to be lower than that of the corresponding laboratory reference (Table 4). The proportion of total variance captured by random effects (batch and farm) were similar across analytical method within trait. Season of birth and parity of dam were never significant, neither in measured nor in predicted traits. The effects of beef pro- duction system was seldom significant across different traits and its effect was always small. The LSMs followed a similar trend (data not shown).

The class of carcass weight was the most important factor affecting all the color traits, regardless of analytical method. Moving from the lightest to the heaviest carcasses, all the color traits predicted by both spectrometers showed the same trend of the corresponding measured traits, with the degree of difference between the two extreme classes almost unchanged across different analytical methods.

In the case of the other meat traits (Table 5), the general means were also unaffected by analytical method, whereas the decrease in the standard deviation of the predicted values compared with the reference values was greater than in the case of color traits. The variance in slaughter batch as a percentage of the total variance was in general higher than the variance in color traits (with the exception of purge loss) and always lower in the predictions from Micro-NIRS than in those from Vis-NIRS. The effect of fattening farm within beef production system was small in all traits regardless of analytical method and the effects of season of birth and parity of dam were never significant. The only exception was the effect of birth season on measured purge loss. The effect of beef production system was significant only for meat pH predicted by Micro-NIRS and for cooking loss predicted by Vis-NIRS.

The results for class of carcass weight were more variable in these traits than in the color traits. In the case of meat pH, the effect was always negligible, although it was significant in the case of the re- ference values. In the case of purge loss, the effect was highly sig- nificant and increased from the lightest to the heaviest carcasses with all analytical methods, although the difference between the extreme classes was greater (+1.03%) for the reference than for the Vis-NIRS (+0.59%) and Micro-NIRS (+0.61%) predicted values. Carcass weight had a significant effect only on laboratory measured cooking loss, but had an erratic pattern. Lastly, shear force increased with increasing carcass weight in a similar way with all three methods, but the effect was significant only in the case of the predicted values.

DISCUSSION

Use of portable and hand-held NIRS instruments for beef quality prediction in the abattoir

The large majority of studies on NIRS prediction of meat quality traits have been carried out in the laboratory using bench-top spec- trometers (Prieto et al., 2017). Automatic at-line evaluations in the abattoir, often using image analysis, are limited to the size and con- formation of carcasses (Craigie et al., 2013). In this study, portable and hand-held NIR spectrometers were used in the abattoir on the Long- issimus thoracis muscle sectional area exposed after dividing the car- cass halves into fore and rear quarters. Spectra were manually acquired, but this operation could be robotized in large slaughterhouse plants.

The Vis-NIR portable spectrometer used in this study is a top-of-the- range instrument characterized by a wide spectrum spanning from the visible to the infrared sections and yielding a very large number of data for each spectrum (1481 absorbance measures). There are very few direct comparisons of different instruments in the scientific literature, especially focusing on portable vs bench-top NIR spectrometers. In a previous study (De Marchi, Penasa, Cecchinato, & Bittante, 2013) the Vis-NIRS was compared with a bench-top spectrometer specifically designed for analyzing food samples (Foss, Foodscan) based on pre- dictions of the meat quality of different beef samples. The Vis-NIRS yielded R2 for the prediction of meat quality traits that were almost identical to those obtained in this study and, except for cooking loss and shear force, yielded RMSE larger than those of the present study. Fur- thermore, De Marchi et al. (2013) showed that prediction performance of the bench-top spectrometer was always lower than that of the Vis- NIRS as a consequence of a narrow spectral range and a lower number of measured absorbance values (100) per sample. The same results were obtained comparing the predictions of color, texture and composition traits by the same instruments on a large number of cheeses belonging to 37 different categories (Stocco, Cipolat-Gotet, Ferragina, Berzaghi, & Bittante, 2019).

