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CICLO XXXI

INNOVATIVE TOOLS FOR PHENOTYPIC CHARACTERIZATION AND GENETIC IMPROVEMENT

OF MEAT QUALITY IN PIEMONTESE BREED

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SUMMARY

The main objective of this thesis was to perform a comprehensive investigation of the possibilities for the improvement of meat quality traits focusing on the development of innovative tools.

The study was carried out sampling 1,327 Piemontese young bulls. Animals were fattened in 115 farms and slaughtered at the same commercial abattoir. Information about farms fattening system was collected through on field surveys.

After slaughter, the following carcass traits were recorded: carcass weight, carcass conformation, age at slaughter and carcass daily gain. Individual samples of the *Longissimus thoracis* muscle were collected and transferred to the laboratory for meat quality analyses: muscle pH, colour parameters of lightness (L*), redness (a*), yellowness (b*), hue angle (h*), chroma (C*), purge losses, cooking losses, and Warner Bratzler shear force, all measured at 7 d after slaughter.

On each meat sample, 5 spectra were collected in a reflectance mode at the abattoir with a portable top-ranking visible near-infrared spectrometer and an hand-held spectrometer. Calibration equations were developed after conventional meat quality assessment using Bayesian methods. The ability of spectroscopy in predicting meat quality traits was evaluated comparing the performances of the two spectrometers.

Genetic correlations between carcass and measured meat quality traits were investigated, as well as genetic relations of meat quality traits with predictions obtained by spectral data. All the sampled young bulls were genotyped with the "GeneSeek Genomic Profiler Bovine LD" (GGP Bovine LD) array containing 30,111 SNPs. A combination of Genome-Wide Association and Pathway Analysis was performed to identify the genomic regions and biological pathways contributing to the variability of carcass and meat quality traits. Genomic variance components and SNP effects were estimated with bayesian methodology and a SNP-BLUP model. Genomic heritability and direct genomic breeding values were computed, assessing the possibility of

implement genomic selection for meat quality traits.

Six main typologies of fattening system were identified within the Piemontese breed. Carcass traits were deeply affected by production system, while little effects on meat quality, limited to colour traits, were observed. The small effect of beef production system showed that the variability of meat quality traits mainly depends on individual animal factors, shifting the possibilities for their improvement to genetic aspects.

All the meat quality traits showed not negligible heritabilities, allowing their improvement through selection. They also displayed genetic relationships with carcass traits, indicating a possible modification as a correlated response to selection for growth rate and muscularity, traits currently included in the breeding goal of the Piemontese breed. However, the establishment of a direct selection procedure relies on the availability of phenotypes collected within a routine recording scheme. Predictions of colour traits and purge losses were satisfactory, whereas pH, cooking losses and shear force predictabilities were rather poor. However, all the predicted traits except shear force showed moderate heritabilities and were highly correlated with measured traits, allowing their use for selection purposes.

From genetic architecture point of view of carcass and meat quality traits, our investigation highlighted that, besides myostatin, other genes contribute to explain the variability in carcass and growth characteristics. Moreover, association of pathways related to transporter activity (oxygen, calcium, ion and cation) was found with meat color parameters.

Genomic heritabilities were higher than pedigree-based heritabilies for purge losses and all colour traits, while they were similar for the other meta quality traits. The accuracy of prediction of genomic breeding values was satisfactory and allows to consider genomic selection as a valid tool to improve meat quality traits in Piemontese breed.

RIASSUNTO

La ricerca alla base di questa tesi di dottorato è stata condotta nell'ambito del progetto "QualiPiem" - Strumenti innovativi per la selezione della qualità della carne nella razza bovina Piemontese. Obiettivo principale è stato valutare la possibilità di migliorare la qualità della carne nei bovini di razza Piemontese, ponendo particolare attenzione agli aspetti applicativi oltre che a quelli conoscitivi. Lo studio ha previsto la comprensione delle basi genetiche dei caratteri di qualità della carne, la messa a punto di strumenti innovativi per il rilievo dei fenotipi, potenzialmente applicabili su larga scala a livello operativo, e l'impiego di informazioni genomiche per la selezione.

Operativamente, il progetto ha previsto il campionamento di 1,327 vitelloni registrati nel Libro Genealogico italiano della razza Piemontese. Gli animali sono stati ingrassati in 115 allevamenti e macellati nella stessa struttura commerciale tra aprile 2015 e febbraio 2017. Le informazioni sui sistemi di ingrasso adottati negli allevamenti sono state raccolte attraverso indagini in campo basate sul colloquio con gli allevatori e analizzate per studiare l'effetto del sistema di allevamento sull'efficienza produttiva e sulla qualità della carne.

Dopo la macellazione, sono stati raccolti i seguenti fenotipi: peso della carcassa a caldo, conformazione della carcassa, età al macello ed accrescimento giornaliero in carcassa. Ventiquattro ore dopo la macellazione, sono stati raccolti campioni individuali del muscolo *Longissimus thoracis* tra la quinta e la sesta vertebra toracica. I campioni sono stati quindi scansionati per effettuare la misura dell'area del muscolo. Inoltre, su ciascun campione di carne, direttamente al macello, sono stati raccolti 5 spettri in riflettanza con due spettrometri portatili: un ASD LabSpec 2500 (range dello spettro tra 350 e 1,830 nm, con acquisizione ogni nm) ed un JDSU (range dello spettro tra 905 e 1,649 nm, con acquisizione ogni 6 nm). Le equazioni di calibrazione sono state sviluppate con metodologie bayesiane e la capacità predittiva della spettroscopia è stata valutata confrontando le prestazioni dei due spettrometri. La valutazione della qualità della carne è stata eseguita con le tradizionali metodologie di analisi in laboratorio 7 giorni dopo la macellazione ed ha incluso il pH,

il colore (L *, a *, b *, h *, C *), le perdite di sgocciolamento, le perdite di liquidi in cottura e la tenerezza.

Sono state studiate le relazioni fenotipiche e genetiche tra i caratteri di efficienza produttiva e quelli di qualità della carne. Inoltre, si è provveduto ad indagare le relazioni genetiche tra i tratti di qualità della carne misurati in laboratorio e le loro predizioni ottenute con la spettrometria nel vicino-infrarosso.

Tutti gli animali campionati sono stati genotipizzati utilizzando il supporto "GeneSeek Genomic Profiler Bovine LD" (GGP Bovine LD) contenente 30.111 SNP. E' stato eseguito uno studio combinando Genome-wide Association e Pathway Analysis per identificare le regioni genomiche e i processi biologici che contribuiscono a spiegare la variabilità dei caratteri di qualità della carne. Le componenti di varianza e gli effetti degli SNP sono stati stimati congiuntamente con la metodologia SNP-BLUP. Sono state stimate le ereditabilità genomiche e predetti gli indici genomici, valutando quindi la possibilità di implementare la selezione genomica per migliorare la qualità della carne nella razza Piemontese.

I risultati ottenuti hanno evidenziato la presenza di sei principali tipologie di ingrasso nel contesto della razza piemontese, ognuna caratterizzata da specifiche tecniche gestionali. I caratteri produttivi sono risultati profondamente influenzati dal sistema di produzione, mentre è emerso un effetto minimo sulla qualità della carne, limitato al colore. L'effetto limitato del sistema di produzione ha dimostrato che la variabilità dei caratteri di qualità della carne dipende principalmente da fattori animale-specifici e che i miglioramenti possono essere apportati agendo a livello dei singoli animali, guardando con particolare attenzione all'aspetto genetico.

E' importante, quindi, che i caratteri di qualità abbiano riportato valori di ereditabilità non trascurabili, lasciandone presupporre un possibile miglioramento attraverso la selezione. Tuttavia, l'inserimento di tali caratteri tra gli obiettivi di selezione dipende dalla disponibilità di fenotipi raccolti all'interno di un processo di registrazione routinario.

Da un punto di vista fenotipico, i caratteri del colore e le perdite di sgocciolamento sono stati predetti in modo soddisfacente con con entrambi gli spettrometri utilizzati in questo studio. La capacità predittiva della spettrometria del vicino infrarosso per il pH, le perdite di cottura e la tenerezza è risultata meno favorevole. Tuttavia, i fenotipi predetti a partire dai dati spettrali sono risultati ereditabili e le elevate correlazioni genetiche tra questi ed i fenotipi osservati potrebbero consentire di utilizzare la spettroscopia a fini selettivi.

Per quanto riguarda l'architettura genetica dei caratteri indagati, il presente studio ha evidenziato che oltre alla miostatina sono presenti diversi geni che contribuiscono a spiegare quote della variabilità esistente, soprattutto per quanto riguarda l'accrescimento in carcassa. Inoltre, è stata messa in evidenza un'associazione tra *pathway* metabolici inerenti all'attività di trasporto cellulare (ossigeno, calcio, ioni e catione) ed i caratteri di qualità della carne relativi al colore.

L'utilizzo delle informazioni genomiche, congiunto alle parentele pedigree, ha prodotto stime di ereditabilità maggiori rispetto a quelle tradizionali per le perdite di sgocciolamento ed il colore della carne, mentre per gli altri caratteri non sono state evidenziate differenze di rilievo. Gli indici genomici che ne sono conseguiti hanno mostrato una capacità predittiva soddisfacente, permettendo di considerare la selezione genomica come un possibile strumento per migliorare i caratteri di qualità della carne nella razza Piemontese.

GENERAL INTRODUCTION

Meat quality has always been important for the consumer, and it is especially a critical issue for the meat industry in the 21st century (Joo et al., 2013). As consumer's demand for high quality meat is increasing in most countries, the meat industry should consistently produce and supply meat that is healthy, safe and tasty for the consumer.

However, the concept of quality referred to beef is intricated as it includes a large number of desirable characteristics in the final product linked to technological, sensory and nutritional aspects. Indeed, meat quality can be defined by the compositional quality (lean to fat ratio, meat percentage, intramuscular fat, marbling, protein, and muscle area), functional quality (water holding capacity, isometric tension, muscle fiber shortening, pH, and cooking loss), and eating quality or palatability (appearance, juiciness, tenderness, and flavour) (AMSA, 2001).

Traditionally, the set of properties used to define the quality of meat are those related to functional and eating quality, associated with our sensory perception: appearance, colour, texture (especially tenderness) and juiciness or water-holding capacity. Particularly, meat colour and water holding capacity are important traits as they represent the primary determinant of consumer acceptance during purchase (Moon et al., 2015), while tenderness is the primary determinant of satisfaction among beef consumers (Mullen et al., 2006).

Conventional traits used to investigate meat quality are related to physical characteristics of meat: pH, colour (usually expressed with cielab coordinates L*, a*, b*, C*, H*), purge and cooking losses and shear force.

The determination of these parameters nowadays is based on a complex sequence: meat sampling at slaughterhouse, laboratory instrumental analyses and, for some traits, sensory description by trained panels of experts. The collection of meat samples at the slaughterhouse causes a depreciation of carcasses and the subsequent analyses are destructive, expensive and time consuming. These conditions strongly limited the collection of a large number of phenotypes with

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the purpose of studying the factors affecting meat quality traits and of investigating the possibilities of their improvement.

It is known that many environmental factors can affect the quality of bovine meat. The way animals are fed, managed, slaughtered and especially carcass handling and post-slaughter processing have an effect on the quality of meat (Mullen et al., 2006). A number of studies assessed the specific effect of management or feeding systems on carcass and meat quality traits during (Daza et al. 2014; Avilés et al. 2015) or before (Dannenberger et al. 2006; Guerrero et al. 2013, Soulat et al., 2018) the fattening period. However very general aspects, such as intensive *vs* extensive system or concentrate *vs* forage feeding, have often been investigated separately. A more comprehensive approach, based on the identification of the fattening systems and the study of their overall effect, would help to better understand the relationship between management and meat quality.

In addition to exogenous causes, meat quality is determinated by animal endogenous peculiarities related to its genetic characteristics. The understanding of the genetic bases of meat quality is scarce (Gao et al., 2007), as little knowledge exists on the genes involved and their interactions. Muscular hypetrophy, due to a single gene mutation (Arthur, 1995; Casas et al., 1998), has been recognized to exert favorable effects on carcass traits and meat quality in terms of tenderness and leanness. Calpain I and calpastatin gene activities have been associated to beef tenderization (Casas et al. 2006; Koohmaraie et al., 1995). Within breed, the existence of genetic variation has been reported, highlighting the possibility for selection (Aass, 1996; Renand, 1985). However, the difficulties concerning the collection of meat samples and the availability of reliable measurements prevented to establish breeding programs focused on the improvement of meat quality.

To overcome the difficulties in obtaining phenotypic information in a routine way, a first step towards genetic improvement of meat quality could be represented by indirect selection. So far,

beef cattle breeding programs mainly concerned production efficiency, focusing in increasing growth rate and live fleshiness (Andersen et al., 1981; Albera et al, 2004). The knowledge of genetic relationships between meat quality and selected beef production traits is required to appraise whether a favorable effect in meat quality can be achieved as a correlated response to selection for the current breeding goal traits (Bonfatti et al., 2013). Boukha et al. (2011) assessed the genetic correlations of meat quality with carcass traits, showing the existence of some antagonistic relationships with carcass daily gain, whereas those with carcass conformation were in general favorable. Some other relevant production traits are likely to display genetic and phenotypic relationships with meat quality. It is the case of carcass weight or age at slaughter, whose expression in turn depends not only on growth potential but also on degree of maturity and fat deposition rate. Currently, these complex relationships have been little investigated. However, yielding durable improvements of carcass and meat quality requires further knowledge on the genetic mechanisms underlying variation in these traits.

Moving the focus on the possibility to directly select for the improvement of meat quality, the study conducted by Boukha et al. (2011) in the Piemontese breed, highlighted the existence of a not negligible genetic variability in all meat quality traits. Apart from the above mentioned difficulties concerning phenotypes availability, meat quality traits are certainly heritable and their inclusion in beef cattle breeding goals is, in principle, possible.

Therefore, it is necessary and essential the development and the application of innovative tools for phenotypes detection on large scale at operational level which can allow to overcome the existing limitations.

Visible and Near infrared spectroscopy (Vis-NIRS), based on the principle that different chemical bonds in organic matter absorb or emit light of different wavelengths when the sample is irradiated, offers a number of important advantages over conventional laboratory instrumental analysis for phenotypes collection. Spectrometers allow rapid and frequent measurements, fast and

simple or no sample preparation, suitability for on-line use and simultaneous determination of different attributes (Prevolnik et al., 2004).

The establishment of a general procedure for the use of Vis-NIR spectroscopy in phenotypes prediction involves the following steps: spectra acquisition on raw samples; traditional laboratory analysis on the samples to provide reference data; development of the calibration equation on a training dataset composed of samples spectra and relative reference phenotypes; validation of the calibration equation using an indipendent testing set; in case of satisfactory results of the validation, routine prediction of phenotypes applaying the validated equations to the spectral data of new samples.

Several studies stressed the capability of reflectance spectroscopy to accurately predict chemical composition of beef (Eichinger & Beck, 1992; Alomar et al., 2003; Tøgersen et al., 2003) and different attributes of meat quality (Leroy et al., 2003; Prieto et al., 2009).

From the perspective of the genetic improvement, only one study investigated the usefulness for selection of NIRS predictions of traditional meat quality traits (Cecchinato et al., 2011). The obtained results suggested that genetic improvement of some of meat quality traits using their predictions from NIR laboratory spectrometers is feasible. Indeed, for colour traits and purge loss the NIRS prediction proved to be heritable and strongly associated to lab measured traits from the genetic standpoint.

The availability of new portable NIR and Vis-NIR spectrometers, able to collect spectra directly from the muscle's surface at slaughterhouse (Prieto et al., 2009) increases the relevance of Vis-NIRS as a phenotyping tool avoiding the need of samples taking and transportation to laboratories. The offer of portable spectrometers with very different characteristics is increasing, placing side by side instruments covering also the visible part of the spectrum to very small and cheaper instruments previously used only for static industrial applications.

Prior to the implementation of these new spectrometers at operational level for phenotypes

collection, it is crucial however, to assess their to predict meat quality traits and to investigate the genetic relationship between traits measured with traditional laboratory analyses and their Vis-NIRS predictions.

As alternative to large scale phenotypes collection and traditional breeding values estimation, genomic selection (GS) can be considered an innovative tool for the genetic improvement of meat quality. GS refers to selection decisions that are based on breeding values predicted using genomewide marker data such as single nucleotide polymorphism (SNP) (Meuwissen et al., 2001). The theoretical basis of GS is that the genetic variance of quantitative trait loci (QTL) for a certain trait can be captured by SNP markers due to linkage disequilibrium between QTL and markers. The estimation of SNP effects is performed in a training population that has been phenotyped for the trait and genotyped for the SNP markers. Effects of all markers are simultaneously estimated and genomic breeding values of selection candidates can then be predicted from genotyping data only. Moreover, GS allows to reduce the generation interval in selected populations and, when the genotyping cost is low, a large number of candidates can be screened, increasing selection intensity. As a result of the combination of these effect, GS can increase the rate of genetic change by a factor of 2 (Schaeffer, 2006). The accuracy of genomic predictions, however, depend on the genetic basis of the trait (namely its heritability), the size of the training population and the extent of the linkage disequilibrium between SNP and QTL (Hayes et al., 2009; VanRaden et al., 2009).

For meat quality traits genomic breeding value predictions are attractive because, once reliable estimates of SNP effects have been obtained, no phenotypes collection is required for candidates for selection. To date, very few investigations concerned the application of GS for the improvement of meat quality traits (Bolormaa et al., 2013) some of them focusing only on meat chemical properties, as lipid content (Pimenetel et al., 2012).

Another application of new genomic technologies involves the identification of genes that have a relevant effect on economic traits, segregating in close linkage disequilibrium with some

detectable markers. Genomewide association study (GWAS) can need to be carried out to describe the marker associations with quantitative trait loci (QTL) for the traits under investigation. In the framework of meat quality traits, GWAS can allow the identification of the SNP markers in linkage disequilibrium with specific genes affecting meat quality, improving the understanding of the trait biology and eventually providing a list of positional candidate genes (Xia et al., 2016). The mapped QTL can be used to implement marker-assisted selection allowing an increase of achievable rate of genetic gain.

Furthermore, GWAS network-based approach could identify causal variants that are caused by variants in multiple genes within a pathway (Leiserson et al., 2013) allowing a better explanation of the genetic architecture of the investigated trait.

The Piemontese breed can be considered a good case study for a comprehensive investigation of the possibilities for the improvement of meat quality traits. Firstly, the Piemontese breed is numerically the most important Italian beef breed, with an overall population of 330,000 heads, including 153,000 cows (Veterinary Information System, 2017), 90% of which registered in the Italian Piemontese Herdbook (ANABORAPI, 2017).Around 60% of the Piemontese beef production is sold as "Piemontese certified" establishing a tight link within the entire production chain. The certification traces back to the Herdbook, ensuring the knowledge of the pedigrees of most of the slaughtered animals. Approximately the 50% of calves born is progeny of selected artificial insemination bulls, allowing a faster and easier spread of genetic superiority compared to conventional beef cattle populations. Furthermore, the meat of Piemontese animals fulfils the requirements of Italian consumers for lean, tender and palatable product (Destefanis and Barge, 1988). Most of these favourable characteristics are due to double muscling, which is inducted by a breed specific mutation of myostatin gene (mh) located on Chromosome 2 (Grobet et al. 1998), which is almost fixed in this population. Piemontese animals display large muscular masses and low fat deposition, reduced weight of the skeleton, reduced feed intake and improved feed conversion

(Fiems, 2012). Compared to conventional animals, the meat of double muscled Piemontese animals has a low levels of intramuscular fat (Barge et al., 1993) and shows a large reduction in muscle collagen, which is also responsible for the increased tenderness (Destefanis et al., 1994).

Lastly, the recent recognition by European Union of the Piemontese meat with the Protected Geographical Indication "Vitelloni Piemontese della coscia" has further increased the interest for enhancing its meat quality attributes.

AIMS OF THE THESIS

The research underlying the thesis was conducted within the project "QualiPiem" - Innovative tools for selection of meat quality in Piemontese breed.

The thesis was aimed to explore the possibility of the improvement of meat quality in Piemontese cattle, with a particular concern to application and to cognitive aspects. The objectives of this thesis were: 1) the investigation of the environmental factors related to fattening system affecting meat quality and carcass traits; 2) the analysis of genetic parameters of meat quality traits and their genetic relations with carcass traits; 3) the development of innovative tools for large scale phenotypes detection based on visible and near-infrared spectroscopy (Vis-NIRS) by portable spectrometers; 4) the investigation of the genetic relations between meat quality traits and their predictions obtained by spectral data collected with portable spectrometers; 5) the implementation of genomic tools for selection of meat quality traits.

The first objective involved meat quality and carcass traits of Piemontese young bulls and their connections with information about farms fattening system. A phenotypic analysis was carried out to characterize the fattening systems adopted in Piemontese breed and to assess their effect on carcass and meat quality traits.

The second objective involved again meat quality and carcass traits of Piemontese young bulls. An overall genetic analysis was conducted in order to calculate genetic parameters for these traits. The relationship of meat quality with carcass traits, which are closely related to the most relevant breeding goal traits in beef cattle, were also considered in this study.

For the third objective spectral data directly collected on muscle's surface at the abbatoir by portable spectrometers were involved. The predictions of meat quality traits were obtained developing calibration equations with Bayesian methodology and a deep investigation on results was performed. The aims were: a) to test the use of portable and hand-held spectrometers at the abattoir on a large number of carcasses; b) to compare, through cross and external validation, a

top-ranking portable instrument with a wide spectrum range (Vis-NIRS) with a very small hand-held one (Micro-NIRS); c) to analyse the sources of variation and repeatabilities of measured meat quality traits, of meat infrared absorbance spectrum, and of NIRS predicted meat quality traits; and d) to test at field level the ability of NIRS predictions to provide reliable information on several meat quality traits.

Aforementioned predictions were involved in the fourth objective in order to: a) investigate the genetic variations in meat quality traits predictions obtained by portable spectrometers; b) compare estimates of genetic parameters of meat quality traits obtained with Vis-NIRS and Micro-NIRS predictions'; c) assess the genetic relationships between traditional measures of meat quality traits and their NIRS predictions.

For the last objective, GWAS and genomic predictions were performed exploiting the genomewide marker data available for all the Piemontese young bulls included in the study. The aims were: a) to identify genomic regions and metabolic pathways contributing to explain the variability in meat quality and carcass traits; b) to investigate the accuracy of prediction of genomic breeding values in order to explore the possibilities for the improvement of meat quality traits by genomic selection.

CHAPTER 1.

