Innovative tools for phenotypic characterization and genetic improvement of meat quality in the Piemontese breed

S. Savoia[1,2,3], G. Bittante[2] and A. Albera[3]

[1]Interbull Centre, Department of Animal Breeding and Genetic, SLU, Uppsala, Swede
Corresponding author: simone.savoia@slu.se
[2]Department of Agronomy, food, natural resourses, animals and environment (DAFNAE),
University of Padova, Legnaro (PD), Italy
[3]Associazione Nazionale Allevatori dei Bovini di Razza Piemontese (Anaborapi),
Carrù (CN), Italy

Abstract

The aims of this study were: to predict meat quality traits (pH, color, purge and cooking losses, shear force) of Piemontese young bulls comparing two portable spectrometers (Micro-NIRS and Vis-NIRS); to estimate genetic parameters for measured meat quality traits and their predictions; to assess the possibility of the improvement of meat quality traits by genomics.

The study was carried out sampling 1,327 Piemontese young bulls, all registered in the Italian Piemontese Herdbook. Twenty-four hours after slaughter, absorbance spectra were collected directly at the abattoir with two very different portable spectrometers after the division of carcasses in two quarters on the exposed *Longissimus thoracis* muscle. Then, individual samples of the *Longissimus thoracis* muscle were collected between the fifth and sixth thoracic vertebrae and transferred to the laboratory. After 8 d of ageing physical attributes of meat samples were assessed by measurement of lightness, redness, yellowness, pH, purge losses, cooking losses and Warner Bratzler shear force. All young bulls were genotyped with the “GeneSeek Genomic Profiler Bovine LD” array containing 30,111 SNPs.

Micro-NIRS and Vis-NIRS predicted colour traits and purge losses satisfactorily, whereas pH, cooking losses and shear force predictabilities were rather poor, as a consequence of the large slaughter batch and residual variances affecting reference analyses. All the predicted traits, except shear force, showed moderate heritabilities and were highly genetically correlated with measured traits, allowing their use for selection purposes. The very simple, small, and cheap spectrometer (Micro-NIRS) yielded results not much inferior to the reference one (Vis-NIRS).

The accuracy of prediction of genomic breeding values was large enough, ranging from 0.216 (pH) to 0.380 (shear force), to consider genomic selection as a valid tool to improve meat quality traits in the Piemontese breed.

The general results indicate that the genetic improvement of meat quality traits which are difficult to select with traditional methodologies could take advantage from the application of new phenotyping technologies, such as Vis-NIR sprectroscopy, and by genomics.

Key words: Portable Near-infrared spectrometers, Genomics, Meat quality, Piedmontese.

Introduction

A selection programme for meat quality traits could be better established if easy routine phenotypes recording, directly at the slaughterhouse and without samples collection, is possible.

Portable Visible and Near infrared (Vis-NIR) spectrometers, allowing rapid and frequent measurements, fast and simple or no sample preparation, suitable for on-line use and simultaneous determination of different attributes (Prevolnik *et al.*, 2004), offer a number of important advantages over conventional laboratory instrumental analysis for phenotypes collection of meat quality traits. From a genetic point of view, beside calibration parameters, heritabilities of predicted traits and their genetic correlations with reference analyses must be investigated to determining the effectiveness of their use as indicator traits for selective breeding.

As alternative to large scale phenotypes collection and traditional breeding values estimation, genomic selection (Meuwissen *et al*., 2001) can be considered an innovative tool for the genetic improvement of meat quality.

The main aim of this study was then to perform a comprehensive investigation of the possibility for the improvement of meat quality traits in the Piemontese breed, focusing on the application of innovative tools as portable Vis-NIR spectrometers and genomics.