It is difficult to compare the results from different studies using single instruments due to the many sources of variation (Prieto et al., 2017) affecting the NIRS predictions (animals slaughtered, type of muscle and position within muscle, slaughter and dissection processes, ambient conditions, ageing, reference analyses carried out, pretreat- ment of spectra, calibration methods, validation procedure, etc.). In a previous study on Piemontese young bulls, a bench-top spectrometer characterized by a wide spectrum (Foss NIRSystem 5000; 1100 to 2498 nm) was used on ground meat samples to investigate the possi- bility of predicting meat quality traits (Cecchinato et al., 2011). The prediction abilities obtained for color traits (R2 ranging from 0.44 to 0.81) were similar to those found in our study with Vis-NIRS (0.62 to 0.88) and Micro-NIRS (0.51 to 0.81). The ability of NIRS to predict meat color has been reported in a number of studies using both ground (Prieto, Andrés, Giraldez, Mantecon, & Lavin, 2008) and intact samples (Leroy et al., 2003; Prieto et al., 2009). Although the spectra were collected under operational conditions and directly in the abattoir from the carcass the day after slaughtering and the color was measured on aged meat, the predictions of color traits obtained in this study had higher R2 and lower RMSE values than those obtained in studies conducted in laboratory conditions (Andrés et al., 2008; Magalhaes et al., 2018). The predictions of the other meat quality traits obtained in this study were less accurate and very similar for the two spectrometers. For pH, the R2 was lower than most of the literature reports (Andrés et al., 2008; De Marchi et al., 2013; Prieto et al., 2008), although RMSEEXT in this study was better than that reported by De Marchi et al. (2013). The very low variation in the pH measurements (CV 0.9%) may account for the modest prediction ability of the spectra (Prieto et al., 2009). The low R2 values for purge and cooking losses found in our study are in the range of the published literature (Andrés et al., 2008; Leroy et al., 2003) and slightly higher than the findings of Cecchinato et al. (2011). The NIRS technology generally has poor prediction ability for water-holding traits as they are indirectly predicted from their as- sociation with the wavelengths of chemical compounds, which is often weak (Prieto et al., 2017).

The accuracy of NIRS prediction of meat shear force was also very limited (R2 0.34 for the Vis-NIRS and 0.16 for the Micro-NIRS, RMSEEXT 10.96 for both instruments) but similar to the results obtained in a previous trial on Piemontese young bulls using a bench-top in- strument (Cecchinato et al., 2011). Muscle heterogeneity makes shear force a difficult trait to predict by infrared spectroscopy, particularly when NIR spectra are collected on ground samples (Prieto et al., 2017). In the literature there is a large variation in the estimates of prediction ability of NIRS for this trait: some authors report moderate values (around 0.5) of R2 of cross-validation (Andrés et al., 2008; Magalhaes et al., 2018), but in most cases the predictions had much lower values (Leroy et al., 2003; Prieto et al., 2008).

Unlike Vis-NIRS, Micro-NIRS has seldom been used in the analysis of different types of meat (Wiedemair et al., 2018; Zamora-Rojas et al., 2013, 2012), and has never been used on large surveys of beef physical traits.

In general, the results reveal that the ability of portable or hand- held spectrometers to predict meat quality traits in the abattoir is comparable to that of bench-top instruments in laboratory conditions.

Comparison of predictions obtained from vis-NIRS and micro-NIRS

We tested two very different NIR spectrometers in this study. The hand-held Micro-NIRS was developed for industrial use, in particular for at-line monitoring of materials during processing. Its average linear size is about one sixth that of the Vis-NIRS, it is almost one hundredth of the weight, and its cost is much lower. The spectrum extension of the Micro-NIRS (wavelengths 905 to 1649) is about half that of the Vis- NIRS, and the frequency of measurement is one sixth (every 6 vs 1 nm), so that the total number of absorbance measures per spectrum is about 12 times smaller (125 vs 1481 measures per sample). The R2 values were always better with the Vis-NIRS, but the R2 were about the same.

Comparing 5 different sources of information in a discrimination analysis of different farming systems on milk and cheese samples, Bergamaschi, Cipolat-Gotet, Cecchinato, Schiavon, and Bittante (2020) found that the methods that yielded a larger number of data per sample generally had much better R2 values, but this effect disappears with external validations on different datasets.

The different measures of repeatability obtained with the two in- struments were quite similar; this was true also in the case of the color traits, although the Vis-NIRS would be expected to perform better be- cause of the extension of the spectrum to the visible light. When the spectra in the range of visible light is missing, the absorption in the NIR region related to chemical bonds of organic matter (protein and lipids) could explain the ability of Micro-NIRS in the prediction of color (Cecchinato et al., 2011). Indeed, as described in detail by Hernández, Sáenz, Alberdi, and Diñeiro (2016), although hue and b* color para- meter of meat are depended on oxidation status of myoglobin, Light- ness, a* and Chroma parameters are dependent, respectively, on total myoglobin content and on oxymyoglobin content.