Characterisation of beef production systems and their effects on carcass and meat quality traits

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ABSTRACT

Using the Piemontese breed as a case study, we characterised beef production systems within the EU classification, and investigated their effects on carcass and meat quality traits. The research involved 1,327 young bulls fattened on 115 farms. The production systems identified by hierarchical cluster analysis were: traditional (restricted feeding and either tie-stalls or loose-housing), modern breeders and fatteners and specialised fatteners (the last two were divided in those using or not using total mixed rations). Despite the large variability in management techniques within production systems, production systems affected (P < 0.05) farm size, animal density, environmental scoring, diet, slaughter age and all carcass traits except weight. Lightness (L*) of *Longissimus thoracis* was the only meat quality trait affected (P < 0.05), with values greater in the traditional tie-stall system (+0.9 L*). Carcass weight (438±44 kg) affected (P < 0.01) all meat quality traits except shear force, with colour traits, pH and water losses greater (P < 0.05) in heavy carcasses.

Keywords*: meat colour traits; lightness; tenderness; cooking losses; Piedmontese.

INTRODUCTION

A study by the European Commission (2011) on the structure of EU beef farms identified three main specialised beef cattle production systems. Suckler cow farmers who produce calves to be sold to other farms for fattening were classified as "breeders", farmers who fatten calves born on their farms were classified as "breeders and fatteners" (B&F), and farmers who purchase weanlings to fatten in their farms were classified as "specialised fatteners".

According to a Farm Accountancy Data Network (FADN) survey, the large majority of European suckler cows are raised in specialised beef operations: 35% by beef breeders and 39% by beef B&F, with the remaining 26% reared on mixed dairy-beef cattle or sheep-beef cattle farms (European Commission, 2011). Only around 50% of male cattle sold at between 1 and 2 years of age are raised on specialised beef farms with specialised fatteners (35%) predominating over B&F (17%). The distribution of suckler cows across farming systems in Italy is similar to that of the EU, whereas specialised beef fatteners have by far the largest share of male cattle marketed at between 1 and 2 years of age (71%) (European Commission, 2011).

In Italy, Piemonte is a suitable case study region because it is the only one that appears on the list of the most important European regions for each of the three beef livestock systems classified by the EU: breeders, B&F and specialised fatteners (European Commission, 2011). Moreover, Piemonte, along with Belgium, obtains the highest prices per male sold, due to their double-muscled breeds: Piemontese and Belgian Blue, respectively.

The Piemontese (Piedmontese) is the most important Italian beef breed, with an overall population of 330,000, including 153,000 cows (Veterinary Information System, 2017), 90% of which are registered in the Italian Piemontese Herd Book (ANABORAPI, 2017).

The beef farming systems in Piemonte are evolving away from very traditional practices (tied animals fed mainly hay and restricted amount of concentrates) to modern ones (loose-housed animals, *ad libitum* feeding and use of total mixed rations [TMR]), and the different systems now

coexist (Sgoifo Rossi, Pastorello, Caprarotta, & Compiani, 2011).

A few studies have looked at the effects of production system or feeding techniques during the fattening period on carcass and meat quality traits (Daza, Rey, Lopez-Carrasco, & Lopez-Bote, 2014; Avilés, Martínez, Domenech, & Peña, 2015). Other studies have also focused on the possible impact of type of animal management before the fattening period on production and meat quality (Dannenberger, Nuernberg, Nuernberg, & Ender, 2006; Guerrero et al., 2013). However, most of the studies investigated very general effects, such as intensive *vs* extensive systems or concentrate *vs* forage feeding.

More recent studies highlighted that individual information on rearing conditions of animals can be exploited with the aim of identifying management techniques affecting carcass and meat quality traits (Gagaoua, Monteils, Couvreur, & Picard, 2017) or to develop predictive models for the same traits (Soulat, Picard, Léger, & Monteils, 2016; Soulat, Picard, Léger, & Monteils, 2018).

Currently no detailed analyses of the fattening systems classified as B&F and specialised fatteners, especially in relation to their effects on animal performance and meat quality traits, and particularly with regard to hypertrophic breeds exist.

The aim of this study, therefore, was to characterise the beef production systems and assess their effects on carcass and meat quality traits using Piemontese breed as a case study.

This knowledge is important for: making a sound economic and technical comparison of the different systems, for predicting future trends in carcass and meat quality trait at population level in the light of the evolution of beef production systems, and for setting future selection goals for genetic improvement of the breed.

MATERIAL AND METHODS

This study is part of the "Qualipiem" project, which is aimed at analysing the phenotypic and genetic sources of variation in meat quality traits in the Piemontese breed and at proposing

innovative selection strategies for their improvement.

Farm sampling and data collection

Information on the farms was collected through an interview-based field survey. A total of 115 farms in the Piedmont region (north-west Italy) were selected according to the following criteria: they were interested in the aims of the research, their cows were registered in the Piemontese Herd Book (only for B&F farms), they were using, at least in part, artificial insemination, and were delivering their slaughter animals to the largest local cooperative slaughterhouse. Trained technicians visited each farm selected and assisted the farmer in filling out a questionnaire designed to elicit information on farm size (land area in ha and number of fattened animals per year), management practices and animal welfare. Information about management practices included: beef production system (B&F *vs* specialised fatteners), housing system (tie-stalls *vs* loose-housing), feed supply (restricted *vs ad libitum*), feed distribution (TMR *vs* separate distribution of concentrates and forage) and feed composition (as proportions in rations). In order to obtain chemical composition of feeding, analytical information of purchased commercial feeding was registered, whereas for farm produced feedstuffs, chemical composition of feeds used was derived from the chemical analysis of each feed ingredient (Sauvant et al. 2004). The animal welfare information included space allowance (m^2 per head) and assessments of building adequacy, cleanliness, aeration, water availability and animal docility on a scale of 1 to 3 (1=insufficient, 2=sufficient, 3=good). Farms were also given an overall evaluation by averaging the above-mentioned scores with the exception of animal docility.

The questionnaire was tested before its application for data collection on a sample of farms during the training of technicians.

Animals and beef sampling

The study was carried out sampling 1,327 Piemontese young bulls progeny of 204 A.I. purebred sires and 1,286 dams, all registered in the Italian Piemontese Herd Book. All the animals were

slaughtered at the same commercial abattoir (Operti, Centallo (CN), 12044, Italy) from April 2015 to February 2017. The young bulls were stunned using captive-bolt pistol prior to exsanguination and dressed according to standard commercial practices. Slaughtering was performed in compliance with the Italian welfare regulations and respecting EU regulations (Council Regulation (EC) No. 1099/2009). After slaughter, hot carcass weight (CW) and carcass conformation class according to the EU linear grading system (Commission of the European Communities 1982) were recorded. In order to better differentiate carcass conformation, the six main grades (S, E, U, R, O, P, from best to worst) were further divided into three subclasses (+,= or -). Prior to statistical analysis, the categories of carcass conformation were rearranged into numerical scores (EUS) ranging from 1, corresponding to class "P-", to 18, corresponding to class "S+". Fatness was not scored in this study as the carcasses of double-muscled breeds are known to be lean and hence exhibit little variation in fatness.

Age at slaughter (AS) was calculated from date of birth to date of slaughter. As individual live weights of animals were not available, daily carcass gain (CDG) calculated as the ratio of carcass weight to age at slaughter, was used as measure of young bulls growth rate (Juniper et al., 2005; Boukha et al., 2011).

The carcasses were not electrically stimulated and they were chilled at 4 °C until twenty-four hours post-mortem. Twenty-four hours after slaughter, individual samples (4.0 cm thick) of the *Longissimus thoracis* (LT) muscle were collected from between the fifth and sixth thoracic vertebrae. The muscle and the excision area were chosen because displayed during the routine slaughterhouse procedures of subdivision of half-carcasses into quarters according to pistol cutting.

The beef samples were scanned with a HP Scanjet 5590 Digital Flatbed Scanner (Hewlett-Packard; Palo Alto, California) to obtain images for subsequent measurement of the rib eye area (REA, cm^2). For image calibration, a ruler marked in centimeters was scanned with meat samples. Then, samples were individually vacuum packed and transferred under refrigerated

conditions to the laboratory, where they were stored in a chilling room at 4°C for 7 days, after which meat quality traits were measured.

Analysis of meat quality traits

After ageing (7 days), purge losses (PL) were determined according to the following procedure: the steaks were first weighed in the bag (packaged weight, W1), then after removal from the bag (unpackaged weight, W3), and then the bag was rinsed, dried and weighed (bag weight, W2). PL (%) was calculated as (W1-W2-W3)/(W1-W2) × 100.

Ultimate pH (7 days) was measured with a portable Crison pH-meter PH 25+ (Crison Instruments S.A.; Alella, Barcelona) equipped with a glass electrode Crison 52 32 suitable for meat penetration and an automatic temperature compensator. Before analysis, the pH-meter was calibrated using standard buffers (pH 4.0 and 7.0). The electrodes were inserted approximately 1 cm into the muscle (Boccard et al. 1981).

The digital images of the samples were processed with the Image Pro Plus 4.5.1. software (Media Cybernetics, 2001) in order to measure the rib eye area (REA, cm^2). Before each measurement, the image was calibrated with a ruler. The surrounding edge of the sample was automatically traced. However, a manual trace by the operator was added when errors in the automatically trace occurred. Only one experienced operator performed all the measurements.

Colour was measured with a Konica Minolta CR-331C colourimeter (Konica Minolta Sensing Americas, Inc; Ramsey, New Jersey) on the freshly-cut surface of each steak after blooming for 1 h at 4°C. The CR-331C colourimeter features 45° circumferential illumination/0° viewing geometry, a Ø25mm measuring area and 2° standard observers. The instrument was calibrated on its own white reference tile supplied by the manufacturer and set with the illuminant D65 (colour temperature 6500 K), which represents average daylight. CIELAB coordinates (CIE 1976), lightness (L*), redness (a*) and yellowness (b*) were recorded, and hue angle (h*) was calculated as $h^* = tan^{-1}$ (b*/a*), Chroma (C*) as $C^* = (a^{*2} + b^{*2})^{0.5}$. Three random readings were taken at different locations

on the meat surface and averaged.

After colour measurements had been taken, cooking losses (CL) were determined. Each steak was sealed in a polyethylene bag and cooked in a water bath preheated at 75°C to an internal temperature of 70°C. The cooking temperature was monitored with a thermometer inserted into the geometric centre of the steak. When the set temperature was reached, the steak was removed from the water bath and cooled for 30 min under tap water to prevent further cooking. It was then removed from the bag, blotted and reweighed (Honikel, 1998). Cooking Losses (%) were calculated as the weight difference between the raw and the cooked samples as percentage of the weight of the raw meat sample. The steaks used to determine cooking losses were also used for the Warner Bratzler shear force (WBSF) test after overnight chilling at 4°C. Six cylindrical cores of cooked meat 1.27 cm in diameter, taken parallel to the muscle fibres were sheared perpendicularly to the longitudinal orientation of the muscle fibres with a V shaped Warner-Bratzler cutting blade fitted to an Instron 5543 Universal Testing Machine (Instron; Norwood, Massachusetts). WBSF was measured as the maximum force (Newtons) required to shear the cylindrical core at a crosshead speed of 200 mm per min (A.M.S.A., 2015).

Statistical analyses

Identification and characterisation of beef production systems

Recent studies (Gagaoua et al., 2017; Soulat et al., 2018) implemented innovative statistical methodologies combining factorial or principal component with cluster analyses for the study of meat quality traits. These methods were able to efficiently categorize animals into management groups and proved to be particularly useful when a very high number of quantitative or qualitative variables were involved. In the present study, the limited number of parameters related to structural and technical features of the investigated farms and the categorical nature of these parameters suggested the authors to perform an agglomerative hierarchical cluster analysis.

The systems were then classified by cluster analysis (Lin & Lin, 1994) on the basis of the

following four variables: beef production system (B&F *vs* specialised fatteners), housing system (tie-stalls *vs* loose-housing), feed supply (restricted *vs ad libitum*) and feed distribution (TMR *vs* separate distribution of concentrates and forage).

A dissimilarity matrix was created with the SAS DISTANCE procedure (2013) using the general dissimilarity coefficient of Gower (1971), an appropriate index for handling nominal, ordinal and (a)symmetric binary data. It was then analysed with the SAS CLUSTER procedure (2013) to create agglomerative hierarchical clusters using Ward's minimum variance method (Murtagh & Legendre, 2014). The optimal number of clusters was chosen on the basis of visual inspection, pseudo T-squared (quantification of the difference in the ratio of between-cluster to within-cluster variance by merging clusters), semi-partial R^2 statistics and validate by calculating average silhouette width (Si) criterion (Rousseeuw, 1987; Gagaoua, Picard, Soulat & Monteils, 2018).

Land area, number of fattened animals per year, number of fattened animals per ha, and animal welfare traits (space allowance, building, cleanliness, aeration, water supply, animal docility and overall environmental evaluations) were subjected to a one-way ANOVA with the identified production systems as the source of variation. Orthogonal contrasts based on the effects of production system were used to compare general management strategies across the identified systems (tie-stalls *vs* all loose-housing; within loose-housing: traditional restricted *vs* modern *ad libitum* feeding; within modern systems: B&F *vs* fatteners, TMR *vs* separated diet and their interaction).

Statistical analysis of carcass and meat quality traits

The effects of production system on carcass and meat quality traits were assessed on the basis of individual data from the 1,327 young bulls sampled after removing observations falling outside the range of 3 standard deviations from the mean for each trait. Age at slaughter and carcass traits were analysed with the SAS MIXED procedure (2013) adopting the following statistical model:

y = birth season + parity of dam + production system + farm(production system) + batch + ε

where y represents the observation of each of the investigated traits; birth season (4 classes: January-March, April-June, July-September, October-December), parity of dam (4 classes: 1st, 2nd, 3rd-8th, >8) and production system are all fixed effects, farm is the random effect of the fattening farm nested within production system (98 levels), batch is the random effect of the day of slaughter (117 levels) and ε is the random residual term. Farms, batch and ε were assumed to be normally and independently distributed $\sim N(0, \sigma^2)$. A minimum cell size of 3 observations was required for both the batch and farm effects.

The fixed effect of carcass weight, (5 classes: <350kg, 350-400kg, 401-450kg, 451-500kg, >500kg) was added to the previous model for the analysis of meat quality traits.

The effects of different management strategies were evaluated with orthogonal contrasts, whereas comparisons of the least square means of the other fixed effects were performed with a Tukey-Kramer test (P<0.05).

RESULTS

Beef production systems

Six clearly recognizable production systems with a good assignment of farms to groups (best silhouette width criterion S_i=0.73) were identified from the cluster analysis (Table 1). Farms characterised by restricted feeding without the use of TMR were classified as traditional systems, with a distinction made between tie-stall and loose-housing of animals. Modern farms, characterised by loose-housed animals fed *ad libitum*, were divided into B&F or specialised fatteners, each further differentiated according to whether not they used TMR.

Traditional farms represented almost 40% of the units surveyed, followed by modern fatteners (33%) and B&F (28%). Nevertheless, almost 41% of the animals sampled came from modern

specialised fatteners, 32% from B&F systems and only 27% from traditional farms, reflecting the smaller size of the latter. The distribution of the farms across the criteria used for the cluster analysis allowed us to describe the main characteristics of the beef production systems identified. In half of the farms in the study, cow-calf and fattening operations were integrated, and loose-housing predominated over tie-stalls (77% of farms). The majority of farms (66%) adopted modern a*d libitum* distribution of feed, but only 30% of farms used TMR, probably due to farm size.

Comparison of farm size traits across the identified production systems is shown in Table 2. On average, the farms were 39 ha in size, fattened 82 animals per year with a very large variation across herds, and allocated around 5.00 m^2 of space to each fattening animal. The production system affected ($P < 0.05$) all the traits investigated. Traditional farms with tie-stalls were the smallest ($P < 0.05$), with an average size of 27.4 ha. Within the systems adopting loose-housing, traditional systems with restricted feeding were smaller (32.1 ha) ($P < 0.05$) than modern ones. Farms using TMR were the largest ($P < 0.01$), with an average of 51.5 ha for B&F, 58.7 ha for fatteners. As expected, traditional farms with tied animals allocated the least space ($P < 0.01$) to fattening animals at 2.00 m^2 per head. Average space allowance for loose animals was lower ($P < 0.01$) in specialised fattener systems than in B&F systems, reflecting the former's more intensive management and higher ($P < 0.01$) animal density. As a consequence of purchasing calves from other farms, specialised fatteners produced about three times more slaughter animals per year than B&F, despite their similar size in terms of land area.

Table 3 shows the effects of production system on some of the indices of animal welfare and environmental conditions. Traditional farms with tie-stalls were the worst overall, with lower ($P < 0.05$) scores for all the traits evaluated (not significant only for animal docility). Although the two traditional systems shared certain management features, those with loose-housing were more similar in structural traits to the modern systems. Among the modern systems, overall conditions were better ($P < 0.01$) in those using TMR, mainly as a consequence of more modern buildings.

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Diet compositions in the different production systems are compared in Table 4. Ground corn was the main feed in every system, accounting for between 30 and 40% of the concentrate mix or TMR supplied to fattening animals. Corn silage was very seldom part of the diet in the systems analysed, and even when it was detected (11 of the 115 farms), as in some TMR specialised fattener units, it was never the main component of the diet. Ear corn silage was widely used in TMR systems ($P < 0.01$) and was an important part of the diet, especially in B&F units. Furthermore, in TMR systems, individual feed ingredients were far more widely used than purchased compound feeds ($P < 0.01$). In all the other systems, the use of commercial compound feed was common, with average proportions in the diets ranging from 30% (traditional, pens) to 49% (B&F, without TMR). In the traditional and modern systems that didn't use TMR, hay distributed *ad libitum* was always used as forage, while a mixture of hay and wheat straw was included in the feed in both TMR systems.

Table 5 shows the estimated chemical composition of the concentrates supplied in the different beef production systems, expressed as percentage of raw feed. On average, concentrates contained 13% crude protein (CP), 6% crude fibre (CF), 4% ether extract (EE) and 5% ashes (AS). The CP content differed ($P < 0.01$) across production systems, with the lowest proportions found on farms using TMR, probably due to ear corn silage being a substantial component of the diet.

Carcass and meat quality traits

Descriptive statistics, the ANOVA results and the effects of beef production system on the carcass traits of Piemontese young bulls are presented in Table 6. The average carcass weight of the Piemontese young bulls sampled was 438.1 (±43.6) kg, while the average age at slaughter was 540.9 (±63.2) days, giving an average daily carcass gain of 0.818 (±0.107) kg/d. Average EUS was 14.66, corresponding to an average evaluation approaching "E+" in the EU linear grading system.

The effect of individual farm within production system explained a proportion of the total variance that varied greatly according to the trait considered, from only about one twentieth in the

case of the rib eye area, one tenth for EUS, a quarter for carcass weight and daily carcass gain, to about half for age at slaughter (Table 6), highlighting that the large variability in management techniques still exists even within a given production system. The results show that the batch effect, i.e. animals slaughtered on the same day, explained a much smaller amount of variance than farm, ranging from 4.7% for daily carcass gain to 17.6% for the rib eye area. In the case of the latter trait, it is possible there was an influence of slaughterhouse operator during the sample collection.

Production traits (age at slaughter and daily carcass gain), but not carcass quality (EUS and rib eye area), were affected by the young bulls' birth season ($P < 0.01$) (results were most favourable in the January/March season, the least in the April/June season) and the parity of their dam ($P < 0.01$) (the most favourable results were obtained for 3rd-8th parity class).

Production system affected age at slaughter ($P < 0.05$), carcass daily gain ($P < 0.01$) and SEUROP ($P < 0.05$). The results for the traditional beef system with tied animals, in particular, were much worse than all the systems with loose-housed animals: tied young bulls grew more slowly ($P < 0.01$) (-0.070 kg/d), over a longer ($P < 0.01$) fattening period (+40 d) to reach a similar weight (-10 kg), and their carcasses had lower ($P < 0.01$) muscularity scores (-0.64) and less ($P < 0.05$) rib-eye area (-3.2 cm^2). The production traits of the 5 beef systems rearing loose-housed animals did not differ much, except that the rib-eye of carcasses produced on modern B&F farms had larger ($P < 0.05$) cross-sectional area than those produced by specialised fatteners (+3.2 cm^2).

As shown in Tables 7 and 8, colour and meat quality traits were consistently affected by batch, which explained an average of around 30% of total variance, ranging from 14% for PL to 63% for pH, the latter trait characterised by little overall variability. The amount of variance explained by the effect of farm within beef system, instead, was almost negligible, with L* and PL having the highest values at around 7% of total variance.

Colour traits, pH and CL were not affected by the young bulls' season of birth nor by the parity of their dams, although both of them had a moderate ($P < 0.05$) influence on PL and the former on

shear force ($P < 0.05$).

The class of carcass weight was a very important source of variation in all meat quality traits, with the only exception of shear force as the LSMs of the heaviest class were always higher (P < 0.05) than those of the lightest.

After taking into account the effects of all the other sources of variation included in the model, beef production system was found to have very little influence on the quality traits. In fact, the differences between the production systems were significant only for one of the colour traits, L*. The traditional tie-stall system produces meat with the highest (P < 0.05) L* value, probably due to the animals' lack of physical activity, underlined by the contrast between tie-stalls and all loose-housing systems. Feeding was also found to influence (P < 0.05) the L* of meat, which was darker from animals fed TMR than from animals fed separated concentrates and hay. We did not find production system to have any influence on pH, shear force and the two water loss traits, PL and CL.

DISCUSSION

Beef production systems

Few previous studies have directed attention to characterising the farming systems used in beef production. Some recent studies are focused on geographical areas and production techniques far from EU conditions (Asem-Hiablie, Rotz, Stout, & Fisher, 2017; Asem-Hiablie, Rotz, Sandlin, Sandlin, & Stout, 2018; Cavalcante et al., 2018). The use of the Piemontese breed as a case study, allowed to analyse in detail the beef production system in an European context. Due to the characteristics of the present research, the obtained results could be of interest also for other European beef breeds characterised by lean carcasses and for European production systems characterised by intensive fattening.

The beef production systems characterised in the present investigation on the basis of a

combination of operation cycle, housing and feeding techniques, showed that traditionally managed farms still coexist with more advanced units using modern technologies. B&F and specialised fattener systems are equally represented in our study in terms of both farms and animals. The loose-housing system has been widely adopted, substantiating Sgoifo Rossi et al.'s (2011) findings, which showed an increase in this management system since a previous study on Piemontese breed carried out by Destefanis et al. (2005), who reported an incidence of 56.2%. The structural investments needed for traditional farms with pens to move from tie-stalls to loose-housing allows them to provide their animals with better environmental conditions. Changes in feeding strategies also reveal a tendency towards modernisation: a*d libitum* increased from 46% (Destefanis et al., 2005) to 66% of feed supply system, and TMR was used on 30% of farms against negligible use in previous studies. More advanced type of feed, such as TMR, go hand in hand with a management technique characterised by modern technology and structures. Although fatteners using TMR had the highest density of animals among all the loose-housing systems, their results were the best in most of the subjective environmental evaluations.