Material and methods

Data collection

The study was carried out on 1,327 Piemontese young bulls. Animals were fattened in 135 farms and slaughtered at the same commercial abattoir (Operti, Centallo [CN], Italy) from April 2015 to February 2017. The young bulls selected were sired by 204 artificial insemination purebred sires on 1,286 dams, all registered in the Italian Piemontese Herd Book. The beef farming systems, feeding regimes, fattening conditions and slaughter performances of the young bulls are described in detail in Savoia *et al.* (2019a). Twenty-four hours after slaughter, Vis-NIRS spectra were collected directly at the abattoir with two different portable spectrometers (Vis-NIRS: wavelength: 350 to 1,830 nm measured every 1 nm 1,481 data points per sample, weight 5,600g; Micro-NIRS: 905 to 1,649 nm measured every 6 nm 125 data points per sample, weight 60g), after the division of carcasses in two quarters on the exposed *Longissimus thoracis* muscle. Then, individual samples of the *Longissimus thoracis* muscle were collected between the fifth and sixth thoracic vertebrae and transferred to the laboratory. The collection, ageing and laboratory analyses of beef samples are described in detail in a previously published work (Savoia *et al.*, 2019a). Briefly, after 8 d of ageing physical attributes of meat samples were assessed by measurement of lightness (L*), redness (a*), yellowness (b*), pH, purge losses (PL), cooking losses (CL) and Warner Bratzler shear force (WBSF, N).

The 1,327 Piemontese young bulls were genotyped by using the array “GeneSeek Genomic Profiler Bovine LD” (GGP Bovine LD) containing 30,111 SNP. Quality control was performed both on SNP markers and animals.

Statistical analyses

A Bayesian model (Bayes B) implemented in the BGLR library of the R software (Pèrez and De Los Campos, 2014) was used to develop calibration equations for each beef quality trait as described by Ferragina *et al.* (2015). As Savoia *et al.* (2019a) reported that the most important source of variation of meat quality traits was the sample batch (animals slaughtered on the same date, the meats aged together and analyzed on the same day), external validation was carried out. This was done by predicting the observations for all the animals slaughtered on a given batch from the regression equations developed using the data from all the other batches, and repeating this procedure for every slaughter batch. Determination coefficients, calculated as the square of the correlation between the observed and predicted values in the calibration set (R^2_{CAL}) and in the external-validation set (R^2_{EXT}), were used to evaluate the accuracy of the predictions. For each of the meat quality traits, estimation of (co)variance components was performed using the VCE software (version 6.0, Groeneveld *et al.*, 2010) through separate bivariate analyses including the measured trait and its prediction obtained by Vis-NIR or Micro-NIR spectrometers, respectively. In matrix notation, the 2-traits statistical model utilized can be written as:

$$y = X\beta + W1c + W2q + Zu + e$$

where y contains observations for measure trait and NIRS pred ictions, β is the vector of non-genetic fixed effects, c is the vector of random herd effects (98 levels), q is the vector of random effect of slaughter batch (106 levels), u is the vector of animal additive genetic effects, e is the vector of random residual effects, and X, W1, W2 and Z are incidence matrices of proper dimensions. Except for shear force, for all meat quality traits the model included the effect of carcass weight. For pH and for L* the model included also parity of dam effect and age at slaughter effects. To facilitate comparisons with literature estimates, we estimated intraherd heritability defined as:

$$h^2 = \sigma^2_a/(\sigma^2_a + \sigma^2_e)$$

where σ^2_a is the additive genetic variance and σ^2_e is the residual variance.

Meat quality phenotypes were pre-corrected for all the non-genetic effects and used as dependent variables in a SNP-BLUP model (GS3 software by Legarra *et al.*, 2016). For each trait, the entire data-set was randomly splitted into training population (80% of animals) and validation population (20% of animals), for 15 times. Using the estimated SNP effects, direct genomic breeding values (DGV) were calculated for the young bulls in the validation populations. To evaluate the prediction ability of DGV, the mean of the correlation coefficient between pre-corrected phenotypes and DGV of validation populations, divided by the square root of the heritability (h) of the trait (Pryce *et al.*, 2012), was used.