The two spectrometers compared in this study produced similar results in terms of prediction accuracy in external validation. However, there are differences in their suitability for practical use in the abattoir. The Vis-NIRS requires a physical support, and, for prolonged use, connection to an external power source or to a supplementary battery, whereas the Micro-NIRS is similar in size and weight to a computer mouse, and is operated directly on the muscle surface, being connected to a portable computer or a tablet through an USB cable.

Sources of variation and repeatability of measured and infrared predicted beef quality traits

The authors are unaware of any study comparing the sources of variation in laboratory reference meat quality traits, in infrared ab- sorbances and in infrared-based meat quality predictions on the same carcasses. Meat is a very heterogeneous material, subject to continuous modifications, and largely influenced by environmental conditions and processing procedure, which explains why meat quality traits are af- fected by sampling factors (day of sampling, samples from different sides, muscle, portions within muscle, etc.), and by analytical factors (sample processing, laboratory conditions, exposure to air and light, instrument calibration, etc.). In this study sample repeatability of the reference analyses on 7-day-aged meat samples ranged between 75 and 93% (Table 3), while animal repeatability, with the exception of purge loss, ranged between 52 and 83%. These figures are much lower than those usually obtained from chemical composition analyses. A NIR spectrum taken in the abattoir 24 h after slaughter on the intact cross- sectional surface of the muscle after quarter separation can probably predict the "native" characteristics of meat. However, the subsequent steps, involving the collection, vacuum-packaging, chilling, transportation, ageing, preparation and analysis of samples, as well as instrument calibration and the operator's skill, cannot be predicted by NIRS at abattoir. The expected maximum repeatability of a NIRS pre- diction is not 100% because it cannot exceed animal repeatability. However, the animal component is the information of interest for both the commercial and genetic uses of the predictions. The most important source of variation in the predictions of meat quality traits obtained from NIR spectra was related to the heterogeneity of the composition of the meat, which means that different position on the muscle surface have different reflecting abilities. While waiting for external probes or spectrometers able to acquire spectra on a wider area to become available, the only possibility to overcome this problem is to collect more spectra from different position on the muscle. Near-infrared hy- perspectral imaging could be another way to obtain a more re- presentative picture of muscle quality. This technique is based on the construction of a three-dimensional "hyper-cube spectral image" composed of one NIR spectrum for each of the many thousands of pixels in the entire image of the cross-section of the muscle sample being analyzed (Xiong, Sun, Zeng, & Xie, 2014). Using this complex method, ElMasry, Sun, and Allen (2012) obtained R2 values in the range 0.73 to 0.88 for meat lightness, yellowness, pH and shear force from 27 young bulls of dairy breeds.

Moreover, a large variation in the relative importance of the variance components, particularly animal and individual muscle site, was observed along the different sections of the electromagnetic spectrum. As a consequence, the relative importance of the variance components of the predicted traits also varies according to the most important in- dividual wavelengths in the prediction equations.

However, using multiple spectra per animal we were able to obtain higher animal repeatability of the meat quality predictions than that of the reference methods with both instruments. This means that NIRS predictions have the potential to better capture animal "native" characteristics related to genetics, as these are not influenced by the post- sampling conditions of the meat.

Field factors testing at slaughterhouse and implications for commercial and genetic purposes

It has often been observed that R2 has a tendency to overestimate the effective reliability of instruments yielding a great number of data points per sample analyzed (Bergamaschi et al., 2020), meaning that this parameter is not very useful for evaluating the predictive ability of NIR spectra. Furthermore, R2 is not always a good indicator of the actual prediction accuracy, particularly when samples of different ori- gins (farms, batches, abattoirs, cuts, etc.) from those included in the calibration dataset are to be predicted. For complex traits, such as those related to meat quality, even R2 (and related RMSEEXT and RER ratio) is not sufficient for adequately evaluating predictive performance. As outlined by Lo, Chernoff, Zheng & Lo, Chernoff, Zheng, and Lo (2015), significant variables are not necessarily good predictors. The determi- nation coefficient is a rather rough statistic, unable to decompose the prediction errors according to their possible source of variation. A field factors testing at slaughterhouse to evaluate the ability of a predictive equation to capture the effects of the major sources of variation can provide further information about possible incomplete or biased esti- mations. A previous study on methane emissions from dairy cows predicted from milk infrared spectra (Bittante & Cipolat-Gotet, 2018) also showed that prediction equations of modest accuracy (R2 of about 0.50) were able to fully capture the effects of the main sources of variation (dairy farming system, individual farm, parity, lactation stage) and that this capability did not always correlate with the de- termination coefficient. A similar test carried out in this study showed that the predictions of color traits and purge loss were able to capture the effects of the main sources of variation in traits in a manner very similar to those yielded by statistical analysis of the reference values. This allows us to speculate that these predictions, from both spectrometers, may be able to also capture genetic variability, as confirmed for laboratory instruments (Boukha et al., 2011; Savoia et al., 2019a). This hypothesis, to be confirmed by further specific research, could open new perspectives for the genetic improvement of meat quality. Indeed, Vis-NIR spectra data collected at slaughterhouse, being easily and cheaply achievable on large number of carcasses, could be used for progeny testing the sires and dams of slaughtered animals. Then, once a reference population consisting of animals with both reliable breeding values and genotypes is available, genomic breeding values for meat quality traits could be estimated for genotyped young selection candi- dates.