Concerning feeding, we were unable to compute the exact nutritional composition of the rations, as we did not have any information on the amount of hay and straw distributed *ad libitum* to fattening animals. However, we were able to calculate the nutritional composition of the concentrate mix by deducting the amount of hay and straw from TMR. The average dietary content of crude protein (CP) varied little among production systems, ranging from 133 to 144 g/kg dry matter (DM).

Boucqué, Fiems, Cottyn, & Buysse (1984) suggested that a CP content exceeding 140 g/kg DM was required for Belgian Blue young bulls. De Campeneere, Fiems, Cottyn, & Boucqué (1999) suggested a CP concentration decreasing from 163 to 120 g CP/kg of dry matter intake (DMI) for the same breed at different stages of life as a function of the animal's body weight. Reducing the CP content in the diet of purebred Piemontese young bulls from 145 g/kg to 108 g/kg of DMI across

the whole fattening cycle was not found to affect growth performance (Schiavon, Tagliapietra, Dal Maso, Bailoni, & Bittante, 2010), nor carcass and meat quality traits (Schiavon et al., 2011), but it improved the efficiency of dietary nitrogen use (Schiavon, Tagliapietra, Dalla Montà, Cecchinato, & Bittante, 2012).

The crude fibre content of the concentrate mix was also relatively low (6.0±1.6% as fed), especially when the modest level of corn silage and dry roughage included in the rations compared with other beef systems was considered (Cozzi, Mazzenga, Contiero, & Burato, 2008). In assessing the high energy content of the diets used in all the beef production systems studied here, consideration should be given to the very low fat deposition ability of all double-muscled breeds (Fiems, 2012) and the difficulty in reaching the minimal level of carcass fatness required by the beef market.

Carcass and meat quality traits

Piemontese cattle are highly specialised for beef production as they are double-muscled, a specific mutation of the myostatin gene (mh) located on Chromosome 2 (Grobet et al., 1998), which is almost fixed in this population. Moreover, this breed is heavily selected for improvement in growth rate and carcass conformation (Albera, Mantovani, Bittante, Groen, & Carnier, 2001) and also, unlike the Belgian Blue, for ease of calving (Kizilkaya et al., 2003). Like other double-muscled breeds, Piemontese cattle have large muscular masses and low fat deposition, and reduced incidence of the skeleton, lower feed intake and better feed conversion (Fiems, 2012) than non-double-muscled specialised beef breeds.

Average values for carcass traits were consistent with those reported in a previous study on the carcass and meat quality traits of Piemontese young bulls (Boukha et al., 2011). In our study, carcass weight was slightly higher, as was the age at slaughter, hence average daily carcass gain was very similar. The average EUS we obtained, close to the "E+" class, is greater than the "E-" average score reported in the aforementioned study (Boukha et al., 2011) and also had slightly

lower variability. There is very little information in the literature on the rib eye area of purebred Piemontese animals. Although the values we obtained were comparable to those reported by Tatum, Gronewald, Seideman, & Lamm (1990) and by Wheeler, Cundiff, Koch, & Crouse (1996) for crossbred Piemontese steers.

The quality of meat from double-muscled Piemontese animals meets the requirements of Italian consumers. It has higher water and protein contents and lower levels of intramuscular fat, usually about 1% (Barge, Brugiapaglia, Destefanis, & Mazzocco, 1993), than meat from conventional animals while the low collagen content is responsible for its greater tenderness (Destefanis, Barge, & Brugiapaglia, 1994). Meat from the Piemontese young bulls and heifers is also in the European Union's register of protected geographical indications (PGI) as "Vitelloni Piemontesi della Coscia" (Reg. no. 703/2017, 5th April 2017).

The results on meat quality in this study also largely agreed with those reported in previous studies on the Piemontese breed (Boukha et al., 2011; Cecchinato, De Marchi, Penasa, Albera, & Bittante, 2011). As in those studies, pH values displayed very small variability and did not exceed 5.87, the value proposed by Page, Wulf, & Schwotzer (2001) as the approximate cut-off between normal and dark-cutting beef carcasses. The average pH value obtained in this study was also very close to those reported by Boukha et al. (2011) for Piemontese young bulls and by Fiems, De Campeneere, Van Caelenbergh, De Boever, & Vanacker (2003) for Belgian Blue bulls.

Meat colour results were very similar to those found by Page et al. (2001) from 1,062 beef carcasses, both in terms of average values and variability, but differed from those reported by Boukha et al. (2011) probably due to the different instruments used for colour detection. We obtained lower average values for CL and shear force compared with Destefanis, Brugiapaglia, Barge, & Lazzaroni's (2003) results for Piemontese young bulls and steers, but we found greater variability in both traits, as expected when comparing a large field survey with an experimental trial.

Effects of carcass weight on meat quality traits

In general, our results revealed a marked effect of carcass weight on colour and meat quality traits. It should be noted that carcass weight is not to be taken as resulting from prolonging or shortening the fattening period of a given young bull, but is rather a measure of young bulls heavier or lighter carcasses. Carcass weight was related to age at slaughter (r = +0.24), but especially to daily carcass gain (r = +0.56), and probably also to dressing percentage and fat deposition. Indeed, young bulls with higher daily carcass gains were of a lower age at slaughter and reached commercial maturity faster.

The heavier carcasses were associated with brighter meat resulting from a combination of higher values in all the three colour coordinates. The relationship between carcass weight and L* was reported by Murray (1989), who analysed 7,695 beef carcasses produced in field conditions and found that carcass weight was inversely related to the incidence of dark meat: carcasses weighing less than 272 kg had twice the incidence of dark meat (5.1%) than those weighing more than 318 kg (2.6%). Furthermore, in a study on Charolais, Limousin and dairy-cross animals, Craigie et al. (2010) found that a* and b* were associated with carcass weight.

Irrespective of the production system, carcass weight also had a strong effect on most of the other quality traits. We found a significant effect of carcass weight class on pH, but we do not expect any practical implications as the variability in this trait was very small. The water holding capacity of meat tends to be higher in the lighter carcasses. The trend for PL to increase with carcass weight at slaughter may be related to the slower cooling rates in heavier carcasses. Relationships between carcass weight or live weight and tenderness have sometimes been found in experimental studies on the effects of prolonging the duration of fattening. In a study on Charolais heifers, Ellies-Oury et al. (2017) reported a significant effect of slaughter weight on meat tenderness only with older animals, the lower values associated with greater carcass weight, whereas no effect was found with younger animals. Similar results were obtained by Sañudo et al. (2004), who reported that the meat of young bulls slaughtered at 550 kg live weight was more tender than that of

young bulls slaughtered at 300 kg. In our study, however, tenderness was the only quality trait unaffected by carcass weight.

Effects of beef production system on carcass traits

Our analyses revealed some unusual aspects as we tried to assess the effects of production system on carcass and beef quality attributes within a single breed, a specific geographical area and relatively homogeneous conditions. All the six production systems identified were characterised by on-farm intensive fattening of animals, large use of cereal-based concentrate feeds and a lack of clear separation between fattening and finishing periods.

In general, we observed a strong effect of production system on all carcass traits except carcass weight. The weight at which animals are slaughtered is determined according to a combination of animal characteristics, such as degree of maturity and body composition, and specific market requirements. With conventional beef breeds and crossbred animals, diet composition and restricted feeding may result in a large variation in fat deposition and, consequently, in the live weight at which optimal carcass fatness is reached. This is not very evident in the case of double-muscled animals (Schiavon & Bittante, 2014), which are not prone to becoming too fat. Weight at slaughter may, therefore, be considered the main target, and the time needed to reach that target is the variable reflecting the degree of efficiency of the production system. The traditional system with tied animals seems to be highly disadvantaged compared with all the beef production systems using loose-housing, as reflected by the lower daily carcass gain, which delays the age at which the animal is slaughtered, and gives rise to a less favourable SEUROP classification and a smaller rib eye area. No significant differences were found between the other beef production systems.

While our analyses were based on field data, most investigations into the effects of management system on production traits have been carried out on experimental stations, often with a small number of animals. A few practices have been compared, such as intensive vs extensive feeding systems (Dannenberger et al., 2006; Guerrero et al., 2013; Daza et al., 2014) or, more specifically,

the effects of different diet compositions among conventional breeds (Johnson, Van Horn, West, & Harris, 1992; Avilés et al. 2015). The results obtained were, therefore, then closely related to the design of the experiment rather than answering a need to comply with market requirements. Conversely, a recent study by Soulat et al. (2018) focused on the possibility to predict carcass quality of beef crossbred heifers showing the importance of whole life rearing factors over carcass traits.

Effects of beef production systems on meat quality traits

In this study, the batch effect explained a large proportion of total variance in meat quality traits, with the exception of pH. As this effect regards animals slaughtered on the same day, it encapsulates the effects of pre-slaughter and slaughter conditions (Adzitey, 2011) and post-mortem handling of carcasses (Warris, 2000). It also includes possible effects of calibration of the equipment used for the physical analyses and of laboratory operators, which are important sources of variation in several meat quality traits. In general, the effects of beef production system and of individual farm within production system on meat quality traits were of a small magnitude. These findings agree with the principles adopted by Meat Standards Australia (MSA) system (Bonny et al., 2018), which puts more focus on slaughter conditions, carcass characteristics, ageing time and cooking techniques than on rearing factors to deliver an eating quality guarantee to consumers.

In our study the effect of production systems was appreciable only for L*, reflecting the possible influence of stall and feeding system on meat colour. Our results agree with Brugiapaglia & Destefanis (2012), who found that the meat of tie-stalled Piemontese young bulls had higher L* and b* values than those of animals fattened in pens. Indeed, the darker meat of animals reared in loose conditions could be related with the muscle's greater oxidative capacity resulting from physical activity (Vestergaard, Oksbjerg, & Henckel, 2000; Juriel, Ortigues-Marty, Picard, Micol & Hocquette, 2006). The anatomical position of the muscle and its involvement in movement also play an important role in colour variation (Dunne, O'Mara, Monahan, French, & Moloney, 2005).

Regarding feeding system, our study revealed that meat from animals on a TMR diet was associated with lower L* values, resulting in a slightly darker colour, in agreement with the findings of Avilés et al. (2015), who reported that meat from calves fed traditionally was paler than meat from TMR-fed calves, although feeding system did not have a significant effect on the other colour traits. Juriel et al. (2006) highlighted a combined effect of pasture and grass diet *vs*. maize silage-based diet on meat colour, while other studies found no clear relationships between feeding system and meat colour (French et al., 2001; Daza et al., 2014).

Apart from colour traits, the scientific literature provides no clear evidence of a relationship between farm management and meat quality. Even when comparison have been made between management systems with greater differences than those examined in this study, such as intensive *vs* extensive fattening, results have often been inconsistent or conflicting. This tendency was also observed in our study where no influence of production system on pH, shear force, PL and CL could be detected. Consistent with our findings, studies by Daza et al. (2014), Cerdeño, Vieira, Serrano, Lavín, & Mantecón (2006) and French et al. (2001) found no effect of feeding system on PL. Guerrero et al. (2013) reported an effect of pre-finishing management of young bulls on PL but not on CL. More recently, Gagaoua et al. (2018) highlighted an effect of carcass fatness over young bulls' meat tenderness, juiciness and flavour. Moreover, they also reported that animals with shorter fattening duration and lower body weight at the beginning of the fattening period were able to produce meat with better eating quality. Soulat et al. (2018) showed that the prediction of meat quality traits obtained from rearing factors were less accurate than those of carcass traits. Nevertheless age at slaughter, ease of birth and genetic potential in muscular development could explain the eating quality of heifers' meat appraised by a tasting panel.

How carcass and meat quality traits will change in future

As already mentioned in this study, the main change to the beef production systems in the case study area has been the gradual replacement of the traditional system of tied animals with systems

using loose-housing. The results obtained here confirm that, aside from improvement in animal welfare and production ethics which are key issues for the European consumers (Hocquette et al., 2018), this trend would greatly improve the animal's production efficiency (daily carcass gain and conformation) without having any undesired effect on meat quality, with the only exception of meat lightness. This goes in the direction of a broader concept relative to a sustainable efficient livestock production, as highlighted by Scollan et al. (2011) and by Hocquette et al. (2018) analysing the future research priorities for animal production. Among the 5 beef systems using loose-housing, there were many differences in terms of number of the manageable animals, capital investments, labour requirements, feeds and welfare issues, with modern systems using TMR fed *ad libitum* being the more efficient. However, the carcass and meat quality traits did not differ much between systems, so no major changes in these traits should be expected in the future as a consequence of changes in farming systems.

Within beef production systems, however, there was large variability among individual farms in carcass weight and daily carcass gain, and particularly for age at slaughter, but not for EUS. A great variability among farms was highlighted also in other studies regarding economic and environmental performances (Veysset, Lherm, & Bébin, 2010; Veysset, Lherm, & Bébin, 2011). This variability should be studied in greater detail to understand which factors, not considered in the present study, may be affecting carcass traits and how they could be exploited to improve production efficiency.

The small effect of beef production system and of individual farm within beef system makes it clear that the variability in carcass conformation and meat quality traits depends mainly on individual animal factors and that improvements to them can be made by taking action at the level of individual animals. As the most important individual factor explaining meat quality was carcass weight class, it is important to understand the extent to which this effect depends on growth rate potential, fat deposition rate, length of fattening period and carcass yield. As only genetics can yield

durable improvements in carcass and meat quality, further knowledge of the genetic mechanisms underlying the variations in these traits also needs to be acquired.

CONCLUSIONS

This study provides a detailed description of beef production systems using as a case study the Piemontese breed, which exemplifies the main beef production systems classified by the European Union. Six main types, according to specific management strategies were identified. Traditional systems coexist alongside more advanced systems using modern technologies. Within the production systems identified, there is still a considerable variation among farms. Carcass traits are strongly affected by production system, with traditional management conditions having lower production efficiency. However, production system exerts only a very small effect on meat quality, limited to colour traits. It appears that meat quality may be conditioned by other factors related to individual animals within farms, suggesting that future improvement should look, in particular, to genetics.

Table 1. Profiles of beef production systems identified by hierarchical cluster analysis on the basis of the following binary variables: beef production system (Breeders&fatteners *vs* specialised fatteners), housing system (tie-stalls *vs* loose-housing), feed supply (restricted *vs ad libitum*) and feed distribution (TMR *vs* separate distribution of concentrates and forage).

Cluster:	Sampled (n):		Incidence on farms (%):			
	Farms	Young bulls	Integrated cow-calf and fattening	Ad libitum feeding (ad lib)	Total Mixed Ration (TMR)	Loose housing system (pens)
All farms	115	1,327	50	66	30	77
Traditional systems[#]:						
• tie-stalls	24	160	63	25	0	0
• pens	21	196	48	0	19	100
Breeders-fatteners, *ad lib*:						
• TMR	14	218	100	100	100	100
• no TMR	18	208	100	100	0	100
Fatteners, *ad lib*:						
• TMR	16	200	0	100	100	88
• no TMR	22	345	0	100	0	100

[#]: adopting restricted feeding.

Table 2. Descriptive statistics and effect of beef production system on farm size, yearly production and space allowance of animals.

	Farm size, ha	Slaughtered animals, $n \times ha^{-1}$	Slaughtered animals, $n \times yr^{-1}$	Space allowance, $m^2 \times head^{-1}$
General mean	39.2	2.55	82.3	4.66
Standard deviation	26.4	4.20	111.5	2.44
Traditional systems[#]:				
- tie-stalls	27.4	1.77	46.0	2.00
- pens	32.1	1.92	63.9	5.20
Breeder-fatteners, *ad lib*:				
- TMR[ç]	51.5	1.41	59.6	6.20
- no TMR[ç]	44.0	0.83	34.8	6.01
Fatteners, *ad lib*:				
- TMR[ç]	58.7	3.76	163.7	4.62
- no TMR[ç]	33.7	5.43	137.0	5.00
Contrasts (estimate):				
- tie-stall *vs* loose-housing[1]	-13.0*	-0.62	-27.8	-3.60**
- restricted *vs* ad lib[2]	-14.9*	-0.94	-34.9	-0.25
- breeders-fattener *vs* fatteners[3]	1.5	-3.48**	-103.1**	1.30**
- TMR[ç] *vs* no TMR[ç4]	16.3**	-0.54	25.7	-0.10
- interaction[5]	8.8	-1.12	0.9	-0.29
RMSE	24.6	3.97	103.3	2.00

[#]: restricted feeding
[ç]: total mixed ration
* $P<0.05$; ** $P<0.01$
[1]:Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[2]:Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[3]:(breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)
[4]:(breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)
[5]:(breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

Table 3. Descriptive statistics and effect of beef production system on subjective evaluation of animal facilities by technicians (1=poor, 2=average, 3=good).

	Building adequacy	Cleanness condition	Aeration efficiency	Water availability	Animal docility	Overall evaluation
General mean	2.11	2.16	2.04	2.73	2.78	2.29
Standard deviation	0.75	0.65	0.77	0.52	0.46	0.52
Traditional systems[#]:						
- tie-stalls	1.38	1.79	1.42	2.54	2.62	1.81
- pens	2.14	1.95	2.10	2.52	2.71	2.20
Breeder-fatteners, *ad lib*:						
- TMR[ç]	2.43	2.29	2.43	3.00	2.93	2.56
- no TMR[ç]	2.06	2.33	2.22	2.72	2.89	2.35
Fatteners, *ad lib*:						
- TMR[ç]	2.73	2.47	2.47	2.93	2.87	2.69
- no TMR[ç]	2.29	2.33	2.00	2.81	2.76	2.39
Contrasts (*estimate*):						
- tie-stall *vs* loose-housing[1]	-0.85**	-0.45**	-0.77**	-0.22*	-0.20	-0.56**
- restricted *vs ad lib*[2]	-0.23	-0.40**	-0.18	-0.34**	-0.15	-0.29**
- breeders-fattener *vs* fatteners[3]	-0.27	-0.09	0.09	-0.01	0.09	-0.08
- TMR[ç] *vs* no TMR[ç][4]	0.41**	0.04	0.34	0.20	0.07	0.26*
- interaction[5]	0.04	0.09	0.13	-0.08	0.03	0.04
RMSE	0.62	0.61	0.70	0.50	0.45	0.44

[#]: restricted feeding
[ç]: total mixed ration
* $P<0.05$; ** $P<0.01$
[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)
[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)
[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

Table 4. Effect of beef production system on ingredient composition (% as fed) of concentrate mix and type of forage supply.

	Corn silage	Ear corn silage	Compound feed	Ground corn	Barley, wheat	Wheat bran[1]	Beet pulp	Soybean meal	Other proteins	Fats	M-V mix	Forages supplied
General mean	2.27	8.90	34.12	34.95	2.72	5.89	3.14	5.42	1.14	0.38	0.87	-
Standard deviation	8.37	21.07	37.22	23.95	5.53	7.57	5.18	6.63	3.31	0.92	1.39	-
Traditional systems[#]:												
- tie-stalls	1.75	2.29	48.58	30.71	5.28	6.58	0.42	3.25	0.92	-	0.25	*ad lib*
- pens	1.07	7.89	30.52	39.30	2.30	7.14	3.84	5.35	1.56	0.19	0.83	*ad lib*
Breeder-fatteners, *ad lib*:												
- TMR[ç]	1.79	32.50	10.51	33.53	1.22	4.53	4.39	9.90	-	0.56	1.08	12.6[2]
- no TMR[ç]	-	-	48.72	38.22	1.94	3.06	3.22	2.44	1.22	0.33	0.72	*ad lib*
Fatteners, *ad lib*:												
- TMR[ç]	8.94	18.99	12.19	34.00	1.16	7.45	4.27	7.59	1.78	1.14	1.43	12.2[3]
- no TMR[ç]	1.27	2.00	40.80	34.37	3.06	6.01	3.75	5.87	1.18	0.35	1.18	*ad lib*
Contrasts (*estimate*):												
- tie-stall *vs* loose-housing[1]	0.72	- 8.30	15.94*	- 5.64	3.15*	1.40	- 3.38	- 2.64	-0.07	- 0.36	- 0.71*	-
- restricted *vs ad lib*[2]	- 1.93	- 5.51	2.47	4.27	0.46	1.88	- 0.07	- 1.10	0.51	- 0.41	- 0.27	-
- breeders-fattener *vs* fatteners[3]	- 4.21*	5.76	3.12	1.69	- 0.53	- 2.94	- 0.20	- 0.56	-0.87	- 0.30	- 0.40	-
- TMR[ç] *vs* no TMR[ç4]	4.73*	24.7**	-33.41**	- 2.53	- 1.31	1.45	0.84	4.59**	- 0.31	0.50*	0.30	-
- interaction[5]	2.94	- 7.76	4.80	2.17	- 0.59	- 0.01	- 0.32	- 2.87	0.91	0.28	- 0.06	-
RMSE	8.09	18.58	34.84	24.29	5.45	7.59	5.08	6.34	3.34	0.87	1.37	-

[#]: restricted feeding

[ç]: total mixed ration

[1]: included other cereal byproducts and distillers and soybean hulls

[2]: % of total intake (on average 87.1% meadow hay and 12.9% barley or wheat straw)

[3]: % of total intake (on average 86.2% meadow hay and 13.8% barley or wheat straw)

* $P<0.05$; ** $P<0.01$

[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)

[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)

[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

Table 5. Descriptive statistics and effect of beef production system on nutrient composition of mix concentrates (% as fed). TMR$^{\varsigma}$ net of forages amount.

	Crude Protein	Crude Fibre	Ether Extract	Ashes
General mean	13.1	6.0	3.9	4.79
Standard deviation	1.8	1.6	1.0	1.4
Traditional systems[#]:				
- tie-stalls	13.6	6.1	3.5	4.7
- pens	13.3	5.7	3.6	4.4
Breeder-fatteners, *ad lib*:				
- TMR$^{\varsigma}$	12.8	5.9	3.6	4.1
- no TMR$^{\varsigma}$	13.6	6.1	4.2	6.0
Fatteners, *ad lib*:				
- TMR$^{\varsigma}$	11.9	6.2	3.9	4.1
- no TMR$^{\varsigma}$	13.5	5.8	4.2	5.2
Contrasts (*estimate*):				
- tie-stall *vs* loose-housing[1]	0.3	0.2	-0.4	-0.3
- restricted *vs ad lib*[2]	0.3	-0.3	-0.4	-0.5
- breeders-fattener *vs* fatteners[3]	0.5	0.0	-0.1	0.4
- TMR$^{\varsigma}$ *vs* no TMR$^{\varsigma}$[4]	-1.2**	0.0	-0.4	-1.5**
- interaction[5]	-0.4	0.2	0.2	0.4
RMSE	1.7	1.7	1.0	1.3

[#]: restricted feeding
$^{\varsigma}$: total mixed ration
* P<0.05; ** P<0.01
[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)
[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)
[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)
[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

Table 6. Descriptive statistics, ANOVA, and effects of beef production system on age of Piemontese young bulls at slaughter and carcass traits.