Results and discussion

The prediction abilities obtained for color traits with Vis-NIRS (R^2_{CAL} 0.62 to 0.88) and Micro-NIRS (R^2_{CAL} 0.51 to 0.81) were similar to those obtained by Cecchinato *et al.* (2011) in a previous study on Piemontese young bulls. For pH, the R^2_{CAL} (0.57 with Vis-NIRS and 0.30 with Micro-NIRS) was lower than most of the literature reports (Prieto *et al.*, 2008; De Marchi *et al.*, 2013) while the low R^2_{CAL} values for purge and cooking losses found in our study were in the range of the published literature (Andres *et al.*, 2008; Leroy *et al.*, 2003) and slightly higher than the findings of Cecchinato *et al.* (2011). The accuracy of NIRS prediction of meat shear force was also very limited (R^2_{CAL} 0.34 for the Vis-NIRS, and 0.16 for the Micro-NIRS). As expected, the R^2_{EXT} obtained were always smaller than R^2_{CAL}, and ranged from 0.52 to 0.80 for colour traits while were lower than 0.32 for the other meat quality traits. 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, and the two portable spectrometers compared in this study, although very different in their suitability for practical use in the abattoir, produced similar results in terms of prediction accuracy in the external validation.

Table 1. Descriptive statistics of reference Piemontese beef quality traits and performance of their prediction by Vis-NIRS and Micro-NIRS instruments.

Item	**Color traits:** L*	a*	b*	**Meat pH**	**Meat losses (%):** Purge	Cooking	**Shear force** (N/cm^2)
Carcasses, N	1147	1148	1150	1144	1146	1157	1147
Descriptive statistics							
Mean	39.89	28.59	9.66	5.55	4.51	16.75	27.16
SD	3.49	1.74	1.66	0.05	1.19	3.43	9.61
Vis-NIRS							
R^2_{CAL}	0.88	0.62	0.70	0.57	0.29	0.26	0.34
R^2_{EXT}	0.78	0.55	0.63	0.30	0.31	0.16	0.16
RMSE-$_{EXT}$	1.43	1.22	1.06	0.05	1.05	3.36	10.69
Micro-NIRS							
R^2_{CAL}	0.81	0.51	0.63	0.30	0.20	0.10	0.16
R^2_{EXT}	0.80	0.52	0.61	0.22	0.27	0.19	0.19
RMSE-$_{EXT}$	1.67	1.23	1.04	0.05	1.07	3.20	10.69

Table 2. Variance components and intraherd heritability of colour traits measured with laboratory analyses and their predictions by Vis-NIRS and Micro-NIRS instruments.

	Traits								
	L*			**a***			**b***		
	Meas.	**Vis NIRS**	**MicroNIRS**	**Meas.**	**Vis NIRS**	**MicroNIRS**	**Meas.**	**Vis NIRS**	**Micro NIRS**
Phenotypic variance	11.64	9.96	9,74	3.12	1.83	1.52	2.79	1.88	1.67
Variance components^1									
Additive genetic	0.23	0.32	0.28	0.09	0.04	0.04	0.10	0.02	0.04
Day of slaughter	0.18	0.15	0.15	0.25	0.31	0.22	0.23	0.26	0.16
Herd	0.06	0.06	0.05	0.11	0.11	0.10	0.08	0.08	0.07
Residual	0.54	0.46	0.52	0.55	0.53	0.64	0.59	0.64	0.73
Intraherd h^2	0.30	0.41	0.35	0.14	0.08	0.07	0.14	0.04	0.05
SE intraherd h^2	0.095	0.104	0.107	0.070	0.066	0.050	0.070	0.044	0.060

[1] ratio to phenotypic variance

Heritability of measured meat quality traits (from 0.13 for purge losses to 0.31 for shear force) was in the range of most literature reports (Johnston *et al*., 2003; Riley *et al*., 2003). As shown in Tables 2 and 3, the predictions of meat quality traits by Vis-NIRS and Micro-NIRS displayed heritability values lower than the corresponding traits measured in the laboratory on aged meat samples, with the exception of L* and purge losses. However, heritabilities of meat quality predictions in most of the cases were large enough to be exploited for selection.

The genetic correlations of the measured colour traits and purge losses with both Vis-NIRS and Micro-NIRS predictions were extremely high, and consistent with those reported by Cecchinato *et al*. (2011). However, a superiority of the Vis-NIRS over the Micro-NIRS was observed in the other meat quality traits (pH 0.70 vs 0.45, cooking losses 0.70 vs 0.25 and shear force 0.81 vs 0.42).

Table 3. Variance components and intraherd heritability of meat quality traits measured with laboratory analyses and their predictions by Vis-NIRS and Micro-NIRS instruments.