The partial inability of infrared spectra taken in the abattoir after slaughter in predicting the fate of the meat sample after its collection, ageing, transportation and analysis need not be a disadvantage if the objective of the prediction is to capture the “native” quality of meat for its genetic improvement or for quality-based payments.

CONCLUSIONS

We tested portable and hand-held spectrometers in the abattoir on a large number of carcasses and obtained good results for the prediction of color traits and purge loss, but less reliable results for predicting meat pH, cooking loss and shear force. The inability of the infrared spectra taken after slaughter to predict the fate of the meat post-sampling till analysis, reflected by a reduction in the determination coefficients, need not be a disadvantage if the aim of prediction is to capture the animal's “native” characteristics, which is the case for the genetic improvement of beef cattle or carcass quality-based payments. The classical statistics of regressions of predicted over measured traits (R2, RMSE, RER, etc) cannot be considered good predictors (in the case of calibration and cross-validation statistics) or the only predictors (in the case of external validation) for evaluating the performance of infrared calibration eqs. A field factors testing at slaughterhouse on a large number of farms and animals showed that both spectrometers have a very good ability to capture the major sources of variations in color traits and purge loss, and also perform acceptably for predicted pH, cooking loss and shear force. Further research is needed to test the use of these predictions for the genetic improvement of beef cattle populations.

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Table 1
Main characteristics of the portable spectrometers used for predicting quality traits of beef.

	Vis-NIRS	Micro-NIRS
Instrument:		
Denomination	LabSpec 2500	Micro NIR Pro
Producer	ASD Inc.	JDSU
Address	Boulder (CO)	San Jose (CA)
Country	USA	USA
Characteristics:		
Type	portable	hand-held
Spectrometer size	12.7 × 36.8 × 29.2 cm	4,5 × 4,4 × 4.0 cm
Spectrometer weight	60 g	60 g
Sample preparation	none	none
Method	reflectance	reflectance
Operating temperature	0 + 40 °C	−20 + 40 °C
Spectra storage	internal	external PC or tablet
Connectivity/interface	10/100Base T Ethernet	USB 2.0, high speed (480 Mbps)
Power source	internal battery or electricity cable	USB 2.0, high speed (480 Mbps)
Illumination:		
Source	halogen	two vacuum tungsten lamps
Aperture	2.0 cm	2.5 cm
Light detection	external probe	internal
External probe size	26 × 10 × 5 cm	–
External probe weight	654 g	–
Detector type	Diode Array (Si,inGaAs)	InGaAs photodiode array
Measurement time	0.1 s	0.5 s
Optical fiber	Yes	–
Scanning method	external reference	external reference
Spectrum:		
Waves range	350–1830 nm	905–1649 nm
Data point interval	1 nm	6 nm
Data point per spectrum	1481	125
Replicates per spectrum	3	3
Spectra collected per sample	5	5
Absorbance calculation[1]	A = log(1/R)	A = log(1/R)