	Age at slaughter d	Carcass weight kg	Carcass gain kg/d	SEUROP score[1]	Rib eye area cm^2
General mean	541	438	0.818	14.66	92.0
Standard deviation	63	44	0.107	1.54	14.3
ANOVA					
Slaughter batch[2] (%)	7.1	8.9	4.7	6.8	17.6
Farm within system[2] (%)	52.5	24.7	27.3	10.2	4.9
Birth season (*F-value*)	13.4**	1.6	4.5**	1.2	0.9
Parity of dam (*F-value*)	7.0**	2.7*	10.9**	0.8	0.4
Beef production system (*F-value*)	3.1*	0.6	4.8**	3.1*	2.3
Beef production system (*LS-means*)					
Traditional systems[#]:					
- tie-stalls	581	426	0.746	14.02	89.5
- pens	539	434	0.815	14.40	91.0
Breeder-fatteners, *ad lib*:					
- TMR[ς]	559	438	0.797	14.92	94.6
- no TMR[ς]	515	432	0.849	14.62	92.8
Fatteners, *ad lib*:					
- TMR[ς]	549	430	0.789	14.46	89.0
- no TMR[ς]	550	438	0.803	14.69	92.2
Contrasts (*estimate*):					
- tie-stall *vs* loose-housing[1]	40**	-10	-0.070**	-0.64**	-3.2*
- restricted *vs ad lib*[2]	-4	2	0.005	-0.28	-1.2
- breeders-fattener *vs* fatteners[3]	-13	1	0.027	0.20	3.1*
- TMR[ς] *vs* no TMR[ς][4]	21	-1	-0.033	0.04	-0.7
- interaction[5]	-22	-7	0.019	-0.27	-2.5
RMSE	42.1	36.1	0.087	1.4	12.4

[1]: Carcass conformation score (from S+=18 to P−=1)

[2]: Random factor variance expressed as % of total variance

* P<0.05;** P<0.01

[#]: restricted feeding

[ς]: total mixed ration

[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)

[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)

[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

Table 7. Descriptive statistics, ANOVA, and effects of beef production system and carcass weight on meat colour traits.

	L*	a*	b*	C*	h*
General mean	39.8	28.6	9.6	30.2	18.5
Standard deviation	3.5	1.7	1.7	2.1	2.0
ANOVA					
Slaughter batch[1] (%)	19.3	24.2	22.4	23.6	21.1
Farm within system[1] (%)	7.1	3.5	3.3	3.3	3.5
Birth season (*F-value*)	0.3	2.3	1.8	2.4	1.2
Parity of dam (*F-value*)	1.3	2.5	2.5	2.5	1.8
Beef production system (*F-value*)	3.1*	1.8	2.3	2.0	2.1
Carcass weight (*F-value*)	14.8**	31.3**	34.7**	33.6**	30.0**
Beef production system (*LS-means*)					
Traditional systems[#]:					
- tie-stalls	40.8	28.98	10.0	30.67	18.86
- pens	39.6	28.57	9.6	30.15	18.36
Breeder-fatteners, *ad lib*:					
- TMR[ç]	39.3	28.72	9.6	30.31	18.37
- no TMR[ç]	40.7	28.80	9.9	30.45	17.78
Fatteners, *ad lib*:					
- TMR[ç]	39.7	28.41	9.4	29.95	18.19
- no TMR[ç]	39.7	28.61	9.6	30.20	18.44
Contrasts (*estimate*):					
- tie-stall *vs* loose-housing[1]	0.9*	0.31	0.3	0.40	0.37
- restricted *vs ad lib*[2]	-0.3	-0.06	-0.1	-0.08	-0.08
- breeders-fattener *vs* fatteners[3]	0.4	0.25	0.3	0.31	0.26
- TMR[ç] *vs* no TMR[ç][4]	-0.8*	-0.14	-0.2	-0.19	-0.33
- interaction[5]	0.6	-0.06	0.1	-0.05	0.08
Carcass weight (*LS-means*)					
< 350 kg	38.8[a]	27.7[a]	8.6[a]	29.0[a]	17.1[a]
351-400 kg	39.4[a]	28.2[a,b]	9.3[b]	29.7[a,b]	18.1[b]
401-450 kg	39.4[a]	28.5[b]	9.5[b]	30.1[b]	18.4[b]
451-500 kg	40.3[b]	29.1[c]	10.1[c]	30.8[c]	19.0[c]

> 500 kg	41.9[c]	29.9[d]	11.0[d]	31.9[d]	20.0[d]
RMSE	2.9	1.4	1.3	1.7	1.7

[1]: Random factor variance expressed as % of total variance.

* $P<0.05$; ** $P<0.01$

[#]: restricted feeding

[ç]: total mixed ration

[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)

[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)

[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

[a,b,c,d]=$P<0.05$

Table 8. Descriptive statistics, ANOVA, and effects of beef production system and carcass weight on meat quality traits.

	pH	Purge losses %	Cooking losses %	Shear force N
General mean	5.56	4.51	16.8	40.97
Standard deviation	0.06	1.20	3.4	10.36
ANOVA				
Slaughter batch[1] (%)	63.1	13.6	43.1	41.8
Farm within system[1] (%)	4.5	7.3	3.1	5.2
Birth season (*F-value*)	0.2	3.9*	0.5	3.4*
Parity of dam (*F-value*)	2.5	2.7*	0.7	0.7
Beef production system (*F-value*)	2.2	0.7	0.9	0.6
Carcass weight: (*F-value*)	3.9*	6.6**	4.5**	0.8
Beef production system (*LS-means*)				
Traditional systems[#]:				
- tie-stalls	5.54	4.24	16.28	39.47
- pens	5.55	4.23	16.54	39.30
Breeder-fatteners, *ad lib*:				
- TMR[ς]	5.56	4.24	16.42	40.68
- no TMR[ς]	5.55	4.47	16.08	40.21
Fatteners, *ad lib*:				
- TMR[ς]	5.55	4.44	16.37	40.00
- no TMR[ς]	5.56	4.38	15.85	41.17
Contrasts (*estimate*):				
- tie-stall *vs* loose-housing[1]	-0.010	-0.086	0.058	-0.87
- restricted *vs ad lib*[2]	-0.006	-0.153	0.360	-1.22
- breeders-fattener *vs* fatteners[3]	0.003	-0.059	0.144	-0.14
- TMR[ς] *vs* no TMR[ς4]	0.004	-0.086	0.430	-0.35
- interaction[5]	-0.009	0.146	0.089	-0.82
Carcass weight (*LS-means*)				
< 350 kg	5.55[a,b]	3.67[a]	15.2[a]	38.52
351-400 kg	5.55[b]	4.45[b]	16.7[b]	40.81
401-450 kg	5.55[b]	4.38[b]	16.8[b]	40.56

451-500 kg	5.56[a]	4.56[b]	16.5[b]	40.21
> 500 kg	5.55[a,b]	4.62[b]	16.1[a,b]	40.59
RMSE	0.03	1.06	2.5	7.68

[1]: Random factor variance expressed as % of total variance.

* P<0.05; ** P<0.01

[#]: restricted feeding

[ç]: total mixed ration

[1]: Traditional tie-stalls *vs* (Traditional tie-stalls + breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[2]: Traditional pens *vs* (breeders-fatteners-noTMR + breeders-fatteners-TMR + fatteners-noTMR + fatteners-TMR)

[3]: (breeders-fatteners-noTMR + breeders-fatteners-TMR) *vs* (fatteners-noTMR + fatteners-TMR)

[4]: (breeders-fatteners-noTMR + fatteners-noTMR) *vs* (breeders-fatteners-TMR + fatteners-TMR)

[5]: (breeders-fatteners-TMR + fatteners-noTMR) *vs* (breeder-fatteners-noTMR + breeders-TMR)

[a,b,c,d]=*P*<0.05

CHAPTER 2.

Heritability and genetic correlations of carcass and meat quality traits in Piemontese young bulls

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ABSTRACT

Age at slaughter (AS), carcass weight (CW), carcass daily gain (CDG), conformation (EUS), and rib eye area (REA, cm^2) were recorded on 1,327 Piemontese young bulls. On the same animals pH, lightness (L*), redness (a*), yellowness (b*), hue angle (h*), chroma (C*), purge loss (PL), cooking loss (CL), and shear force (WBSF) were assessed on the *Longissimus thoracis* muscle. Heritabilities of carcass traits ranged from 0.07 (EUS) to 0.32 (CDG), whereas those of meat quality from 0.12 (PL) to 0.32 (WBSF). Genetically, an increase of AS exerts an unfavorable effect on PL (0.40) and colour traits (L*-0.20, a*-0.32, b* -0.25), whereas CW and CDG exert the opposite effect. EUS is favorably correlated with PL (-0.32) and unfavorably with WBSF (0.53) while REA is unfavorably related to PL (0.41), CL (0.35), a* (-0.58), b* (-0.44) and favorably to L* (0.41). The current selection of Piemontese breed can cause indirect modification of some quality traits of beef, particularly colour and tenderness.

Keywords*: heritability, meat color traits, tenderness, cooking losses, Piemontese*

INTRODUCTION

Beef consumption in the EU has declined over the last 20 years by 12% (Organisation for Economic Co-Operation and Development, 2017). Together with the adverse publicity concerning environmental, health, authenticity and safety issues, inconsistent quality may have contributed to this decline (Farmer & Farrell, 2018). Indeed, consumers want beef that is safe, nutritious and of good-eating quality (Verbeke et al., 2010) and they would be willing to pay a higher price for better-eating quality if this can be assured (Polkinghorne & Thompson, 2010).

The knowledge of meat quality characteristics is then essential for the beef market (Farmer & Farrell, 2018), and their evolution with time is crucial for the future of many beef production systems. However, due to the expensive and laborious operations in obtaining phenotypes at population level, measurements or sensory scoring (Gill et al., 2010; Do et al., 2016) of meat quality traits are very rarely used in the selection of specialised beef breeds.

The knowledge of the genetic relationships between meat quality traits and the traits that are being improved by a breeding program for meat production is crucial to understand in which direction meat quality will evolve on the basis of the current selection.

To reduce the generation interval while maintaining a good level of accuracy of selection, beef breeds are mainly selected according to production traits collected on candidate sires during performance testing on station (Andersen et al., 1981). Production traits (daily gain, live fleshiness) are used as predictors of carcass trait, which are difficult to collect at slaughterhouses and, consequently, rarely available and used (Johnston, Reverter, Ferguson, Thompson, & Burrow, 2003).

Regarding quality of meat, several studies quantified the heritability of some measured or scored meat quality traits in cattle breeds reared in very different farming and market systems (Wolcott et al., 2009; Gill et al., 2010; Rolf et al., 2015; Do et al., 2016), and highlighted that genetic variability exploitable for genetic improvement existed among these traits.

In cattle, some investigations focused on the estimation of genetic correlations between live animal performances and meat quality traits (Marshall, 1999; Burrow, Moore, Johnston, Barendse, & Bindon, 2001) showing few unfavourable relations between growth and meat quality traits. Few results have been published on genetic correlations between traits included in the breeding objective and meat characteristics in the European framework of specialised beef cattle breeds (Bonfatti, Albera, & Carnier, 2013). Despite the higher value of meat from hypertrophied animals (European Commission, 2011), only one attempt has been made to study the heritability of beef quality traits and their genetic correlations with production traits within a double muscled beef breed (Boukha et al., 2011). In this study both the carcass weight and the age at slaughter were considered as environmental effects and included in model to estimate genetic parameters of meat quality traits as fixed effects. As a consequence, the estimated heritabilities of meat quality traits were obtained at equal carcass weight and age. However, the existence of genetic variability for weight and age at slaughter requires to deepen the knowledge of their relationships with meat quality traits in order to provide unbiased predictions of breeding values (Sbarra, Mantovani, Quaglia, & Bittante, 2013).

Then, this study is aimed to investigate the genetic parameters and to analyse the phenotypic and genetic relations within and between carcass and meat quality traits.

MATERIAL AND METHODS

This study is part of the "Qualipiem" project aimed at analysing the phenotypic and genetic sources of variation of meat quality traits in the Piemontese breed in order to propose innovative selection strategies for their improvement.

Animals and beef sampling

A total of 115 farms of the Piemonte region (North-west Italy) enrolled in the Herd Book of Piemontese cattle breed were selected. They belonged to 6 different farming systems, from very traditional to more intensive ones. The criteria of clusterization and selection of farms have been

described in Savoia et al. (2018c).

The study was carried out on 1,327 Piemontese young bulls reared in the aforementioned commercial farms and slaughtered according to the decision of the farm owners at the same commercial abattoir (Operti, Centallo [CN], 12044, Italy), from April 2015 to February 2017. Young bulls were selected for being sired by 204 A.I. purebred sires and by 1,286 dams, all registered in the Italian Piemontese Herdbook.

After slaughter, hot carcass weight (CW) and carcass conformation class according to the EU linear grading system (Commission of the European Communities, 1982) were recorded. In order to obtain a better differentiation of carcass conformation, the six main grades (S, E, U, R, O, P from the best to the worst) were further subdivided into three subclasses (+,= or -). Prior to statistical analyses, categories of carcass conformation were rearranged into numerical scores (EUS) ranging from 1, corresponding to the P- class, to 18, corresponding to the S+ class. Fatness score was not considered in this study because of the lack of variation due to the well-known leanness of carcasses produced by double-muscled breeds and the need to fulfil local market requirements. Age at slaughter (AS) was calculated from the dates of birth and the dates of slaughter. As individual live weights of animals were not available, daily carcass gain (CDG) calculated as the ratio of carcass weight to age at slaughter, was used as a measure of young bulls growth rate (Juniper et al., 2005; Boukha et al., 2011). The carcasses were not electrically stimulated and they were chilled at 4 °C until twenty-four hours post-mortem.

Twenty-four hours after slaughter, individual samples (4.0 cm thick) of the *Longissimus thoracis* (LT) muscle were collected between the fifth and sixth thoracic vertebrae. Beef samples were scanned with a HP Scanjet 5590 Digital Flatbed Scanner (Hewlett-Packard; 132 Palo Alto, California) to obtain images for subsequent measurement of the rib eye area (REA, cm^2), then were individually vacuum-packaged and transferred under refrigerated condition to the laboratory. Upon arrival, samples were stored at 4°C in a chilling room for 7 days *post-mortem* until measurement of

meat quality traits.

Analysis of Meat Quality Traits

As described in details in the previous study (Savoia et al., 2018c), after ageing (7 days), the following meat quality characteristics were analysed:

- purge losses (PL, %) were determined by the difference between weight at packaging and weight after ageing;

- ultimate pH was measured using a portable Crison pH-meter PH 25+ (Crison Instruments 142 S.A.; Alella, Barcelona) equipped with a glass electrode Crison 52 32 suitable for meat penetration and an automatic temperature compensator (Boccard et al. 1981);

- rib eye area (REA, cm^2), were measured using the digital images of the samples processed using Image Pro Plus 4.5.1. software (Media Cybernetics, 2001);

- colour traits were measured on the freshly-cut surface of the steak after 1 h of blooming at 4°C using Konica Minolta CR-331C colorimeter (Konica Minolta Sensing 152 Americas, Inc; Ramsey, New Jersey) according to CIELAB coordinates (CIE 1976),: lightness (L*), redness (a*) and yellowness (b*) were recorded and hue angle (h*) and Chroma (C*) were calculated as h* = tan^{-1} (b*/a*) and C* = (a*^{2} + b*^{2})$^{0.5}$ (three random readings at different locations on the meat surface were taken and averaged);

- cooking losses (CL, %) were obtained from sealing the steak in a polyethylene bag and cooking in a water bath preheated at 75°C, to an internal temperature of 70°C (Honikel, 1998);

- Warner Bratzler shear force (WBSF, N) was obtained from 6 cylindrical cores 1,27 cm in diameter of cooked meat, with a V-shaped cutting Warner-Bratzler blade, fitted to an Instron Universal Machine model 5543 (Instron, Norwood, Massachusetts) (A.M.S.A., 2015).

Statistical Analyses

Prior to statistical analyses, observations falling outside the range of three standard deviations

from the mean of each carcass or quality trait were excluded from the data-set.

Estimation of (co)variance components and genetic parameters

(Co)variance components were estimated by REML procedures using the VCE software (version 6.0; Groeneveld, Kovac, & Mielenz, 2010). Estimation of (co)variance components for carcass and meat quality traits was performed through multiple-traits analyses within each group of traits. The estimation of (co)variance components between the two group of traits was performed through a series of multiple-traits analyses including all the carcass traits and one of the meat quality traits at a time. The general model, in matrix notation, can be written as:

$$y = X\beta + Wc + Wq + Zu + e$$

where y contains observations for carcass traits and the meat quality trait concerned, β is the vector of nongenetic fixed effects, c is the vector of random herd effects (98 levels), q is the vector of random effect of the day of slaughter (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. Preliminary analyses suggested the inclusion of the fixed effects of birth season (4 classes: January-March, April-June, July-September, October-December) for WBSF (N) and of parity of dam (4 classes: 1st, 2nd, 3rd-8th, >8) for carcass weight. For PL (%), age at slaughter and daily carcass gain both the aforementioned effects were included in the model.

Effects of different herds were assumed to be normally and independently distributed c~N(0, C ⊗ I); the effect of the day of slaughter was assumed to be normally and independently distributed q~N(0, Q ⊗ I). A minimum cell size of 3 observations was required for both the slaughter day and farm effects. Animal additive genetic effects were assumed to be normally distributed u~ N(0, G ⊗ A), where G is the (co)variance matrix between animal genetic effects, in the different traits and A is numerator of Wright's relationship matrix. Additive relationships were computed using a pedigree file including the phenotyped animals and all their known ancestors (13,122 animals). Residuals were assumed to follow the normal distribution, e~N(0, R ⊗ I).

To facilitate comparisons with literature estimates, we calculated intraherd heritability defined as:

$$h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_e^2)$$

where σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance. Genetic correlations were computed as:

$$r_a = (\sigma_{a1;a2})/(\sigma_{a1} \cdot \sigma_{a2})$$

where $\sigma_{a1;a2}$ is the additive genetic covariance between traits 1 and 2 and σ_{a1} and σ_{a2} are the additive genetic standard deviations of traits 1 and 2, respectively. Phenotypic correlations were computed as:

$$r_a = (\sigma_{p1;p2})/(\sigma_{p1} \cdot \sigma_{p2})$$

where $\sigma_{p1;p2}$ is the phenotypic covariance between traits 1 and 2, and σ_{p1} and σ_{p2} are the phenotypic standard deviations of traits 1 and 2, respectively.

RESULTS

Heritability of carcass and meat quality traits

The quantification of the variance components (Table 1) revealed that the slaughter batch represented the minor source of variation for carcass traits (4 to 9% of phenotypic variance) with the only exception of rib-eye area (18% of variance explained by slaughter batch). The effect of the fattening farm was much more variable, explaining from 6% of phenotypic variance for rib-eye area to 52% for the age at slaughter. The additive genetic variance represented a proportion of phenotypic variance varying from 6% in the case of SEUROP score and 22% of carcass daily gain, leading to an estimate of intra-herd heritability low for muscularity (7%), medium-high for carcass daily gain (32%) and intermediate for the other traits (18 to 21%).

Differently from carcass traits, all meat colour traits presented a very low effect of fattening farm, a greater effect of the slaughter batch and an additive genetic variance slightly lower than

10% for all traits except lightness (23%) (Table 2). This led to an estimate of the intra-herd heritability of about 30% for lightness and 13% for the other colour traits. Likewise, the other meat quality traits were characterized by a low effect of the fattening farm (from 4 to 6%), but by a very high incidence of the slaughter batch from 14% for purge loss to more than 60% in the case of pH (the latter was characterized by a very low phenotypic variance). Additive genetic variance represented 18% of phenotypic variance in the case of shear force and about 10% for the other traits (Table 3). The resulting intra-herd heritabilities were about 15% for purge and cooking losses and above 30% for muscle pH and shear force.

Phenotypic and genetic correlations among carcass traits

The phenotypic and genetic correlations among the carcass traits are summarized in Table 4. All phenotypic correlations were positive, ranging from almost null to intermediate values (+55%), with the only exception of the strong negative (-66%) correlation observed between the age at slaughter and the daily carcass gain.

The genetic correlations were often larger and more variable in sign than the phenotypic ones. Age at slaughter was negatively correlated not only with carcass daily gain but also with carcass weight, whereas it was positively correlated with SEUROP score and, to a much lower extent, with rib-eye area.

Carcass weight and carcass daily gain were positively correlated, while both were negatively correlated with SEUROP score and independent from rib-eye area. Lastly, a negative correlation was observed between SEUROP score and rib-eye area.

Phenotypic and genetic correlations among meat quality traits

Table 5 summarizes the phenotypic and genetic correlations among the meat quality traits. Among colour traits, L* and b*, and a* and b* were strongly correlated with each other both phenotypically and genetically, whereas L* and a* were independent.

Meat pH was positively correlated with shear force and cooking losses, and negatively with

a* and b*. High PL (%) were associated to increased L* values and, only from the genetic standpoint, with higher shear force and b* and lower CL (%). Cooking losses showed a moderate positive genetic correlation with shear force while it was strongly correlated with a* and b* phenotypically. Lastly, shear force was almost independent from colour traits.

Phenotypic and genetic correlations between carcass and meat quality traits

Phenotypic and genetic correlations between carcass traits and meat quality traits are summarized on Table 6. All the phenotypic correlations were generally low with the only notable exception of the moderate positive correlations between carcass weight and a* and b* and between carcass daily gain and L*.

The genetic correlations were in general more important and variable in sign. Age at slaughter displayed moderate positive correlations with PL and shear force, whereas it was negatively associated with a* and b*. Both carcass weight and carcass daily gain were markedly associated to L*. Carcass weight showed also moderate negative correlations with pH and a positive relationship with PL. SEUROP scores were negatively correlated with PL, and positively with shear force. Lastly, rib-eye area was correlated positively with PL, CL, and L*, and negatively with a* and b* indices.