	Traits											
	pH			**Purge Losses, %**			**Cooking Losses, %**			**Shear Force, N**		
	Meas.	**Vis NIRS**	**Micro NIRS**	**Meas.**	**Vis NIRS**	**Micro NIRS**	**Meas.**	**Vis NIRS**	**Micro NIRS**	**Meas.**	**Vis NIRS**	**Micro NIRS**
Phenotypic variance	0.30[3]	0.13[3]	0.06[3]	1.39	0.36	0.28	11.78	2.07	0.57	113.14	23.33	15.41
Variance components[1]												
Additive genetic	0.08	0.08	0.06	0.10	0.17	0.10	0.10	0.03	0.01	0.16	0.00	0.05
Slaughter day	0.61	0.48	0.49	0.14	0.21	0.16	0.42	0.54	0.13	0.42	0.53	0.40
Herd	0.06	0.07	0.05	0.05	0.04	0.15	0.04	0.04	0.03	0.06	0.07	0.07
Residual	0.25	0.37	0.40	0.71	0.58	0.69	0.44	0.40	0.83	0.37	0.41	0.48
Intraherd h^2	0.25	0.18	0.13	0.13	0.22	0.13	0.19	0.07	0.01	0.31	0.00	0.10
SE intraherd h^2	0.087	0.077	0.087	0.072	0.103	0.070	0.085	0.057	0.043	0.097	0.036	0.074

[1] ratio to phenotypic variance
3 × 10^{-2}

Table 4. Additive genetic correlations of colour and meat quality traits measured with laboratory analyses with their predictions (SE in parentheses) obtained with Vis-NIR and Micro-NIR spectrometers.

Traits	**Vis-NIRS**	**Micro-NIRS**
L*	1.000 (0.001)	1.000 (0.001)
a*	0.958 (0.173)	0.783 (0.225)
b*	1.000 (0.001)	0.930 (0.189)
pH	0.701 (0.164)	0.448 (0.256)
Purge Losses, %	0.979 (0.085)	0.879 (0.162)
Cooking Losses, %	0.703 (0.168)	0.248 (0.271)
Shear Force, N	0.805 (0.187)	0.418 (0.316)

Table 5. Accuracies of genomic predictions measured by Pearson's correlation between pre-corrected phenotypes and direct genomic breeding values (r(y,DGV)) divided by the square root of the heritability (h) of the trait and regression coefficient of the pre-corrected phenotypes on direct genomic breeding values (b(y,DGV)) for meat quality traits of Piemontese young bulls based on SNP-BLUP methods.

Trait[1]	**n. training**	**n. validation**	**r/h**	**b**
L*	910	246	0.324	1.00
a*	910	246	0.290	1.34
b*	909	250	0.357	1.73
pH	915	242	0.231	1.25
Purge Losses, %	905	249	0.305	1.48
Cooking Losses, %	919	247	0.216	1.54
Shear Force, N	897	249	0.380	1.65

The accuracy of genomic predictions was 0.23 for pH, 0.31 for purge losses and 0.22 for cooking losses. Colour traits showed similar accuracies. The highest accuracy was reported for shear force with a value of 0.38. Considering all traits together, the gain of raw accuracy, calculated as the correlation between direct genomic breeding values and pre-correct phenotypes, was associated with an increase of the heritability of the trait as supported by the findings of Bolormaa *et al.*(2013). Except for Lightness, direct genomic breeding values underestimated the pre-corrected phenotypes of animals in validation populations.

Conclusions

Portable and hand-held spectrometers have been tested at the abattoir level on a large number of carcasses. Good results have been obtained for the prediction of colour traits and purge loss, but with less reliable results for meat pH, cooking loss and shear force. The estimated genetic parameters showed that NIRS predictions of colour traits, pH and purge losses can be used as indicator traits of the corresponding measurements for selection purposes. For cooking losses results were more controversial, while estimates for shear predictions were not reliable. The accuracies reached by genomic breeding values in all the investigated traits suggested that young candidates for selection could be evaluated for meat quality traits using genotype information.

Acknowledgements

Authors acknowledge financial support from Fondazione Cassa di Risparmio di Cuneo, project "QualiPiem – Metodi innovativi per la selezione della qualità nella razza Piemontese".