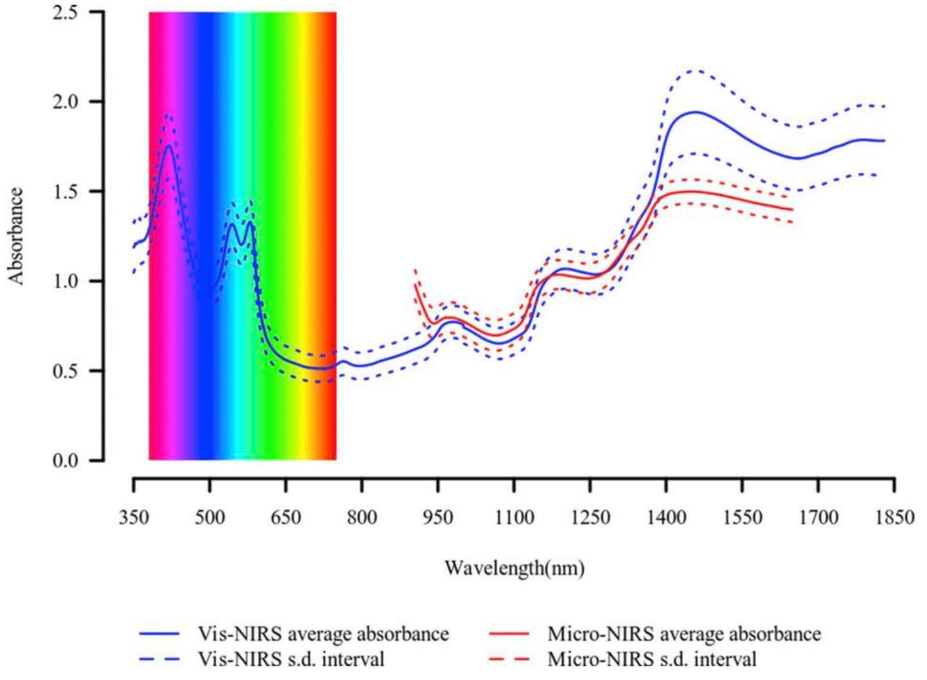

Fig. 1. Calibration: average (solid line) and standard deviation interval (between dotted lines) of absorbance spectra of 5-6th rib cross-sectional area of Longissimus thoracis muscle of 1157 Piemontese young bulls obtained using Vis-NIRS (blue color) and Micro-NIRS (red color) instruments (the spectrum of each animal was obtained as average of 5 spectra taken in different sites of the muscle sectional area, each one with three replicates).

Table 2
Descriptive statistics of reference Piemontese beef quality traits and performance of their prediction by Vis-NIRS and Micro-NIRS instruments.

Item	Meat pH	Color traits:					Water holding capacity (%)		Shear force
		L*	a*	b*	C*	H*	Purge loss	Cooking loss	(N)
Meat samples, N	1144	1147	1148	1150	1148	1146	1146	1157	1147
Mean	5.55	39.89	28.59	9.66	30.20	18.53	4.51	16.75	27.16
SD	0.05	3.49	1.74	1.66	2.14	2.04	1.19	3.43	9.61
Min	5.43	30.47	23.22	4.84	23.60	12.20	1.68	7.83	8.92
Max	5.72	50.80	33.92	14.44	36.67	23.60	8.04	26.83	56.98
Vis-NIRS									
R^2_{CAL}	0.57	0.88	0.62	0.70	0.64	0.72	0.29	0.26	0.34
R^2_{CV}	0.44	0.84	0.55	0.65	0.58	0.65	0.23	0.17	0.20
R^2_{EXT}	0.30	0.84	0.55	0.63	0.58	0.64	0.31	0.16	0.16
$RMSE_{EXT}$	0.05	1.43	1.22	1.06	1.48	1.27	1.05	3.36	10.69
Micro-NIRS									
R^2_{CAL}	0.30	0.81	0.51	0.63	0.55	0.65	0.20	0.10	0.16
R^2_{CV}	0.20	0.78	0.49	0.62	0.53	0.62	0.17	0.08	0.12
R^2_{EXT}	0.22	0.80	0.52	0.61	0.55	0.63	0.27	0.19	0.19
$RMSE_{EXT}$	0.05	1.67	1.23	1.04	1.45	1.23	1.07	3.20	10.69

Table 3
Variance components (as fractions of total variance), and sample and animal repeatability of quality traits of Piemontese beef measured in the laboratory or predicted using infrared spectra from two NIRS instruments (30 young bulls × 2 sides × 2 lab replicates or 5 spectra taken on different sites of the muscle section = 120 analyses or 300 spectra, each one obtained from 3 replicates on the same muscle site).