DISCUSSION

Genetics of carcass traits

The date of slaughter of the Piemontese young bulls included in this study was chosen according to farmers decision taken for each individual animal. In such a situation, the age at slaughter is not only due to "environmental" factors (farm management and financial strategies, market requirement, etc.), but it is partially under the control of animal genetics, being moderately heritable (0.18). A previous study (Sbarra et al., 2013) on three Italian beef breeds not characterized by double muscling (Chianina, Marchigiana and Romagnola), reported for this trait h^2 values

ranging from 0.28 to 0.39. The age at slaughter was then interpreted as a measure of slaughtering/market precocity. Indeed, in farming systems in which the optimal slaughtering date is decided for each animal and not on a pen/group basis, this decision is often strongly affected by the live weight and fattening condition of animals in relation to the local market requirements. This interpretation is confirmed by the very limited variability of carcass fatness observed in this study on the basis of the SEUROP scoring system, that did not allow to estimate genetic parameters. Also in other studies focusing on the estimation of genetic parameters for carcass traits, carcass fatness was not considered or produced heritability estimates lower than the other traits (Minick, Dikeman, Pollak, & Wilson, 2004; Hornyak, Frickh, & Furst-Waltl, 2008; Gill et al., 2010; Kluska et al., 2018). So the age at slaughter could be considered as an indicator of precocity in achieving the optimal conditions in terms of fat deposition and protein accretion. As in double-muscled breeds fat deposition is very limited and less variable than in conventional beef breeds (Fiems, 2012), a lower heritability of slaughtering precocity in Piemontese than in conventional beef breeds could be expected.

The results of this study confirm that carcass weight is moderately heritable (0.19). This value falls in the interval (from 0.13 to 0.24) reported by Sbarra et al. (2013). In a previous survey on Piemontese young bulls (Boukha et al., 2011) the heritability of carcass weight was found to be much larger (0.33), but it is worth noting that in that case the age at slaughter was not considered as a trait, like we did in our investigation. In fact, Boukha et al. (2011) included the age at slaughter in the statistical model as an "environmental" fixed factor to adjust carcass weight, and this could have led to the higher heritability found then. Indeed, the study of Sbarra et al. (2013) demonstrated that the inclusion of the age at slaughter in the statistical model as a covariate increased the heritability estimates of carcass weight from 4 to 6 points, but also lead to biased estimation of the breeding values. The regression of carcass weight on age at slaughter do not reflect the growth rate of an individual animal if its slaughter date is delayed, but only differences between animals of good

growth potential, slaughtered earlier, and animals of lower genetic potential, slaughtered later. This concept is demonstrated by the consistent (-0.53) negative genetic correlation between age at slaughter and carcass weight and gain obtained in this study (Table 4). A similar problem was previously studied in the case of age and weight of young calves sold at auctions (Bittante, Cecchinato, Dal Zotto, De Marchi, & Penasa, 2011) yielding similar results, because also in that case the farmer's decision to anticipate or delay the presentation of the calf to the auction was based on the expression of traits partly under genetic control (growth rate, conformation, etc.).

The heritability of carcass gain, that is the ratio between carcass weight and age at slaughter, is almost identical in this study (0.32), in the previous survey on Piemontese breed (0.33), and in the study on 3 conventional beef breeds (0.27 to 0.42). As expected, being a ratio, carcass daily gain is strongly correlated (both phenotypically and genetically, Table 4) positively with its numerator (carcass weight) and negatively with its denominator (age at slaughter).

Estimate of heritability of SEUROP carcass conformation in Piemontese young bulls was poor (0.07). The Piemontese is a double muscled breed whose muscularity is largely dependent on the myostatin gene (mh: muscular hypertrophy) mutation almost fixed in the population (Grobet et al., 1998; Bellinge, Liberles, Iaschi, O'Brien, & Tay, 2005). This result is very different from the value, around 0.3, obtained in a previous survey on the same breed (Boukha et al., 2011), where e a different model, including also carcass weight was used. SEUROP scores of Piemontese carcasses presented modest positive phenotypic correlations with all the other carcass traits, whereas they were strongly and negatively correlated from the genetic point of view with all carcass traits except age at slaughter (that in turn was negatively correlated with carcass weight and gain, Table 5). Rib-eye area showed a heritability value larger than that of the SEUROP score and closer to that found for carcass weight, that is the trait to whom rib-eye area is more phenotypically correlated (Table 5).

A large number of studies were focused on the estimation of genetic parameters of carcass

traits (Moser, Bertrand, Misztal, Kriese, & Benyshek, 1998; Johnston et al., 2003; Crews, Lowerison, Caron, & Kemp, 2004; Do et al., 2016). Variable results have been obtained depending on the investigated breeds and farming systems, but in most cases the estimated genetic parameters were in the range of those found in this study.

Genetics of beef quality traits

Colour is the first quality aspect influencing the consumer's purchase choice. Lightness was much more heritable than the other two colour traits, confirming the results generally found in other studies (Johnston et al., 2003). Lightness was phenotypically and genetically independent from a* but highly correlated with b*, that in turn was highly correlated with a* (Table 5). Our results partly agree with literature reports as generally all the colour traits have been found to be highly associated (Page, Wulf, & Schwotzer, 2001). Similarly to the findings of Boukha et al. (2011), colour traits were independent from shear force both from the genetic and phenotypic standpoints. In more extensive farming systems with conventional breeds L* has been sometimes found to be favourably correlated with shear force (Johnston et al., 2003), and this could be related to the darker colour induced by the increase in oxidative activity often caused by pasture rearing (Dannenberger, Nuernberg, Nuernberg, & Ender, 2006). Differently from Boukha et al. (2011), genetic associations of colour traits with cooking losses were weaker than the corresponding phenotypic correlations. Results of this study also highlight that a paler meat is likely to be associated with increased purge losses especially from the genetic point of view.

For these traits a comparison with literature data is very difficult, for the high heterogeneity within and between studies with respect to breed, sex, environment, slaughter endpoint and finishing feeding regime (Johnston et al., 2003; Minick et al., 2004). Especially for shear force, it should be considered that some methodological aspects (ageing length and data editing) can widely affect the estimates of genetic parameters (Johnston, Reverter, Robinson, & Ferguson, 2001; Zwambag et al., 2013).

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The two water holding traits, purge and cooking losses presented moderate correlations with each other. They displayed a moderate positive genetic association with shear force indicating that a better water holding capacity is associated to a tender meat. Genetic and phenotypic correlations of shear force with ultimate pH were positive. They were in the same direction but of higher magnitude compared with the findings of Boukha et al. (2011), while differing from the negative phenotypic correlation reported by Destefanis, Barge, Brugiapaglia, & Tassone (2000).

Overall, it is evident that the estimates of genetic parameters for meat quality traits are largely variable in different cattle beef populations in relations to the breed characteristics, the prevalent farming system and the market requirements.

Effects of carcass traits on beef quality traits and perspectives for genetic improvement

From the phenotypic point of view, the correlations between carcass and meat traits were not relevant, with few exceptions regarding L*, that was correlated positively with carcass daily gain and negatively with age at slaughter, and a* and b* that were positively associated with carcass weight (Table 6). From the genetic point of view, factors increasing the age at slaughter seem to exert an unfavourable effect on purge losses and colour traits, whereas those affecting carcass weight and gain exert the opposite effect. These results be explained by the genetic negative correlations existing between age at slaughter and carcass weight. The estimated genetic correlations between SEUROP scores and meat quality indicate that the improvement of carcass conformation can affect favorably the purge losses but negatively the tenderness of meat. Lastly, the rib-eye area showed unfavourable genetic correlations with most of the meat quality traits, namely water holding capacity and a* and b* colour indices, whereas it was independent on shear force and favourably associated to L*.

The Piemontese breed is selected for muscularity and growth rate during performance testing on station of candidate sires (Albera, Mantovani, Bittante, Groen, & Carnier, 2001), and for direct and maternal ease of calving during progeny testing of selected sires (Carnier et al., 2000). The selection of young sires through performance testing is aimed at improving carcass weight and gain

and SEUROP scores measured in commercial abattoirs (Albera, 2015). Summarizing the genetic correlations reported in Table 6, this procedure could result in a modest favourable effect on beef lightness, but unfavourable on beef tenderness. The selection for maternal traits at population level could reduce the muscularity of cows (Bittante et al., 2018), increasing the sexual dimorphism, although cow muscularity is also evaluated through type scoring in the Piemontese breed (Mantovani, Cassandro, Contiero, Albera, & Bittante, 2010). Few studies focused on the relationships between maternal performance and carcass (Kluska et al., 2018) or beef production traits (Albera, Groen, & Carnier, 2004), but rarely with beef quality traits.

The results obtained in this study confirm that meat quality traits are heritable and their improvement with selection is theoretically possible. The analysis of genetic correlations, also shows that the current selection of beef breeds, based especially on improvement of growth rate and muscularity, can cause indirect modification of some quality traits of meat, particularly colour and tenderness. Due to the cost and the complexity of meat quality evaluation, a selection for these traits based on direct phenotyping of slaughtered animals through golden standard methods is unfeasible. In order to improve meat quality attributes through selection, two alternatives, one for phenotypic and one for genetic evaluations can be exploited.

The first alternative to be evaluated relies on the prediction of meat quality traits at abattoir level by mean of cheap, rapid, high throughput methods (Farmer & Farrell, 2018). Sensory subjective evaluation has some interest, but it is still complex and expensive (Gill et al., 2010). Methods based on near-infrared spectroscopy have shown some promising results for the prediction of meat colour and purge losses (Cecchinato, De Marchi, Penasa, Albera, & Bittante, 2011) and for chemical composition and fatty acid profile (Cecchinato et al., 2012). Results on cooking losses and tenderness were less satisfactory (Farmer & Farrell, 2018). Most of the mentioned studies are based on laboratory benchtop near-infrared spectrometers requiring the up-taking of meat samples from the carcass (that could be depreciated), but recently portable instruments are available for a direct

use in the abattoirs (Craigie et al., 2010). So, new research is needed to test the feasibility of selection for meat quality traits directly predicted at the abattoir level on intact carcasses, quarters, or anatomical joints.

The second alternative relies on a genome-wide selection based on the use of genomic breeding values predicted from estimates of the SNP marker effects for the meat quality traits. This strategy, exploiting the existence of linkage disequilibrium between the SNP markers and the QTL affecting the investigated traits (Meuwissen, Hayes, & Goddard, 2001), requires the establishment of a calibration procedure for SNP effects estimation performed on an "experimental" dataset and its subsequent use at population level (Rolf et al., 2015). As genomic calibration needs to be repeated in time to take into account the possible decline in the association between SNP markers and QTL for the traits of interest, the phenotyping based on spectroscopy predictions could be coupled with genomic approach for a reliable program of genetic improvement of beef quality.

CONCLUSIONS

The results obtained in this study highlighted that carcass traits are heritable, and that the age at slaughter could be considered an indicator of precocity of fat deposition and protein accretion. Moreover, meat quality traits showed that genetic variability theoretically exploitable to genetic improvement among animals exists.

From the phenotypic point of view, the correlations between carcass and meat quality traits were not relevant, with few exceptions. From the genetic point of view, factors increasing age at slaughter seem to exert an unfavourable effect on purge losses and colour traits, whereas those affecting carcass weight and gain exert the opposite effect. These results can be explained by the negative genetic correlation between age and weight. Then, the current selection of beef breeds, based especially on improvement of growth rate and muscularity, could cause indirect modification of some characteristics of meat, mainly those related to colour and tenderness.

Due to the cost and the complexity of meat quality traits' collection, a selection for these traits based on traditional phenotyping of slaughtered animals appears not to be feasible. Near-infrared spectroscopy and genomic selection seem to be possible alternatives for the genetic improvement of meat quality traits.

Funding and conflict of interest statement

Table 1. Descriptive statistics, variance components and heritability of carcass traits of Piemontese young bulls.

	Age at slaughter d	Carcass weight kg	Carcass gain kg/d	Muscularity SEUROP score (1-18)	Rib eye area cm^2
Young bulls, N	1166	1159	1161	1166	1154
General mean	539.0	438.8	0.820	14.69	92.27
Standard deviation	61.9	44.1	0.106	1.54	14.3
Minimum	382.0	309.0	0.536	10.00	52.43
Maximum	728.0	564.0	1.097	18.00	142.67
Phenotypic variance	4167	1996	0.0115	2.369	205
Variance components[1]					
- additive genetic	0.072	0.122	0.217	0.058	0.160
- slaughter batch	0.075	0.093	0.039	0.076	0.177
- fattening farm	0.516	0.265	0.280	0.103	0.064
Intra-herd heritability:	0.175	0.189	0.319	0.070	0.211
SE intra-herd h^2	0.076	0.083	0.085	0.064	0.095

[1]: Ratio between each variance component and phenotypic variance.
[2]: SEUROP class with +/- subclasses transformed in numerical values.

Table 2. Descriptive statistics, variance components and heritability of meat colour traits of Piemontese young bulls.

	Lightness L*	Redness a*	Yellowness b*	Chroma C*	Hue h*
Young bulls, N	1156	1157	1159	1158	1155
General mean	39.89	28.61	9.66	30.21	18.54
Standard deviation	3.49	1.74	1.66	2.15	2.03
Minimum	30.47	23.22	4.84	23.60	12.20
Maximum	50.80	33.92	14.44	36.93	23.60
Phenotypic variance	11.87	3.11	2.77	4.74	4.13
Variance components[1]					
- additive genetic	0.234	0.085	0.090	0.091	0.099
- slaughter batch	0.178	0.250	0.224	0.243	0.210
- fattening farm	0.057	0.101	0.080	0.102	0.059
Intra-herd heritability:	0.306	0.132	0.129	0.139	0.135
SE intra-herd h^2	0.095	0.070	0.070	0.075	0.074

[1]: Ratio between each variance component and phenotypic variance.

Table 3. Descriptive statistics, variance components and heritability of meat quality traits of Piemontese young bulls.

	pH	Purge losses %	Cooking losses %	Shear force N
Young bulls, N	1165	1155	1166	1147
General mean	5.56	4.51	16.76	41.03
Standard deviation	0.06	1.19	3.43	10.45
Minimum	5.43	1.68	7.83	15.89
Maximum	5.77	8.04	26.83	75.22
Phenotypic variance	334[2]	1.39	11.62	111
Variance components[1]				
- additive genetic	0.102	0.101	0.097	0.176
- slaughter batch	0.618	0.140	0.416	0.404
- fattening farm	0.050	0.049	0.040	0.055
Intra-herd heritability:	0.308	0.124	0.179	0.325
SE intra-herd h^2	0.087	0.072	0.085	0.097

[1]: Ratio between each variance component and phenotypic variance.

[2]: Phenotypic variance multiplied by 10^6

Table 4. Phenotypic and genetic correlations among carcass traits of Piemontese young bulls (SE in parentheses).

	Phenotypic correlation	Genetic correlation
Age at slaughter, with:		
• carcass weight	0.269 (0.061)	-0.530 (0.199)
• carcass gain	-0.663 (0.046)	-0.865 (0.066)
• SEUROP score	0.015 (0.052)	0.716 (0.188)
• rib eye area	0.002 (0.039)	0.183 (0.107)
Carcass weight, with:		
• carcass gain	0.533 (0.032)	0.883 (0.059)
• SEUROP score	0.357 (0.035)	-0.432 (0.284)
• rib eye area	0.323 (0.038)	0.003 (0.116)
Carcass gain, with:		
• SEUROP score	0.258 (0.057)	-0.653 (0.210)
• rib eye area	0.237 (0.049)	-0.101 (0.117)
SEUROP score, with:		
• ribeye area	0.077 (0.042)	-0.539 (0.226)

Table 5. Phenotypic and genetic correlations among meat quality traits of Piemontese young bulls (SE in parentheses).

	Phenotypic correlation	Genetic correlation
Meat pH, with:		
• purge losses	-0.025 (0.029)	0.002 (0.094)
• cooking losses	0.079 (0.036)	0.291 (0.093)
• shear force	0.404 (0.030)	0.450 (0.067)
• L*	-0.176 (0.038)	-0.128 (0.076)
• a*	-0.132 (0.030)	-0.549 (0.083)
• b*	-0.223 (0.033)	-0.546 (0.086)
Purge losses, with:		
• cooking losses	0.110 (0.043)	-0.366 (0.128)
• shear force	0.074 (0.037)	0.262 (0.098)
• L*	0.303 (0.035)	0.775 (0.051)
• a*	-0.071 (0.037)	0.024 (0.199)
• b*	0.203 (0.036)	0.368 (0.168)
Cooking losses, with:		
• shear force	0.002 (0.051)	0.299 (0.169)
• L*	-0.190 (0.049)	0.116 (0.114)
• a*	-0.803 (0.033)	-0.034 (0.067)
• b*	-0.791 (0.036)	0.057 (0.074)
Shear force, with:		
• L*	0.041 (0.043)	-0.022 (0.086)
• a*	-0.141 (0.036)	0.004 (0.106)
• b*	-0.108 (0.037)	-0.008 (0.116)
L*, with:		
• a*	-0.002 (0.039)	0.012 (0.156)
• b*	0.788 (0.027)	0.469 (0.110)
a*, with:		
• b*	0.580 (0.007)	0.889 (0.032)

Table 6. Phenotypic and genetic correlations[1] between carcass and meat quality traits of Piemontese young bulls.

	Age at slaughter d	Carcass weight kg	Carcass gain kg/d	Muscularity SEUROP score	Rib eye area cm^2
Phenotypic correlations:					
Meat pH	0.053	0.029	-0.023	0.030	0.003
Purge losses	-0.023	0.129	0.127	0.137	0.125
Cooking losses	-0.018	-0.031	0.005	0.012	0.043
Shear force	0.060	0.020	-0.034	0.054	0.023
L*	-0.216	0.137	0.304	0.123	0.096
a*	0.142	0.346	0.139	0.169	0.016
b*	0.064	0.348	0.213	0.197	0.061
Genetic correlations:					
Meat pH	-0.041	-0.305	-0.157	-0.113	0.143
Purge losses	0.403	0.332	-0.027	-0.316	0.668
Cooking losses	0.048	0.009	-0.016	-0.003	0.345
Shear force	0.206	0.134	-0.035	0.532	0.090
L*	-0.199	0.569	0.471	-0.054	0.410
a*	-0.324	-0.261	0.005	0.015	-0.579
b*	-0.248	0.0004	0.133	0.251	-0.444

[1] SE of phenotypic correlations range from 0.030 to 0.082, SE of genetic correlations range from 0.044 to 0.317.

CHAPTER 3.

Prediction of meat quality traits in the abattoir using portable and hand-held near infrared spectrometers: validation, repeatability and field testing

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ABSTRACT

A study has been implemented to evaluate the use of portable near-infrared spectrometers (NIRS) directly at the abattoir without up-taking of meat samples, for predicting physical quality of meat. Spectra have been acquired with portable visible-near infrared (Vis-NIRS) and hand-held (Micro-NIRS) instruments on 1,207 Piemontese young bulls after slaughtering. From the carcass of the same animals a sample of *Longissimus thoracis* muscle was collected and analyzed by gold standard methods. Calibration equations were developed using a Bayesian approach. Vis-NIRS showed better calibration statistics but validation statistics similar to those of Micro-NIRS. Meat colour traits and purge loss showed good predictability with both instruments, whereas for meat pH, cooking loss and shear force prediction abilities were much less favourable. This was a consequence of the large slaughter batch and residual variances affecting reference analyses of meat quality traits related to several causes (sampling, chilling, ageing, processing, instrument calibration). These factors cannot be predicted by NIR spectra collected at the abattoir which in turn are able to predict the animal "native" characteristics. A field testing showed the very good ability of both spectrometers to capture the major source of variation of meat colour traits and purge loss and the acceptable ability for pH, cooking losses and shear force.

Keywords*: meat quality, NIRS, meat colour, meat purge loss, meat cooking loss, meat tenderness*

INTRODUCTION

The quality of meat is referred to many different aspects whose determination is based on meat sampling, instrumental analyses in the laboratories and/or sensory description by trained panels of experts (Przybylski and Hopkins, 2016). This implies that the analysis of meat quality traits is normally adopted almost only for research purposes (Hocquette et al., 2016).

Quality control in the beef industry, beyond hygienic aspects, is often limited to carcass quality evaluation based on muscularity and fatness (Brad Kim et al., 2016). When meat is evaluated on a freshly cut muscle section, as in the case of the division of a carcass side in two quarters, subjective scoring or computer aided vision of some traits (muscle development, color, marbling) is performed (Jackman et al., 2011). In practice, beef industry lacks of reliable methods for objective, rapid, cheap predictions of meat quality applicable at line in the abattoir. As a consequence, an affordable payment system based on meat quality at commercial level and a phenotyping procedure needed to establish a selection program for the genetic improvement of meat quality traits are currently unvailable.

Some attempts have been made in different species to use the infrared spectroscopy for predicting meat quality traits, as reviewed by Karoui et al. (2010) and by Prieto et al. (2017), and to use these predictions for genetic purposes. In the case of beef production, a previous study (Cecchinato et al., 2011) dealt with the use of near-infrared spectroscopy (NIRS) at laboratory level after ageing on meat samples collected at abattoir. That investigation showed that NIRS application could be a valuable method for phenotyping beef carcasses, estimating genetic parameters, and predicting breeding values of sires of slaughtered animals for meat colour traits and purge loss, but not for cooking losses and meat tenderness.

The use of laboratory NIRS instruments can contribute to reduce the cost of some meat analyses but cannot become a basis for a routine system of meat quality prediction, for both commercial and genetic purposes. The collection of meat samples from each carcass, and the

subsequent transportation and processing in the laboratory causes carcass depreciation and increase of labour requirement and analytical costs.

The availability of portable NIR spectrometers increases the interest for testing their use directly at the abattoir or in the meat processing units without up-taking of samples and their transportation to laboratories (De Marchi, 2013; Wang et al., 2018). The suppliers of spectrometers are increasing their offer providing instruments with very different characteristics. Some instruments cover also the visible part of the spectrum (Vis-NIRS) qualifying them as particularly suitable to predict also meat colour traits (Qiao et al., 2015). Moreover, also very small instruments (Micro-NIRS), previously used for at line industrial applications, were adapted for a hand-held use (Zamora-Rojas et al., 2012 and 2013; Wiedermair et al. 2018), but they have not been widely tested for the prediction of beef quality.

Thus, the objective of this study was: a) to test the use of portable and hand-held spectrometers at the abattoir level on a large number of carcasses; b) to compare a top-ranking portable instrument using a wide spectrum (Vis-NIRS) with a very small hand-held one (Micro-NIRS) through cross-validation and external validation; c) to analyse the sources of variation of measured beef quality traits, of beef infrared absorbance spectrum, and of NIRS predicted beef quality traits; and d) to test at field level the ability of NIRS predictions to investigate several beef quality traits (pH, colour traits, purge losses, cooking losses, shear force).

MATERIAL AND METHODS

Animals and beef samples collection

This study is part of the *Qualipiem* project aimed to investigate the genetic bases of meat quality in the Piemontese breed and to evaluate the possibility of phenotyping beef quality traits directly at the abattoir level. The project involved 1,327 young bulls from 115 farms belonging to 6 different beef farming systems. The clustering and characteristics of the beef systems, the feeding

practices, the slaughtering of animals, and the collection, ageing, and laboratory analyses of beef samples have been described in details in a previous study (Savoia et al., 2018c).