List of References

Andrés, S., A. Silva, A.L. Soares-Pereira, C. Martins, A.M. Bruno-Soares, and I. Murray. 2008. The use of visible and near infrared reflectance spectroscopy to predict beef M. *longissimus thoracis* et *lumborum* quality attributes. Meat Science, 78:217-224.

Bolormaa S., J.E. Pryce, K. Kemper, K. Savin, B.J. Hayes, W. Barendsee, Y. Zhang, C.M. Reich, B.A. Mason, R.J. Bunch, B.E. Harrison, A. Reverter, R.M. Herd, B. Tier, H.U. Graser, and M.E. Goddard. 2013. Accuracy of prediction of genomic breeding values for residual feed intake and carcass and meat quality traits in *Bos Taurus*, Bos Indicus and composite beef cattle. Journal of Animal Science, 91: 3088-3104.

Cecchinato, A., M. De Marchi, M. Penasa, A. Albera, and G. Bittante. 2011. Near-infrared reflectance spectroscopy predictions as indicator traits in breeding programs for enhanced beef quality. Journal of Animal Science, 89: 2687-2695.

De Marchi, M., M. Penasa, A. Cecchinato, and G. Bittante. 2013. The relevance of different near infrared technologies and sample treatments for predicting meat quality traits in commercial beef cuts. Meat Science, 93: 329-335.

Ferragina, A., G. De Los Campos, A. Vazquez, A. Cecchinato, and G. Bittante. 2015. Bayesian regression models outperform partial least squares methods for predicting milk components and technological properties using infrared spectral data. Journal of Dairy Science, 98:8133- 8151.

Groeneveld, E., M. Kovac, and N. Mielenz. 2010. VCE User's Guide and Reference Manual Version 6.0

Johnston, D.J., A. Reverter, D.M. Ferguson, J.M. Thompson, and H.M. Burrow. 2003. Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 3. Meat quality traits. Australian Journal of Agricultural Research, 54:135-147.

Legarra A., A. Ricard, and O. Filangi. 2016. GS3 Genomic Selection – Gibbs Sampling – Gauss Seidel (and BayesCp).

Leroy, B., S. Lambotte, O. Dotreppe, H. Lecocq, L. Istasse, and A. Clinquart. 2003. Prediction of technological and organoleptic properties of beef *Longissimus thoracis* from near-infrared reflectance and transmission spectra. Meat Science, 66: 45–54.

Meuwissen T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using zgenome-wide dense marker maps. Genetics,157: 1819–1829.

Pérez, P., and G. de los Campos. 2014. Genome-wide regression and prediction with the BGLR statistical package. Genetics, 198: 483–495.

Prevolnik, M., M. Candek-Potokar, and D. Skorjanc. 2004. Ability of NIR spectroscopy to predict meat chemical composition and quality – a review. Czech Journal of Animal Science, 49: 500–510.

Prieto, N., A. Andrés, F.J. Giraldez, A.R. Mantecon, and P. Lavin. 2008. Ability of near infrared reflectance spectroscopy (NIRS) to estimate physical parameters of adult steers (oxen) and young cattle meat samples. Meat Science, (79), 692-699.

Pryce J.E., J. Arias, P.J. Bowman, S.R. Davis, K.A. Macdonald, G.C. Waghorn, W.J. Wales, Y.J. Williams, R.J. Spelman, and B.J. Hayes. 2012. Accuracy of genomic predictions of residual feed intake and 250-day body weight in growing heifers using 625,000 single nucleotide polymorphism markers. Journal of dairy science, 95: 2108-2119.

Riley, D.G., C.C. Chase Jr., A.C. Hammond, R.L. West, D.D. Johnson, T.A. Olson, and S.W. Coleman. 2003. Estimated genetic parameters for palatability traits of steaks from Brahman cattle. Journal of Animal Science, 81: 54–60.

Savoia, S., A. Brugiapaglia, A. Pauciullo, L. Di Stasio, S. Schiavon, G. Bittante, and A. Albera. 2019a. Characterization of beef production systems and their effect on carcass and meat quality traits of Piemontese young bulls. Meat Science, 153: 75-85.