Item	Meat pH	Color traits					Water holding capacity (%)		Shear force (N)
		L*	a*	b*	C*	H*	Purge loss	Cooking loss	
Laboratory analyses:									
Total variance	1.4[e]	9.1	3.7	2.9	5.5	3.8	0.8	11.2	135.5
Variance components:									
Slaughter batch (σ^2_{SB})	0.28	0.14	0.05	0.15	0.06	0.24	0.18	0.17	0.55
Animal (σ^2_{An})	0.61	0.71	0.73	0.66	0.72	0.58	0.25	0.44	0.23
Carcass side (σ^2_{CS})	–	0.08	0.04	0.02	0.03	0.0[illegible]	–	0.14	0.13
Residual/muscle site (σ^2_{Re})	0.11	0.07	0.18	0.17	0.18	0.18	0.56	0.25	0.08
Repeatability:									
Sample repeatability[a]	–	0.93	0.81	0.83	0.82	0.82	–	0.75	0.92
Animal repeatability[b]	0.61	0.83	0.77	0.78	0.77	0.76	0.25	0.53	0.52
Vis-NIRS predictions:									
Total variance	3.2[e]	10.4	2.5	3.6	4.8	5.4	0.6	3.5	58.2
Variance components:									
Slaughter batch (σ^2_{SB})	0.09	0.11	0.27	0.33	0.24	0.33	0.26	0	0.26
Animal (σ^2_{An})	0.21	0.59	0.36	0.43	0.47	0.39	0.32	0.07	0.05
Carcass side (σ^2_{CS})	0.08	0.08	0.07	0.07	0.06	0.06	0.14	0.15	0.11
Residual/muscle site (σ^2_{Re})	0.62	0.23	0.30	0.18	0.23	0.23	0.28	0.78	0.58
Repeatability:									
Sample rep. Individual spectra[a]	0.38	0.77	0.70	0.82	0.77	0.77	0.72	0.22	0.42
Animal rep. Individual spectra[b]	0.23	0.65	0.50	0.63	0.62	0.58	0.42	0.07	0.07
Sample rep. Average spectra[c]	0.79	0.89	0.90	0.92	0.92	0.93	0.80	0.32	0.74
Animal rep. Average spectra[d]	0.73	0.88	0.84	0.87	0.89	0.87	0.68	0.32	0.31
Micro-NIRS predictions:									
Total variance	0.6[e]	9.6	2.7	1.8	2.6	2.5	0.3	1.1	13.3
Variance components:									
Slaughter batch (σ^2_{SB})	0.18	0.10	0.25	0.26	0.25	0.27	0.04	0.05	0.19
Animal (σ^2_{An})	0.05	0.55	0.49	0.44	0.43	0.42	0.51	0.44	0.14
Carcass side (σ^2_{CS})	0.22	0.13	0.09	0.10	0.10	0.11	0.15	0.16	0.09
Residual/muscle site (σ^2_{Re})	0.55	0.22	0.17	0.20	0.22	0.21	0.30	0.36	0.58
Repeatability:									
Sample rep. Individual spectra[a]	0.45	0.78	0.83	0.80	0.78	0.79	0.71	0.64	0.42
Animal rep. Individual spectra[b]	0.06	0.61	0.65	0.60	0.57	0.57	0.53	0.46	0.17
Sample rep. Average spectra[c]	0.52	0.83	0.89	0.87	0.87	0.87	0.78	0.76	0.78
Animal rep. Average spectra[d]	0.18	0.81	0.84	0.81	0.81	0.8[illegible]	0.76	0.74	0.60