Briefly, the young bulls selected for the research were all enrolled in the Herd Book of the Italian Piemontese breed, sired by A.I. bulls, reared in commercial farms representative of the Piemonte region (north-west of Italy) farming systems. The six production systems were: traditional farms with tie stalls and restricted feeding based on hay and compound feed; traditional farms with pens and restricted feeding similar to the previous group; modern cow-calf (breeders and fatteners) operations with or without use of total mixed rations (TMR); and modern specialized fatteners with or without use of TMR.

The average age at slaughtering was about 18 months (541±63d), the average carcass weight was 438±44 kg, (corresponding to an average carcass daily gain of 0.82±0.11 kg/d), and the muscularity grading, evaluated according to the SEUROP system (Commission of the European Communities, 1982), classified 66.7% of carcasses in the E class (excellent).

Experimental design

The objectives of the study were pursued organizing three specific trials:

- <u>Calibration trial</u>, to achieve objective a) to test portable NIRS instruments at abattoir on a large number of carcasses, and objective b) to compare a top portable instrument with a very small one;

- <u>Repeatability trial</u>, to achieve objective c) to analyse the sources of variation of measured beef quality traits, of beef infrared absorbance spectrum, and of NIRS predicted beef quality traits;

- <u>Field testing trial</u>: to achieve objective d) to test at population level the ability of NIRS predictions to investigate beef quality traits.

NIRS instruments and spectrum collection

Two very different spectrometers were used in this study. The most important characteristics

differentiating the two instruments are summarized in Table 1. The first is a "top" instrument (Vis-NIRS) collecting an extended spectrum spanning from the visible to the NIR sections of the electro-magnetic waves interval (wavelength: 350 to 1,830 nm), measured every 1 nm (1,481 data points per sample). The second instrument (Micro-NIRS) is characterized by a very small size (the weight is 60g vs 5,600g of the Vis-NIRS), a shorter spectrum limited to the NIR section (905 to 1,649 nm), measured every 6 nm (125 data points per sample).

The collection of the spectra was performed with both instruments at the abattoir after the division of the carcass side in two quarters (pistol cut) the day after slaughtering (about 24 h). Prior to taking meat spectra, instruments were calibrated using a standard white Barium Sulfate surface. The spectra were collected on the cross-sectional surface of the *Longissimus thoracis* muscle between the 5th and 6th -rib. Spectra were collected by applying the scanning head of the fiber-optic contact probe over the surface of the muscle. With both instruments, five spectra were obtained in reflectance (R) mode from different sites of the same muscle cut surface. Each spectrum resulted from the average of three replicates on the same position. The average and standard deviation intervals of the absorbance obtained as log(1/R) from the two spectrometers are illustrated in Figure 1.

Spectral data editing and processing

To better compare the two technologies/instruments, avoiding differences due to data processing, meat spectra were analyzed using the same statistical environment (R studio, version 3.4.1) instead of the native software installed in every instrument. Outlier spectra were detected by using absorbance values obtained from the two instruments. Each spectrum was centered and standardized and then Mahalanobis distance was calculated. Spectra with Mahalanobis distance greater than the square root of the critical value of a Chi-Squared distribution with $\alpha=0.001$ and degrees of freedom equals to number of wavelengths were discarded as outliers. Centered and standardized meat spectra were then used for the development of calibration equations.

Meat quality reference analyses

The collection and processing of the meat samples and the analyses of meat quality traits were described in details in the previous study (Savoia et al., 2018c).

Briefly, the analyses of meat quality traits were carried out for all carcasses on a section of the same muscle (*Longissimus thoracis* at the level of the 6th thoracic vertebra) used for spectral acquisition after one week of under-vacuum ageing at 4°C. The meat quality traits were analysed according to the methods proposed 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 inserted approximately 1 cm into the muscle and an automatic temperature compensator;

- colour traits, measured 3 times and averaged on the freshly-cut surface after 1 h of blooming at 4°C using Minolta CR-331C colorimeter according CIELAB coordinates (CIE 1976): Lightness (L*), redness (a*) and yellowness (b*) were recorded, and Hue angle (H*) and Chroma (C*) were calculated as $H^* = \tan^{-1} (b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{0.5}$;

- purge loss (PL, %), computed as the difference between the weight of the sample cut before (day 1) and after (day 7) vacuum packaging and expressed as percentage of the initial value;

- 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 till the attainment of an internal temperature of 70°C, and expressed as percentage of the initial value (Honikel, 1998);

- shear force (WBSF, N), determined on 6 cylindrical cores 1,27 cm in diameter of cooked meat with a V-shaped cutting Warner-Bratzler blade, fitted to an Instron Universal Machine model 5543, and expressed as the maximum force (Newtons) required to shear the cylindrical core (AMSA, 2015).

Calibration trial

The data from 1,166 Piemontese young bulls were used for the study. For each animal the meat quality traits measured on a meat sample and two NIR average spectra (one per instrument) were used.

A Bayesian model (Bayes B), implemented with the BGLR library of R-software (Pèrez and De Los Campos, 2014) was used to develop calibration equations for each meat quality trait as described by Ferragina et al. (2015). For each instrument, data have been partitioned into a calibration set, containing 80% of the observations randomly selected, and a validation set with the remaining 20% of the data. This procedure was repeated 15 times for each trait to insure independence of the calibration and validation sets, both representing different animals from the same herds and slaughter dates. The determination coefficients, calculated as square of the correlation between observed and predicted values in the calibration set (R^2_{cal}) and in the validation set (R^2_{cv}), were used to evaluate the accuracy of predictions.

Moreover, as the most important source of variation of meat quality traits is the batch of slaughter (samples from animals slaughtered in the same date, aged together and analysed in the same day), an external validation was also performed. This was based on predicting the observations for all the animals slaughtered in a given date from the regression equations developed from the data of all other dates, and repeating this procedure for every date of slaughtering ("Leave One Date Out" procedure). The determination coefficients (R^2_{EXT}) was calculated on the predictions of the excluded dates.

Repeatability trial

To analyse the most important sources of variation and evaluate the repeatabilities of the reference meat quality analyses, of the absorbance of meat spectra at the level of each individual wavenumber, and of the meat quality traits predicted with both spectrometers, the carcasses on 30 young bulls were used. On both sides of each carcass (60 sides) a double thickness meat sample was collected to allow for two replicated analyses per side of each meat quality trait (four data for each

animal, 120 in total). Moreover, for each side 5 spectra from different sites of the cross-sectional area of the muscle were taken (300 spectra in total), each one being the average of three replicates from the same site. The predicted beef quality traits were obtained applying the equations developed in the Calibration Trial on the individual spectra of each cross-sectional muscle position (300 predictions per trait).

The source of variation of the data obtained were quantified using the Mixed Procedure of SAS software (2013) adopting the following statistical model:

$$y = \text{slaughter date} + \text{animal} + \text{carcass side (animal)} + \text{residual}$$

where y is the vector of the considered traits (analytical values for each of the meat quality traits; absorbance of every wavenumber of the spectrum at each muscle site by the two NIR spectrometers; and predicted values for each of the meat quality traits by the two NIR spectrometers). The terms slaughter date, animal, and carcass side (nested within animal) are random variables assumed to have σ^2_{SD}, σ^2_{An}, and σ^2_{CS} variances, respectively and $\varepsilon \sim N(0, \sigma^2_{Re})$ is the random residual term. This represents the variability between the two meat samples of each carcass side for the measured beef quality traits or between the 5 sites of the cross-sectional area of the muscle of each side for absorbancies and predicted meat quality traits. Parameters from the mixed model were estimated using restricted maximum likelihood method (REML).

Different repeatability indices were then computed for measured beef quality traits:

- Sample repeatability = $(\sigma^2_{SD} + \sigma^2_{An} + \sigma^2_{CS})/ (\sigma^2_{SD} + \sigma^2_{An} + \sigma^2_{CS} + \sigma^2_{Re})$

- Animal repeatability = $\sigma^2_{An} / (\sigma^2_{An} + \sigma^2_{CS} + \sigma^2_{Re})$

The same repeatability indices were computed also for predicted meat quality traits obtained from both NIR spectrometers using all individual spectra (5 per muscle section).

Moreover, as it is common to use the average spectrum from the 5 individual spectra to

develop the calibration equations, also the repeatability indices referred to average spectra were computed:

- Sample repeatability = $(\sigma^2_{SD} + \sigma^2_{An})/(\sigma^2_{SD} + \sigma^2_{An} + \sigma^2_{CS})$

- Animal repeatability = $\sigma^2_{An} / (\sigma^2_{An} + \sigma^2_{CS})$

The repeatability of absorbance of individual wavenumbers were computed as sample repeatability.

For measured meat pH and purge loss only one value per side was measured so the sample repeatability could not be estimated.

Field testing trial

To evaluate the ability of the two NIR spectrometers to yield predictions able to capture the effects of the major sources of variation affecting the measured traits, the same dataset with 1,166 Piemontese young bulls used in the calibration trial was analysed using the Mixed Procedure of SAS Software (2013) adopting the following model:

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

where: y represents 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 modeled in 4 classes (January-March, April-June, July-September, October-December), of the parity of the dam modeled in 4 classes (1st, 2nd, 3-8, >8) and of the 6 production systems; carcass weight is a fixed effect modeled in 5 classes (<350kg, 350-400kg, 401-450kg, 451-500kg, >500kg); farm is the random effect of the fattening farm nested within production system (98 levels); batch is the random effect of the day of slaughter (117 levels); and ε is the random residual term. Farms, batch and ε were assumed to be normally and independently distributed N(0, σ^2). For

both the batch and farm effects a minimum cell size of 3 observations was required.

Comparison between least square means has been performed through a Tukey-Kramer test ($P<0.05$).

RESULTS

Calibration trial

Descriptive statistics of the meat traits analysed by the reference laboratory methods are shown in Table 2. We illustrated and discussed these meat quality traits of Piemontese young bulls in a previous survey (Savoia et al., 2018c).

Table 2 reports the performance of meat quality predictions based on spectra of cross-sectional area of *Longissimus thoracis* exposed at the abattoir when the carcass sides were divided according to the pistol cutting the day after slaughtering. The R^2_{CAL} varied from 0.51 to 0.88 for colour traits, whereas it was much lower (from 0.10 to 0.34) for prediction of pH, purge and cooking losses and shear force (with the only exception of the pH predicted by Vis-NIRS: 0.57). The R^2_{CAL} were always greater for predictions based on Vis-NIRS respect to those yielded by Micro-NIRS.

At cross-validation, the R^2_{CV} obtained were always smaller than R^2_{CAL}, especially for equations based on Vis-NIRS. The external validation, based on predicting individual batches on the basis of calibration equations developed using all the other batches, yielded similar values, without notable differences between the two spectrometers. The R^2_{EXT} ranged from 0.52 to 0.80 for colour traits, and were lower than 0.32 for the other meat quality traits (Table 2). Correspondingly, the difference between the SD of measured traits and the $RMSE_{-EXT}$ of the corresponding predictions were related, as expected, to the R^2_{EXT}.

Repeatability trial

To better understand the differences in accuracy of prediction of meat quality traits through

NIRS calibration equations, the sources of variation of reference and predicted traits were quantified and summarized in Table 3.

The effect of slaughter batch on sample variability of meat 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 variability. The effect of carcass side was always very small ($\leq$ 14%) as well as the residual variability among replicates ($\leq$ 25%), with the notable exception of purge loss where it represented more than half of the total variability. As expected, the animal represented the major source of variation for pH and colour traits ($\geq$ 58%), whereas it represented a much smaller proportion of total variance for purge loss (25%), cooking loss (44%) and shear force (23%).

The overall result led to a high sample repeatability for physical analyses of an heterogeneous material like meat samples ranging from 75% of cooking losses to 93% of L*. As expected, animal repeatability was lower than sample repeatability: -5 to -10 percentage points for colour traits and -22 and -40 percentage points for cooking loss and shear force.

Moving to the NIR spectra, the proportion of different sources of variation on total variance of absorbance of individual waves was strongly dependent on the wavelength, as clearly shown in Figure 2. Examining the variability of the spectra yielded by Vis-NIRS (Figure 2,a), it can be seen that the visible light section of electromagnetic spectrum (350 to 750 nm) is characterized by a large heterogeneity among different waves (especially for violet radiations). The visible red radiations showed a pattern similar to the first section of near-infrared section (750 to 1,300 nm), which is much more homogeneous than the rest of the spectrum, and that is characterized by a high variability attributed to individual animals (about 50% of total variance), whereas the remaining variability is explained by the other three sources of variation considered here (slaughter batch, carcass side, and muscle sampling/residual variability), with similar importance. This pattern can explain the good sample repeatability (75-80%) shown by the visible red and the first portion of

infrared radiations.

In the fraction 1,300 to 1,400 nm of the electromagnetic spectrum there was a dramatic change in the proportion of the different sources of variability; over 1,400 nm the absorbance of meat samples was strongly affected by the specific site within the cross-sectional area of the muscle (position depending), and the animal effect and the repeatability of measurement decreased to very low values.

It is worth noting that, in the section of the spectrum in common, Micro-NIRS and Vis-NIRS showed a very similar pattern (Figure 2).

The strong dependence of meat spectrum on the position within muscle sectional area explains the great proportion of residual/muscle site variance on total variance of NIRS predictions of meat quality traits. In fact, this source of variation accounted for 54 to 77% of total variance in the case of predictions obtained from Vis-NIRS and from 25 to 77% of total variance in the case of Micro-NIRS (Table 3). The corresponding values for the reference analyses were from 7 to 25% of total variance. The animal effect represented the second source of variation in order of importance for all meat traits in the case of Micro-NIRS and for the majority of traits obtained from Vis-NIRS. Slaughter date was a source of variation greater than carcass side for almost all traits with both instruments (Table 3).

The high incidence of residual/muscle site variance of the absorbance of many NIR wavelengths explained the lower sample and animal repeatability of predicted traits obtained with both instruments in comparison to those measured with reference analyses. The incidence of residual/muscle site effect on total variability of the predicted traits was almost always greater than in the case of the reference traits, particularly for pH, cooking loss and shear force. The only exception was represented by purge loss.

The above mentioned repeatabilities are referred to the predictions obtained from a spectrum averaging three replicates and taken on a single position of the muscle. To overcome this variability,

in practice the average of several spectra taken in different position of the muscle cross sectional area are often used, as in the calibration trial of this study. In this case, sample and animal repeatabilities calculated after exclusion of the residual/muscle site component are more informative. The sample and animal repeatability based on the average of the spectra from 5 different positions within the muscle sectional area were, with both spectrometers and with few exceptions, similar or also greater than those measured with reference analyses (Table 3).

Field testing trial

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

The general means of colour traits were almost identical across analysis method, whereas the standard deviation tended to decrease in predicted traits with respect to the corresponding laboratory reference (Table 4). The incidence of random effects (batch and farm) on total variability was similar across analysis method within trait. The season of birth and parity of dam were never significant, whereas the effect of beef production system presented some differences in the significance level across different traits and methods. However the differences were of limited size and the trend of LSMs was similar (data not shown).

The class of carcass weight was the most important factor affecting all the colour traits, regardless the analysis methods. For all colour traits an almost linear significant growing pattern was observed moving from the lightest to the heaviest carcasses, with the entity of the difference between the two extreme classes almost unchanged across different analysis methods.

Also in the case of the other meat traits (Table 5), the general means were not affected by

analytical method, whereas the decrease of standard deviation of predicted with respect to reference values was greater than in the case of colour traits. The incidence of the variability of slaughter batch on total variance was in general higher than in colour traits (with the exception of purge loss) and always reduced in Micro-NIRS derived predictions compared to those from Vis-NIRS. The effect of the fattening farm within beef production system was of limited size in all traits across analysis method. Also in this case season of birth and parity of dam effects were always non-significant, with the only exception of the effect of birth season on measured purge loss. The effect of beef production system was significant only for the meat pH predicted by Micro-NIRS and the cooking loss predicted by Vis-NIRS.

The class of carcass weight displayed more variable results in these traits than in colour traits. In the case of meat pH the effect was always negligible, even tough significant in the case of the reference values. For the purge loss it was highly significant and increased moving from the lightest to the heaviest carcasses with all analysis methods, even though the difference observed between the extreme classes was greater (+1.03%) for reference than for Vis-NIRS (+0.59%) and Micro-NIRS (+0.61%) predicted values. Carcass weight effect had a significant effect only on laboratory measured cooking loss but displayed an erratical pattern. Lastly, the shear force increased while increasing carcass weight in a similar way with all the three methods, but the effect was significant only in the case of the predicted values.

DISCUSSION

The discussion is organized according to the order of the objectives of this study.

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

The large majority of studies on NIRS prediction of quality traits of meat have been carried out at laboratory level using bench top spectrometers (Prieto et al., 2017). Automatic at-line evaluations at the abattoir are limited to the size and conformation of carcasses, often using image

analysis (Craigie et al., 2013). These techniques are mainly aimed at evaluating muscularity and fatness of carcasses and not meat quality because of the modest correlations with these traits. A problem for at-line evaluation of beef quality traits is that the exposed cut surface of muscles on beef carcasses or halves are very few and not much representative of the characteristics of the major beef cuts (De Marchi, 2013).

In this study portable and hand-held NIR spectrometers have been used at the abattoir on the sectional area exposed of the *Longissimus thoracis* muscle after the dividing of the carcass halves in the fore and rear quarters. Spectra up-taking was manually performed, but this operation could be robotized in large slaughterhouse plants.

The Vis-NIR portable spectrometer used in this study is a "top" instrument characterized by a wide spectrum spanning from visible to infrared section of the electromagnetic spectrum (wavelengths: 350 to 1,830 nm) with a frequent measurement of absorbance (one measure every 1 nm) and then yielding a very large number of data for each spectrum (1,481 absorbance measures). Direct comparisons of different instruments, especially those focusing on portable *vs* bench-top NIR-spectrometers, are rare in the scientific literature. In a previous study (De Marchi et al., 2013) the Vis-NIRS was compared with a bench-top spectrometer specifically designed for the analyses of food samples (Foss, Foodscan) on the prediction of meat quality of different meat samples. The Vis-NIRS yielded R^2_{CV} of prediction of meat quality traits almost identical to those obtained in this study. The prediction performance of the bench-top spectrometer was always lower compared to that of the Vis-NIRS as a consequence of a narrow spectral range and reduced number of absorbance values measured per sample. It worth noting that, with both instruments, better results were achieved using spectra collected on the surface of intact cross sectional muscle surface than after grinding and mixing the muscle sample.

Comparison of results yielded by different studies with single instruments are impaired by the many causes of variation (Prieto et al., 2017) affecting NIRS predictions (animals slaughtered, type

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of muscle and position within muscle, slaughter and dissection processes, ambient conditions, ageing, reference analyses carried out, pretreatment of spectra, calibration methods, validation procedure). In a previous study on Piemontese young bulls a bench-top spectrometer characterized by a wide spectrum (Foss NIRSystem 5000, from 1,100 to 2,498 nm) was used on ground meat samples to investigate the possibility to predict meat quality traits (Cecchinato et al. 2011). The prediction abilities obtained for colour traits (R^2_{CAL} ranging from 0.44 to 0.81) were similar to those found on 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 colour has been reported in a number of studies both with ground (Prieto et al., 2008) and more often with intact samples (Leroy et al., 2003; Prieto et al., 2009). Although the spectra have been collected in operational conditions and directly on carcass, the predictions of colour traits obtained in this study displayed R^2_{CAL} higher than studies conducted in laboratory conditions (Magalhaes et al., 2018; Andres et al., 2008). The predictions of the other meat quality traits obtained in this study were less accurate and very similar across the two spectrometers. For the pH, the R^2_{CAL} was lower than most of the literature reports (Andres et al., 2008; Prieto et al., 2008; De Marchi et al., 2013). The very low variability of the pH measurements (CV 0.9%) could probably justify the modest spectra prediction ability (Prieto et al., 2009). For purge and cooking losses the low R^2 values found in our study are in the range of published literature (Andres et al., 2008; Leroy et al., 2003) and slightly higher than the findings of Cecchinato et al. (2011). The NIRS technology has in general a poor predictability of water holding traits as they are indirectly predicted from their association with the wavelengths of chemical compounds which is often weak (Prieto et al., 2017).

Also for shear force the accuracy of NIRS prediction was limited (R^2_{CAL} 0.26 for the Vis-NIRS and 0.10 for the Micro-NIRS) but similar to the results obtained in the previous trial by Cecchinato et al. (2011). Due to the muscle heterogeneity shear force is a difficult trait to predict using infrared spectroscopy, particularly when grounded samples are used (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 reported moderate (around 0.5) values of R^2 of cross-validation (Magalhaes et al. 2018, Andres et al., 2008), but in most of the cases predictions had much lower values (Prieto et al., 2008; Leroy et al., 2003).

Differently from Vis-NIRS, Micro-NIRS was seldom used in meat analysis of different types (Zamora-Rojas et al., 2012 and 2013; Wiedermair et al. 2018), and never used on large surveys on beef meat physical traits.

In general, the results highlight that the prediction ability of meat quality traits obtained from portable or hand-held spectrometers used at the abattoir is comparable to that from bench-top instruments in laboratory conditions.

Comparison of predictions obtained from Vis-NIRS and Micro-NIRS

Two very different NIR spectrometers have been tested in this study (Table 1). In comparison with the Vis-NIRS, the hand-held Micro-NIRS is a spectrometer developed for industrial use, especially for at-line monitoring of materials during processing. Its average linear size is about one sixth than the Vis-NIRS, its weight is almost one hundredth, and also its cost is much lower. The spectrum of the Micro-NIRS has an extension (wavelengths: from 905 to 1,649nm) about half than the Vis-NIRS and the frequency of measurement is one sixth, so that the total number of absorbance measures per spectrum is about 12 times smaller (125 *vs* 1,481 measures per sample). The obtained R^2_{CAL} were always in favour of the Vis-NIRS, but the R^2_{CV} were about the same and the R^2_{EXT} were more often in favour of the Micro-NIRS (Table 2).

Comparing 5 different sources of information in discrimination analysis among different farming systems, Bergamaschi et al. (2018) found that the methods producing a larger number of data per sample generally allow for much better R^2_{CAL}, but this superiority disappears when moving to validations on different datasets.

Also the different measures of repeatability were not much different between the two

instruments, also in the case of the colour traits, although a better performance of the Vis-NIRS would be expected as a result of the extension of the spectrum to the visible light. This was in opposition with De Marchi et al. (2013) who compared predictions of meat colour from NIR transmittance with those from Visible and NIR reflectance showing that a better performance (R^2 of cross-validation two times as high) could be achieved when also the visible wavelenght part of the spectra was included.