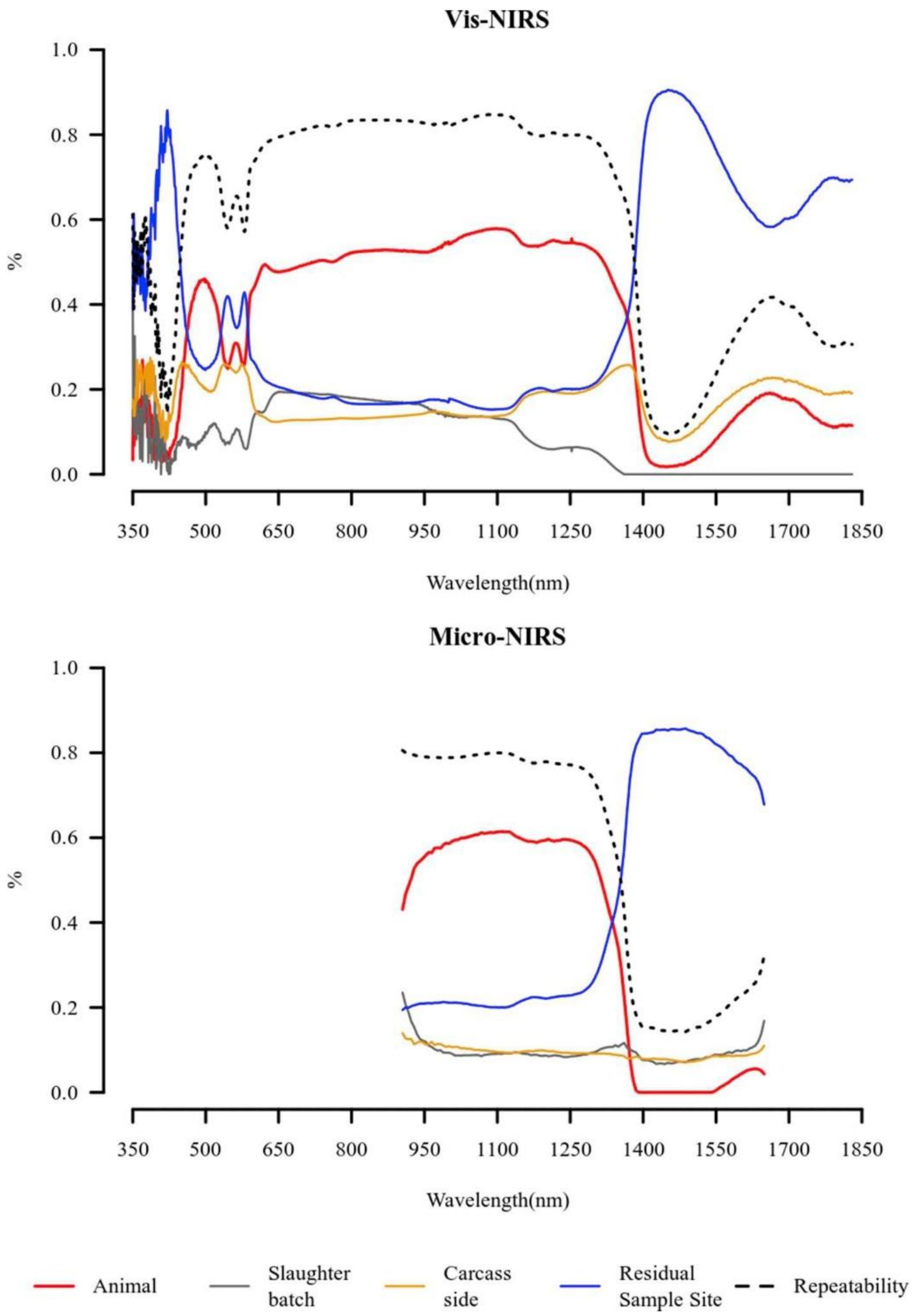

Fig. 2. Repeatability: Animal (red color), slaughter batch (grey color), carcass side (orange color), and site on cross-sectional area (residual, blue color) variance as fractions of total variance, and repeat- ability (black color, dotted line) of absorbance at each wave-number of 5-6th rib cross-sectional area of Longissimus thoracis obtained using Vis-NIRS and Micro-NIRS instruments (30 Piemontese young bulls × 2 sides × 5 spectra taken on different sites of the muscle section = 300 spectra, each one obtained from 3 replicates on the same muscle site).

Table 4
Comparison of the main sources of variation of meat color traits predicted by two NIRS instruments with those measured in the laboratory in terms of descriptive statistics, ANOVA, and effects of carcass weight (1166 young bulls, 1 meat sample analyzed per animal, 1 averaged spectrum per animal obtained from 5 spectra from different sites of the muscle surface, each one with 3 replicates).