The two spectrometers compared in this study produced similar results in terms of accuracy of predictions in external validation. However, a different suitability to their use in practical conditions at the abattoir arises. The Vis-NIRS requires a physical support and a connection to an external power source or to a supplemental battery, whereas the Micro-NIRS has the size and weight similar to the external probe of Vis-NIRS, and is much easily operated in practical conditions in the abattoir.

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

The meat is a very heterogeneous material, subjected to continuous modifications, and largely influenced by environmental conditions and processing procedure. This explains why the meat quality traits are so affected by sampling factors (day of sampling, samples in different side, muscle, portions within muscles, etc.), and by analytical factors (processing of samples, laboratory conditions, exposition to air and light, instrument calibration, etc.). In this study sample repeatability of reference analyses was quantified to be in the range of 75 to 93% (Table3), while animal repeatability, with the only exception of purge loss, was between 52 and 83%. These figures are much lower than those usually obtained for chemical composition analyses. A NIR spectrum taken at the abattoir within 24 h from slaughtering 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 sample up-taking, vacuum-packaging, chilling, transportation, ageing, preparation and analysis of samples, as well as instruments calibration and operator skills

cannot be predicted by NIRS. The expected maximum repeatability of a NIRS prediction is not 100% because it cannot exceed the animal repeatability. However, the animal component is the information of interest for both commercial and genetic use of predictions.

The most important source of variation of meat quality traits predictions obtained from NIR spectra was related to the heterogeneity of composition of the material, which results in different reflecting abilities according to the different position on the muscle surface. Currently, waiting for the availability from the industry of external probes or spectrometers able to acquire spectra on a wider area, the only possibility to overcome this problem is the collection of more spectra on different position of the muscle. Near-infrared hyperspectral imaging could be another way to obtain a more representative picture of muscle quality. This technique is based on the construction of a three-dimensional "hyper-cube spectral image", composed by one NIR spectrum for each of the many thousands of pixels of the entire image of the cross section of the analysed muscle sample (Xiong et al., 2014). Using this complex method ElMasry et al. (2012) obtained R^2_{CV} in the range 0.73 to 0.88 for meat lightness, yellowness, pH and shear force on 27 young bulls belonging to dairy breeds.

Moreover, a large variability of the relative importance of variance components, and particularly of the animal and of individual muscle site, has been observed along the different sections of the electromagnetic spectrum. As a consequence, also the relative importance of variance components of the predicted traits can vary according to the individual wavelengths more represented in the prediction equations.

However the use of multiple spectra per animal allowed to obtain animal repeatability of meat quality predictions higher than that of reference methods with both instruments. This means that NIRS predictions have the potential to better capture the animal "native" characteristics, being not influenced by the fate of meat samples.

Field testing and implications for commercial and genetic purposes

A tendency of R^2_{CAL} to overestimate the effective reliability of instruments yielding a great number of data point per sample analysed is often observed, making this parameter not very useful to evaluate predictive ability of NIR spectra. Also R^2_{CV} cannot always be a good indicator of the achieved prediction accuracy, particularly when samples from different origin (farms, batches, abattoirs, cuts, etc.) than those included in calibration dataset are to be predicted. For complex traits, such as those related to the quality of meat, even R^2_{EXT} (and related $RMSE_{EXT}$ and RER ratio) could not be sufficient for a good evaluation of the predictive performance. As outlined by Lo et al. (2015), significant variables are not necessarily good predictors. The determination coefficient is a rather rough statistic, unable to decompose the prediction errors according to the possible source of variation. A field testing, allowing to evaluate the ability of a predictive equation in capturing the effects of the major sources of variation, can give further information about possible incomplete or biased estimations. A previous study on methane emissions of the dairy cows predicted from milk infrared spectra (Bittante and Cipolat-Gotet, 2018), showed that also prediction equations of modest accuracy (R^2_{CV} of about 0.50) were able to fully capture the effect of the main sources of variation (dairy farming system, individual farm, parity, lactation stage) and that this ability was not always correlated with the determination coefficient. A similar testing performed in this study (Table 4 and 5) showed that the predictions of colour traits and purge loss were able to depict the effect of the main sources of variation of traits in a manner very similar to those yielded by statistical analysis of the reference values. This allows to speculate that probably these predictions, from both spectrometers, could be able to capture also the genetic variability. This hypothesis, to be confirmed by further specific research, could open new perspectives for the genetic improvement of meat quality, making the phenotyping at population level possible and providing reliable calibrations for genomic selection.

Finally, the partial inability of infrared spectra, taken at the abattoir after slaughtering, in predicting the fate of the meat sample after up-taking, ageing, transportation and analysis could be

considered favourably if the objective of prediction is to capture the "native" quality of meat for both its genetic improvement or quality based payment.

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 top-ranking portable Vis-NIRS instrument showed better results in terms of R^2_{CAL}, probably because of the great number of spectra data point, but not in terms of R^2_{CV} and R^2_{EXT} compared to the hand-held Micro-NIRS, which is better in terms of easiness of use at abattoir level.

The values obtained at laboratory level for physical meat quality traits are affected by several causes of variation (sampling, chilling, ageing, transportation, sample processing, instrument calibration, etc.) that increase the variance due to batch of slaughtering/analyses and the residual variance and reduce the repeatability of the reference analyses. The inability of the infrared spectra taken after slaughtering to predict the fate of meat samples till analyses, reflected by the reduction of determination coefficients, could be considered a pro if the aim of prediction is to capture the animal "native" conditions. This happens when predictions are used for genetic improvements of beef cattle or carcass quality based payments. The classical statistics of regressions of predicted over measured traits (R^2, RMSE, RER, etc) cannot be considered good predictors (in the case of calibration statistics) or the only predictors (in the case of external validation) for evaluating the performance of infrared calibration equations. A field testing on a large number of farms and animals proved the very good ability of both spectrometers to capture the major sources of variations of colour traits and purge loss, but also an acceptable performance achieved 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.

TABLES AND FIGURES

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

	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	5,600 g	60 g
Sample preparation	none	none
Method	reflectance	reflectance
Operating temperature	0 +40 ^{0}C	-20 +40 ^{0}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	2.0mm	2.5mm
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 sec	0.5 sec
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	A=log(1/R)	A=log(1/R)

Table 2. NIRS calibration trial: descriptive statistics of Piemontese meat quality traits and performance of their prediction by Vis-NIRS and Micro-NIRS instruments (1 meat sample analysed per animal, 1 averaged spectrum per animal obtained from 5 spectra from different sites of the muscle section area, each one with 3 replicates).

Item	Meat pH	Colour traits:						Meat losses (%):		Shear force (N/cm²)
		L*	a*	b*	C*	H*		Purge	Cooking	
Carcasses, N	1144	1147	1148	1150	1148	1146		1146	1157	1147
Descriptive statistics										
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	0.78	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	9.70

Table 3. Repeatability trial: variance components (as fractions of total variance), and sample and animal repeatability of quality traits of Piemontese meat measured in the laboratory or predicted using infrared spectra from two NIRS instruments taken at 5 individual sites on muscle section or averaged before analysis (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					Meat losses (%)		Shear force
		L*	a*	b*	C*	H*	Purge	Cooking	(N/cm²)
Laboratory									
Total variance	1.40 [e]	9.13	3.73	2.88	5.50	3.77	0.78	11.20	135.49
Variance components:									
Slaughter date (σ^2_{SD})	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	0.00	-	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									
Total variance	3.2 [e]	2.5	3.6	4.8	5.4	0.6	10.4	3.5	58.2
Variance components:									
Slaughter date (σ^2_{SD})	0.09	0.27	0.33	0.24	0.33	0.26	0.11	0	0.26
Animal (σ^2_{An})	0.21	0.36	0.43	0.47	0.39	0.32	0.59	0.07	0.05
Carcass side (σ^2_{CS})	0.08	0.07	0.07	0.06	0.06	0.14	0.08	0.15	0.11
Residual/muscle site (σ^2_{Re})	0.62	0.30	0.18	0.23	0.23	0.28	0.23	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									
Total variance	0.6 [e]	9.6	2.7	1.8	2.6	2.5	0.3	1.1	13.3
Variance components:									
Slaughter date (σ^2_{SD})	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	0.80	0.76	0.74	0.60

[a]: Sample repeatability using individual analysis or muscle site spectra = $(\sigma^2_{SD} + \sigma^2_{An} + \sigma^2_{CS})/ (\sigma^2_{SD} +$

$\sigma^2_{An} + \sigma^2_{CS} + \sigma^2_{Re}$)

[b]: Animal repeatability using individual analysis or muscle site spectra = $\sigma^2_{An} / (\sigma^2_{An} + \sigma^2_{CS} + \sigma^2_{Re})$

[c]: Sample repeatability using averaged muscle spectra = $(\sigma^2_{SD} + \sigma^2_{An})/ (\sigma^2_{SD} + \sigma^2_{An} + \sigma^2_{CS})$

[b]: Animal repeatability using averaged muscle spectra = $\sigma^2_{An} / (\sigma^2_{An} + \sigma^2_{CS})$

[e]: $\times 10^{-3}$

Table 4. Calibration trial: 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 (1,166 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[1] (%)	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[1] (%)	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	38.6[a]	38.6[a]	38.5[a]	27.7[a]	27.5[a]	27.5[a]	8.6[a]	8.5[a]	8.5[a]	28.7[a]	28.8[a]	29.1[a]	17.0[a]	17.1[a]	17.1[a]
351-400 kg	39.3[a]	39.3[a]	39.3[a]	28.3[a,b]	28.2[b]	28.2[b]	9.3[b]	9.2[b]	9.3[b]	29.7[b]	29.7[b]	29.8[a,b]	18.0[b]	18.0[b]	18.0[b]
401-450 kg	39.4[a]	39.5[a]	39.5[a]	28.5[b]	28.5[c]	28.5[b]	9.5[b]	9.5[c]	9.5[b]	30.1[c]	30.0[c]	30.0[b]	18.4[b]	18.3[c]	18.3[b]
451-500 kg	40.2[b]	40.1[b]	40.2[b]	29.0[c]	29.0[d]	28.9[c]	10.1[c]	9.9[d]	10.0[c]	30.7[d]	30.6[d]	30.7[c]	19.0[c]	18.8[d]	18.9[c]
> 500 kg	41.7[c]	41.3[b]	41.5[c]	29.8[d]	29.6[e]	29.5[d]	10.9[d]	10.6[e]	10.6[d]	31.4[e]	31.4[e]	31.7[d]	19.9[d]	19.7[e]	19.7[d]
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

[1]: Random factor variance expressed as % of total variance.

* *P*<0.05; ** *P*<0.01

a,b,c,d=*P*<0.05

Table 5. Calibration trial: 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 (1,166 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 Losses %			Cooking Losses %			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[1] (%)	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[1] (%)	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	5.55[a,b]	5.56	5.55	3.61[a]	4.11[a]	4.19[a]	15.2[a]	16.6	16.5	39.2	40.3[a]	40.5
351-400 kg	5.54[b]	5.55	5.55	4.44[b]	4.35[a,b]	4.37[a,b]	16.8[b]	16.7	16.6	40.8	40.5[a]	40.7
401-450 kg	5.55[b]	5.55	5.55	4.41[b]	4.40[b]	4.45[b]	16.8[b]	16.7	16.7	40.0	40.9[a]	40.8
451-500 kg	5.56[a]	5.56	5.55	4.59[b]	4.55[c]	4.59[c]	16.5[a,b]	16.9	16.7	39.9	41.6[b]	41.1
> 500 kg	5.56[a,b]	5.56	5.55	4.64[b]	4.70[c]	4.80[d]	16.0[a,b]	16.9	16.6	41.3	42.3[b]	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

[1]: Random factor variance expressed as % of total variance.

* *P<0.05; ** P<0.01*

Figure 1. Calibration trial: 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 1,157 Piemontese young bulls obtained using Vis-NIRS (blue colour) and Micro-NIRS (red colour) 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).
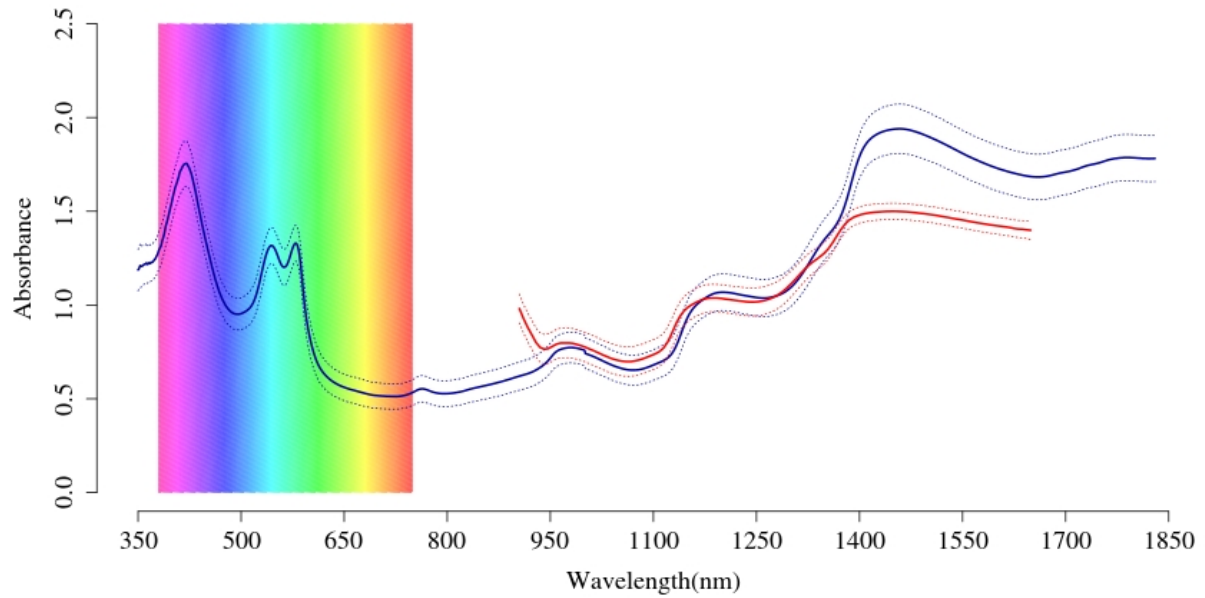
Figure 2. Repeatability trial: Animal (red colour), slaughter batch (green colour), carcass side (orange colour), and site on cross-sectional area (residual, blue colour) variance as fractions of total variance, and repeatability (black colour) of absorbance at each wave-number of 5-6th rib cross-sectional 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).
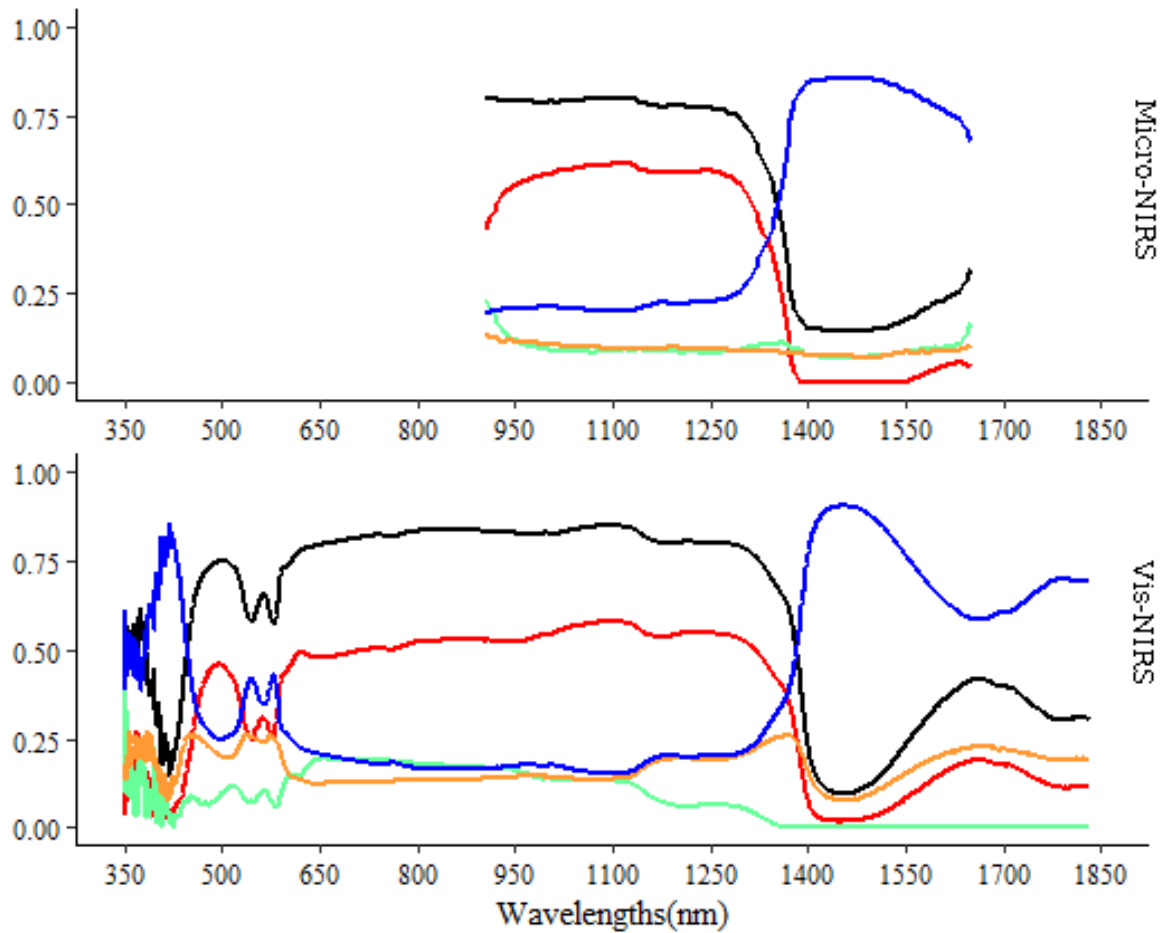
CHAPTER 4.

Genetic correlation between meat quality traits and their prediction at abattoir by different portable visible and near-infrared reflectance spectrometers

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ABSTRACT

The objective of this study was to investigate the possibility of using meat quality traits predictions obtained by two different portable spectrometers (Vis-NIRS and Micro-NIRS) from spectra taken at abattoir on the intact muscle surface one day after slaughtering in comparison with meat sampling, aging, transport, mincing and lab-analysis. 1,327 young Piemontese bulls were raised and fattened on 115 farms and slaughtered at the same commercial abattoir. The meat quality traits evaluated were pH, purge loss (PL, %), cooking loss (CL, %), lightness (L*), redness (a*), yellowness (b*), chroma (C*), hue angle (h*) and shear force (WBSF, N). Predictions for all the investigated traits were obtained with a Bayesaian model, using meat spectra collected at the abattoir using both a Vis-NIRS (350 to 1,830 nm, every 1 nm) and a Micro-NIRS (905 to 1,649 nm, every 6 nm) spectrometers. Estimations of (co)variance for measured traits and their NIRS predictions were obtained through a set of bivariate animal model REML analyses. The prediction performance of calibration equations was satisfactory for all colour traits (R^2 from 0.52 to 0.80), low for pH and PL (R^2 around 0.30), very poor for CL and WBSF (R^2 below 0.20) without differences between the 2 spectrometers. Except for L* and PL, a reduction of heritability in most of the predicted traits respect to the measured ones was observed. The genetic correlations between measured and predicted traits were high with an average value of 0.81 and a superiority of Vis-NIRS over Micro-NIRS for some trait. Results showed that NIRS predictions of colour traits, pH and PL can be used as indicator traits of the corresponding measurements for selection purposes. For CL results were more controversial, while estimates for WBSF predictions were not reliable.

Keywords*: genetic parameter, meat quality, near-infrared spectroscopy, Piedmontese*

INTRODUCTION

Improving meat quality attributes through genetic selection proved to be theoretically feasible as many quality traits display moderate to medium heritability values (Boukha et al., 2011). However, the establishment of a selection procedure relies on the availability of phenotypes collected within a routine recording scheme. For meat quality traits this step can be a serious limitation. Indeed, nowadays, it requires the collection of meat samples at the slaughterhouse, causing a depreciation of carcasses, and the subsequent laboratory analyses are destructive, expensive and time consuming.

Visible and Near infrared spectroscopy (Vis-NIRS), based on the principle that different chemical bonds in organic matter absorb or emit light of different wavelengths when the sample is irradiated, offers a number of important advantages over conventional methods such as rapid and frequent measurements, fast and simple or no sample preparation, suitability for on-line use and simultaneous determination of different attributes (Prevolnik, Candek-Potokar, & Skorjanc, 2004).

Several studies assessed the application of reflectance spectroscopy to predict accurately chemical composition of beef (Eichinger & Beck, 1992; Alomar, Gallo, Castaneda, & Fuchslocher, 2003; Tøgersen et al., 2003) and different attributes of meat quality (Leroy et al., 2003; Prieto et al., 2009).

From the perspective of the genetic improvement, scientific knowledge is almost absent. In a previous study (Cecchinato, De Marchi, Penasa, Albera, & Bittante, 2011), which is the only one dealing with genetic comparison between laboratory measured and infrared predicted meat quality traits we are aware of, medium-high genetic relationships have been found between some of the measured and the corresponding predicted meat quality traits. High genetic correlations were found for all colour traits and purge losses, greater than the corresponding phenotypic correlations, whereas both the phenotypic and genetic correlations for tenderness and cooking losses were negligible. These findings suggest that genetic improvement of some of the meat quality traits using their predictions obtained by NIR spectrometers is feasible. The study by Cecchinato et al. (2011)

howvere, was based on the use of a laboratory bench-top NIR spectrometer and on the acquisition of spectra from the same sample, in the same site and day, of laboratory analyses, after sampling, aging, transport, dissection and mincing of muscle portion. 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.

The availability of new portable NIR and Vis-NIR spectrometers, able to collect spectra directly from the muscle's surface at slaughterhouse (Prieto et al., 2009), increases the relevance of these instruments as phenotyping tools in programs focusing on selection for improving meat quality traits.

In this direction goes the study by De Marchi (2013) which investigated the application of Vis-NIR spectroscopy to predict beef quality traits at the slaughterhouse, by directly applying a fiber-optic probe on an exposed part of muscle. The study showed that prediction models were satisfactory for pH and colour indices, and promising for cooking losses. Since no large-scale study have been carried out on the use of portable NIR spectrometers to test the prediction of meat quality, Savoia et al. (2018b) compared two spectrometers very different for dimension, easiness of use and cost in the prediction of meat quality directly at abattoir on the muscle surface without the need of meat sampling.

Both instruments proved to be useful for predicting some meat traits, and also the very small, cheap and portable NIR spectrometer (Micro-NIRS) can be used at operational level for predicting meat quality traits without performance losses when compared to more large and expensive transportable Vis-NIR spectrometers (Savoia et al., 2018b).