	L^*			a^*			b^*			C^*			H^*		
	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS
General mean	39.8	39.8	39.8	28.6	28.6	28.6	9.7	9.6	9.7	30.2	30.2	30.2	18.5	18.5	18.5
Standard deviation	3.4	3.1	3.1	1.7	1.3	1.2	1.6	1.4	1.3	1.7	1.6	2.1	2.0	1.7	1.6
ANOVA															
Slaughter batch[a] (%)	19.7	18.0	15.8	24.0	34.0	21.3	22.2	27.3	15.6	23.7	21.3	23.5	21.6	25.9	18.2
Farm within system[a] (%)	7.3	7.6	8.3	5.0	5.3	3.4	4.3	4.8	3.2	2.9	3.8	4.8	3.7	3.6	5.4
Birth season (*F-value*)	0.2	0.2	0.3	2.0	1.1	1.8	1.6	1.5	1.3	1.0	2.2	2.6	1.3	0.4	0.8
Parity of dam (*F-value*)	0.5	0.8	0.6	1.2	2.6	0.3	1.3	0.8	0.4	2.7	0.2	1.2	0.8	0.4	0.3
Beef production system (*F-value*)	3.0*	2.4*	1.3	1.6	2.0	1.6	2.2	2.4	2.6*	1.9	1.6	1.7	1.9	2.5*	2.2
Carcass weight (*F-value*)	12.6**	10.0**	12.7**	23.7**	37.1**	38.0**	28.7**	33.3**	34.2**	37.8**	37.7**	24.3**	26.6**	32.2**	31.9**
Carcass weight (*LS-means*)															
< 350 kg	38.6a	38.6a	38.5a	27.7a	27.5a	27.5a	8.6a	8.5a	8.5a	28.7a	28.8a	29.1a	17.0a	17.1a	17.1a
351–400 kg	39.3a	39.3a	39.3a	28.3a,b	28.2b	28.2b	9.3b	9.2b	9.3b	29.7b	29.7b	29.8a,b	18.0b	18.0b	18.0b
401–450 kg	39.4a	39.5a	39.5a	28.5b	28.5c	28.5b	9.5b	9.5c	9.5b	30.1c	30.0c	30.0b	18.4b	18.3c	18.3b
451–500 kg	40.2b	40.1b	40.2b	29.0c	29.0d	28.9c	10.1c	9.9d	10.0c	30.7d	30.6d	30.7c	19.0c	18.8d	18.9c
> 500 kg	41.7c	41.3b	41.5c	29.8d	29.6e	29.5d	10.9d	10.6e	10.6d	31.4e	31.4e	31.7d	19.9d	19.7e	19.7d
RMSE	2.8	2.7	2.7	1.4	1.0	1.0	1.3	1.0	1.1	1.3	1.2	1.7	1.7	1.3	1.3

Table 5
Comparison of the main sources of variation of meat quality traits predicted by two NIRS instruments with those measured in the laboratory in terms of descriptive statistics, ANOVA, and effects of carcass weight (1166 young bulls, 1 meat sample analyzed per animal, 1 averaged spectrum per animal obtained from 5 spectra from different sites of the muscle surface, each one with 3 replicates).

	pH			Purge Loss (%)			Cooking Loss (%)			Shear force (N)		
	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS	Lab	Vis-NIRS	Micro-NIRS
General mean	5.55	5.56	5.55	4.51	4.46	4.50	16.7	16.8	16.7	40.6	41.0	40.9
Standard deviation	0.05	0.04	0.02	1.19	0.59	0.53	3.4	1.4	0.8	10.3	4.8	3.9
ANOVA												
Slaughter batch[a] (%)	64.8	50.8	48.1	14.0	24.3	19.0	40.4	55.8	14.7	42.6	54.1	39.5
Farm within system[a] (%)	4.2	6.3	4.7	7.8	4.6	4.4	3.8	2.3	2.3	6.8	5.3	7.0
Birth season (*F-value*)	0.1	0.4	1.4	6.3**	0.3	1.3	1.3	0.7	0.9	2.0	0.8	0.1
Parity of dam (*F-value*)	2.4	2.0	1.8	2.3	0.7	0.6	0.3	0.2	0.6	0.9	0.1	1.2
Beef production system (*F-value*)	2.0	1.7	2.7*	0.6	1.6	1.2	1.0	3.9**	1.9	0.4	1.0	0.2
Carcass weight (*F-value*)	4.4*	1.3	1.2	6.5**	12.5**	16.8**	4.6**	1.6	1.7	0.9	5.8**	2.4*
Carcass weight (*LS-means*)												
< 350 kg	5.55a,b	5.56	5.55	3.61a	4.11a	4.19a	15.2a	16.6	16.5	39.2	40.3a	40.5
351–400 kg	5.54b	5.55	5.55	4.44b	4.35a,b	4.37a,b	16.8b	16.7	16.6	40.8	40.5a	40.7
401–450 kg	5.55b	5.55	5.55	4.41b	4.40b	4.45b	16.8b	16.7	16.7	40.0	40.9a	40.8
451–500 kg	5.56a	5.56	5.55	4.59b	4.55c	4.59c	16.5a,b	16.9	16.7	39.9	41.6b	41.1
> 500 kg	5.56a,b	5.56	5.55	4.64b	4.70c	4.80d	16.0a,b	16.9	16.6	41.3	42.3b	41.9
RMSE	0.03	0.02	0.02	1.04	0.50	0.50	2.5	0.9	0.7	7.6	3.1	2.9