The main objective of this study was, therefore, to investigate the suitability of portable infrared spectrometers for phenotyping beef cattle as a base for the genetic improvement of meat quality. The specific aims were: to analyse the genetic variation in meat quality traits predictions obtained by two very different portable spectrometers directly at abattoir on the intact muscle surface in comparison with the measurements obtained in the laboratory after sampling, aging,

transport and analyses in the laboratory; to assess the genetic relationships between laboratory measures of meat quality traits and their spectra-based predictions.

MATERIAL AND METHODS

This study is part of the "Qualipiem" project, which is aimed at analysing the phenotypic and genetic sources of variation in meat quality traits in the Piemontese breed and at proposing innovative selection strategies for their improvement.

Animals

The study was carried out sampling 1,327 Piemontese young bulls slaughtered at the same commercial abattoir from April 2015 to February 2017. Young bulls were progeny of 204 A.I. purebred sires and 1,286 dams, all registered in the Italian Piemontese Herd Book.

Animals were fattened in 115 farms representative of the beef production systems of the Piedmont region (north-west Italy). The beef farming systems, feeding regime, fattening conditions and slaughtering performances of young bulls were described in detail by Savoia et. al. (2018c). In brief, the young bulls were reared in farms belonging to one of the following beef production systems: traditional with restricted feeding and either tie stalls or loose housing management of animals, modern breeders and fatteners, and specialised fatteners with *ad libitum* feeding and loose housing (the last two systems further subdivided into those using or not using total mixed rations).

The average carcass weight of the Piemontese young bulls sampled was 438.1 (±43.6) kg, while the average age at slaughter was 541 (±63) days, giving an average daily carcass gain of 0.818 (±0.107) kg/d. Average carcass conformation score (SEUROP systems with each category divided in 3 subclasses, 1–18 point scale) was 14.66, corresponding to an average evaluation approaching "E+" in the EU linear grading system and average rib eye area measured at the 5th rib was 92 cm^2 (±14.3).

Spectra collection

Spectra collection was described in detail by Savoia et al. (2018b) as well as the technical

characteristics of the instruments used. Briefly, the collection of the spectra was done with the following spectrometers:

- Vis-NIRS: LabSpec 2500 (ASD Inc., Boulder, CO, USA) having a spectral range in the section of visible and near-infrared electromagnetic radiations (wavelengths 350 to 1,830 nm), measured every 1 nm, producing 1481 data points per sample; the size of the instrument is 12.7 × 36.8 × 29.2 cm and the weight is 5,600 g; the spectra is collected through a probe (26 × 10 × 5 cm) connected to the instrument through an optical fiber;

- Micro-NIRS: Micro NIR Pro (JDSU San Jose, CA, USA) having a spectral range in the section of near-infrared (wavelengths 905 to 1,649 nm), measured every 6 nm, producing 125 data points per sample; the size of the instrument is 4.5 × 4.4 × 4.0 cm and the weight is 60 g; the spectra is collected directly by the instrument that should be connected to a lap-top or a tablet through a USB cable.

after the division of the right carcass side in two quarter (pistol cut) at the abattoir the day after slaughtering (about 24 h post-mortem). The spectra were collected on the cross sectional surface of the *Longissimus thoracis* muscle between the 5th and 6th rib by applying the scanning head of the fiber-optic contact probe (10 mm of diameter) of Vis-NIRS or directly the Micro-NIRS over the surface of the muscle. Five *spectra* for each instrument were obtained from different position of the same muscle cut surface.

Beef samples collection and meat quality analyses

Twenty-four hours after slaughter, immediately after spectra collection, individual samples (4.0 cm thick) of the *Longissimus thoracis* (LT) muscle were collected from between the 5th and 6th rib, then were individually vacuum packed and transferred under refrigerated conditions to the laboratory, where they were stored in a chilling room at 4°C for 7 days, after which meat quality traits were measured on all samples.

Assessment of meat quality included muscle pH, lightness (L*), redness (a*), yellowness (b*), hue angle (h*), Chroma (C*), purge losses (PL, %), cooking losses (CL, %), and Warner

Bratzler shear force (WBSF, N), all measured at 7 d after slaughter. Details on procedures used to assess meat quality traits can be found in Savoia et al. (2018c).

Statistical analyses

Spectral data editing and validation procedure

The editing and the processing of spectral data were performed according to the model described in details in the previous study by Savoia et al. (2018b). In brief, the two original data sets of the *spectra* collected with the Vis-NIR and the Miscro-NIR portable spectrometers were edited to discard records with errors (e.g., individual identification spectra not matching reference samples) and *spectra* outliers identified through Mahalanobis distance. Before the development of calibration equations, spectral data were centered and standardized to improve the goodness of fit of chemometric modelling.

A Bayesian model (Bayes B), implemented with BGLR library of 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).

In order to reproduce the operational conditions, an external validation procedure was carried out to estimate (co)variance components and to evaluate the magnitude of genetic correlations between meat quality traits measured in the laboratory and their predictions from calibration equation based on Vis-NIR and Micro-NIR spectra. As the most important source of variation was frequently the batch (all animals slaughtered in the same day), the external validation was performed predicting the observations of one batch by the calibration equation developed using the measured meat quality data of all animals slaughtered in all the other days.

The final dataset contained the measured and predicted observations for 1,166 animals.

Estimates of (co)variance components and genetic parameters

(Co)variance components were estimated by REML procedures using the VCE software (version 6.0, Groeneveld et al., 2010). For each of the meat quality traits, estimation of (co)variance components was performed through separate bivariate analyses including the measured trait and its

prediction obtained with Vis-NIR or Micro-Nir spectrometers respectively.

The general model, in matrix notation, can be written as:

$$y = X\beta + W1\ c + W2\ q + Zu + e$$

Where y contains observations for traits 1 and 2, β is the vector of nongenetic fixed effects, c is the vector of random herd effects (98 levels), q is the vector of random effect of the day of slaughter (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. Effects of different herds were assumed to be normally and independently distributed c~N(0, C $\otimes$ I); effects of the day of slaughter was assumed to be normally and independently distributed q~N(0, Q $\otimes$ I). A minimum cell size of 3 observations was required for both the batch and farm effects. Animal additive genetic effects were assumed normally distributed u~ N(0, G0 $\otimes$ A), where G0 is the (co)variance matrix between animal effects, and A is numerator of Wright's relationship matrix. Additive relationships were computed using a pedigree file including all phenotyped animals and their known ancestors (13,122 animals). Residuals were assumed to follow the normal distribution, e~N(0, R0 $\otimes$ I).

To facilitate comparisons with literature estimates, we estimated intraherd heritability defined as:

$$h^2 = \sigma^2 a/(\sigma^2 a + \sigma^2 e)$$

where $\sigma^2 a$ is the additive genetic variance and $\sigma^2 e$ is the residual variance.

Nongenetic effects

Preliminary univariate analyses, using the SAS GLM procedure (2013), were performed to identify significant (P < 0.05) nongenetic effects to be included in the models to estimate (co)variance components.

For PL (%) the model included the effects of birth season (4 classes: January-March, April-June, July-September, October-December) and of parity of dam (4 classes: 1st, 2nd, 3rd-8th, >8). For pH, L* and WBSF (N) the model included the effects of parity of dam.

RESULTS

Descriptive statistics of meat quality traits measured in the laboratory on aged samples and their predictions obtained using the spectra taken at the abattoir the day after animals' slaughtering are reported in Table 1. Large differences in variability across traits in laboratory analysis measurements were observed. The water losses traits, PL and CL, and WBSF showed the highest variability, followed by colour traits whereas the SD of pH measurements was very limited. For all the considered traits, the average values of predictions obtained with both instruments were very similar to the corresponding laboratory measurements. Conversely, the variability of predicted traits was always much lower than that of measured traits. This was particularly marked for PL, CL and WBSF that showed a decrease in the standard deviation of predictions over measured traits ranging from 50% to 78%. For colour traits the reduction was less pronounced (-10% to -27%). The loss of variability was in general more marked in the predictions obtained with the Micro-NIRS spectrometer (-40%) in comparison with the Vis-NIRS instrument (-30%), The prediction performance of calibration equations, as appraised by the external validation, was satisfactory for all colour traits (R^2_{EXT} from 0.52 to 0.80), low for pH and PL (R^2_{EXT} around 0.30), very poor for CL and WBSF (R^2_{EXT} below 0.20). No relevant differences were observed between the 2 spectrometers in terms of magnitude of R^2_{EXT}. Across traits there was a clear relationship between the loss of variability in the predictions with respect to the measurements and the quality of the prediction performance (R^2 0.89 and 0.79 for Vis-NIRS and Micro-NIRS respectively, data not shown).

Table 2 reports the variance components and heritabilities of colour traits, comparing laboratory measured and spectra predicted traits. The effect of the day of slaughter was the most important source of variation for all traits with the exception of L*, accounting for 15 to 30% of the total variance. The incidence of this effect was always lower in Micro-NIRS predictions compared to Vis-NIRS predictions and to laboratory measured traits. The effect of the fattening herd of the young bulls was of limited size (5 to 10% of total variance according to the trait) and relatively

homogeneous for laboratory measured and spectra predicted traits.

The animal additive genetic effect explained from 20 to 30%, according to the instrument, of the total variance of L* with a higher incidence in both the predicted compared to the measured traits. As a consequence, the intraherd heritability values were relatively high ranging from 0.30 in lab-measurements to 0.41 in Vis-NIRS predictions. In all the other colour traits a different behaviour was observed: the proportion of variance of the animal effect was around 10% and consistent across the measured traits while it was much lower with both prediction techniques. Heritability values were therefore quite low and similar in the predictions, with values ranging from 0.04 to 0.08 with the only exception of h* from Micro-NIRS spectrometer, compared to heritability of 0.14 shown by laboratory measured traits.

The variance of the slaughter day was very high for the measured pH, CL and WBSF and for most of the corresponding predictions ranging from 40 to 60% of total variance (Table 3). Only Micro-NIRS predictions of CL were little affected by daily variance (13%), but for this trait a very large part of variation was unexplained. Both measured and predicted PL displayed proportions of slaughter day variance similar to those of colour traits. Likewise, also in meat quality traits a little amount of variability was due to the herd effect, not exceeding 7% of total variance in most of the cases.

The proportion of variance explained by the additive genetic effect was much higher in measured CL and WBSF than in their predictions. Hence heritabilities of measured traits were moderate for CL (0.19) and relatively high for WBSF (0.31) but they considerably dropped in predicted traits. Particularly, the predictions of CL obtained with Micro-NIRS and of WBSF obtained with Vis-NIRS instruments showed almost null incidence of additive genetic variance and resulting heritabilities close to zero. For both pH and PL the estimated heritabilities were higher for the predictions obtained from Vis-NIRS instrument compared to those from Micro-NIRS (0.18 vs 0.13 and 0.22 vs 0.13, respectively). The heritability of measured pH was higher than that of corresponding predictions, whereas for PL it was similar to that of Micro-NIRS predictions and

markedly lower compared to Vis-NIRS results.

Estimates of genetic and residual correlations obtained by bivariate analyses of colour and meat quality traits measured in the laboratory on aged meat samples and their predictions obtained from meat spectra taken at abattoir 24 h after slaughter are presented in Table 4 for both Vis-NIRS and Micro-NIRS. The values of residual correlations reflected the prediction performance of calibration equations. Genetic correlations between lab-measured and spectra-predicted traits were always higher than the corresponding residual correlations. Their average value across traits was 0.81 compared to values around 0.50 for residual correlations for both instruments. Large differences were observed among traits in genetic correlations. For colour traits and PL they were extremely high, almost always 1 for the Vis-NIRS, on average 0.9 for the Micro-NIRS. In the other traits the genetic correlations were of lower magnitude and different estimates for the two spectrometer were obtained. The estimated genetic correlations obtained from Vis-NIRS were greater than those from the Micro-NIRS particularly for pH (0.70 vs 0.45), CL (0.70 vs 0.25) and WBSF (0.81 vs 0.42).

Overall, the two spectrometers produced quite similar results in terms of prediction performance in all the considered traits. Genetic parameters of derived predictions for colour traits were comparable, whereas a superiority of the Vis-NIRS over the Micro-NIRS was observed in the other meat quality traits.

DISCUSSION

The main objective of this research was the evaluation of the possible use of NIRS technology to predict phenotypes for meat quality traits to be used for genetic evaluation purposes. The use of portable instruments at the slaughterhouse and the spectra acquisition on muscle surface naturally exposed during the routine procedures of subdivision of half-carcasses into quarters might avoid the collection of meat samples, the depreciation of carcasses and the subsequent transport, ageing and laboratory analyses. We described, compared and discussed in details in a previous work the

Vis-NIR and Micro-NIR spectroscopic techniques in terms of instrument characteristics, repeatability, calibration, cross-validation and external validation of predictions, and the ability to capture the main phenotypic sources of variation of meat quality traits (Savoia et al., 2018b). In this study we focused the discussion of the two spectroscopic techniques on the estimates of genetic parameters and on their use for genetic improvement of meat quality in beef cattle populations.

In our investigation, to obtain the predictions of meat quality traits from spectral data we used the model of Savoia et al. (2018b), which has been implemented to mimic the operational conditions. The observations of the samples collected in one day are predicted by calibration equations developed using observations collected in all the other days (leave one day out procedure). As a consequence, the evaluation of predictive performance of NIRS has been performed using an external instead of a cross-validation, which led to lower values of R^2 compared to most of the published studies. Bittante et al. (2014) and Eskildsen et al. (2014), showed that the cross-validation procedure tended to overestimate the predictive ability of cheese-yield traits from FTIR spectra in comparison with external validation. Particularly for traits affected by a high environmental variation related to farms, seasons or batches the spectral predictive performance obtained by cross-validation can be inflated. Calibration model parameters, such as cross or external validation R^2 or RMSE, cannot be sufficient to establish the usefulness of spectral predictions for genetic improvement purposes. Even though Soyeurt et al. (2011) stated that R^2 of cross-validation exceeding 0.75 would be required to use predictions of dairy traits for selection, some authors reported satisfactory results also with moderate or even low prediction performances in milk (Rutten et al., 2011; Bittante et al., 2014) and meat quality traits (Cecchinato et al., 2011). Furthermore, in most of the meat quality traits a large day to day variability is observed as consequence of sample heterogeneity and of analytical factors (day of sampling, samples processing, laboratory conditions) reducing the prediction ability of infrared spectra, particularly when these are acquired directly at the abattoir (Savoia et al., 2018b). Indeed, beside the R^2, the suitability of infrared predictions as indicator traits for selection relies on a combination of their

heritability and loss of additive genetic variance with respect to measured traits, and to their genetic correlations with corresponding measurements (Bonfatti et al., 2017; Rutten et al., 2010).

Heritability of measured and predicted meat quality traits

Heritability of measured meat quality traits was in the range of most literature reports (Johnston et al., 2003; Riley et al., 2003). In a previous survey concerning the genetics of meat quality traits in the Piemontese breed Boukha et al. (2011) found heritabilities similar to this study for L*, b* and C*, whereas for a* and h* their estimates were considerably higher. However, in that study a different equipment for the measurement of colour traits was used. The heritabilities of other measured meat quality traits were in some cases lower (CL and WBSF) in other higher (pH and PL) than the findings of this study.

In general, the predictions of meat quality traits based on spectra taken at abattoir 24 after slaughtering of young bulls displayed heritability values lower than the corresponding traits measured in the laboratory on aged meat samples, with the exception of L* and PL. The better accuracy of calibration equations of L* over other colour traits could justify the differences in the ability of predictions to capture the additive genetic variance. The difference in heritabilities between predicted and measured traits was on average 0.08 for both the two spectrometers but variability across traits was observed. Although lower than measured traits, heritabilities of meat quality predictions in most of the cases were large enough to be exploited for selection. Only the predictions of CL obtained with Micro-NIRS and those of WBSF obtained from Vis-NIRS were useless as characterized by almost null additive genetic variance. So far only one study addressed the estimation of genetic parameters for meat quality traits predicted by NIR spectroscopy (Cecchinato et al., 2011). That investigation was performed on animals of the same breed and of similar age to those in our study. However, a bench top spectrometer in laboratory conditions was used to acquire spectra on minced samples and a different procedure for the development of calibration equations, based on partial least squares regression and random assignment of samples to calibration and testing sets, was established. Differently from our study, Cecchinato et al. (2011)

reported that the predictions of all meat quality traits except L* had similar or increased heritabilities in comparison with those of measured traits. In general, their estimates of heritabilities of predicted traits were higher than ours for most of the traits and no null heritabilities were found for CL and WBSF predictions. It should be considered that in that study predictions were based on spectra taken in the laboratory on aged and minced meat samples the same day and on the same material used for meat analyses. In the present study, the spectra have been taken in the abattoir, 6 days before laboratory analyses on the muscle surface exposed during dissection of the carcass side in the two quarters. So, our calibrations predict the quality that meat will present after sampling, ageing, transport, grinding, and analyses.

In our investigation, the reduction in the heritabilities of predictions over measurements resulted from to an average decrease of 70% in the additive genetic variance which exceeded the decrease observed in the phenotypic variance, ranging from 50 to 60% depending on the instrument. These results are consistent with those of Bonfatti et al. (2017) who found a similar pattern for the infrared predictions at population level of a large number of traits related to milk composition and technological properties. Conversely, other studies on the same traits reported that the reduction observed in additive genetic variance shown by infrared predictions with respect the measured traits was associated also to a reduction in the residual variance leading to increased heritability values particularly for poorly predicted traits (Bittante et al., 2014; Cecchinato et al., 2009).

The reduction in the phenotypic variability of meat quality predictions observed in our study was strongly related to the accuracy of the calibrations measured by the R^2 of external validations (Figure 1). As expected, the use of predictions in place of original traits implies a reduction in their variability which is directly related to the predictive performance of the adopted model. The results were identical for the two spectrometers and in agreement with the findings of Cecchinato et al. (2011). Instead the association of losses in additive genetic variability with the R^2 of external validations of calibration models was less tight, particularly with the Vis-NIR spectrometer (Figure 2). As a consequence, neither the heritabilities of the predictions nor their losses with respect to

those of measured traits displayed a consistent relationship with the predictive performance of calibration models. These findings, in agreement with those of Cecchinato et al. (2011), confirm that also meat quality traits predicted from infrared spectra with moderate or even low accuracy can be heritable and display exploitable genetic variability.

Correlations between measured and predicted meat quality traits and their possible genetic improvement

Genetic correlations of predicted with measured traits are important in determining the effectiveness of their use as indicator traits for selective breeding (Rutten et al., 2010; Cecchinato et al., 2009). Indeed, the rate of genetic gain achievable with indirect selection is affected by the genetic correlations between desired and indicator traits (Falconer and Mackay, 1996). For all the colour traits the genetic correlations of measured traits with Vis-NIRS predictions were extremely high, but also those with Micro-NIRS predictions were very large despite the lack of the visible wavelength part of the spectra. These results are consistent with those of Cecchinato et al. (2011), who found genetic correlations ranging from 0.85 and 0.99 for bench-top NIRS predictions of meat colour traits and the corresponding measurements. For PL the estimated genetic correlations obtained in this study with both the instruments were substantial and larger than those obtained by Cecchinato et al. (2011). In a similar way, also Vis-NIRS predictions of pH displayed rather high genetic correlations with measurements, whereas the corresponding values for the Micro-NIRS predictions were only moderate. In the study of Cecchinato et al. (2011) CL and WBSF proved to be difficult traits to predict from NIR spectra also from the perspective of genetic improvement, as they showed inconsistent values for both the estimated phenotypic and genetic correlations with their laboratory measurements, even though spectra were taken on the same material, site and day of laboratory analyses. A partly different pattern for these traits was observed in the present study. Although being characterised by low predictive abilities of calibration models, the Vis-NIRS predictions correlated well with the measured traits from the genetic standpoint, whereas for the Micro-NIRS the corresponding association was weaker. However, taking into consideration the

considerable loss of the additive genetic variance displayed by Vis-NIRS predictions of WBSF, only the predictions of CL obtained from the same instrument seem to be useful for selection purposes.

Similarly to the findings of Cecchinato et al. (2011), the genetic correlations between predicted and measured traits were positively associated to the predictive ability of the calibration models (Figure 3), but to a lower extent with respect to phenotypic or residual correlations. Traits predicted very accurately by calibration models always show high genetic correlations with their measurements, while more variability in estimated correlations is observed when the prediction performance is moderate or poor (Bonfatti et al., 2017).

The possibility of using for genetic purposes phenotypic predictions obtained with imprecise methods is object of a large debate. It worth noting that recently Bovenhuis et al. (2018), replying to criticisms raised on a technique for predicting enteric methane emissions, stated that "… even if measurements are inaccurate, imprecise, or biased, they might provide valuable information for selective breeding.", and also "When given the choice, accurate and unbiased measurements are preferred. However, such measurements are seldom available on a large scale and at reasonable cost. … However, inaccurate and biased sniffer methane phenotypes do not automatically imply inaccurate and biased methane breeding values". For evaluating the ability of infrared predictions for the genetic improvement of animal product's quality it is clear that heritability of predictions and their genetic correlations with measured traits are much more relevant than phenotypic accuracy, precision and unbiasedness of the technique. From this point of view, both infrared spectrometers proved to be useful tools for establishing a program for indirect genetic improvement of meat quality.

CONCLUSIONS

This study investigated the feasibility of selection for meat quality traits by using their NIRS predictions obtained from spectra collected at the abattoir on intact muscle surface using portable instruments. The accuracy of the predictions was good for colour traits but low for the other

investigated traits. Despite the reduction of additive genetic variance and heritability observed in most of the predicted traits, the estimated genetic parameters showed that NIRS predictions of colour traits, pH and PL can be used as indicator traits of the corresponding measurements for selection purposes. For CL results were more controversial, while estimates for WBSF predictions were not reliable. For the selection of these traits, alternative strategies such as those using genomic approaches are to be investigated.

The two spectrometers compared in this study are very different in terms of dimensions, easiness of use and cost, but showed similar results for the prediction of colour traits and their relative genetic parameters, but a superiority of the Vis-NIRS instrument was highlighted in the estimates of genetic parameters of other predicted meat quality traits. This was probably more related to the higher density in spectral acquisition in comparison with the Micro-NIRS instrument than to the extension of the spectral range also to the visible wavelengths.

Overall, selection of complex traits like those related to meat quality, whose direct phenotyping is difficult, can benefit of the application of NIRS technology.

Funding and conflict of interest statement

Table 1. Descriptive statistics of color and meat quality traits measured in the laboratory on aged samples and of their predictions by Vis-NIR and Micro-NIR spectra taken at abattoir 24 h after slaughtering, and prediction performance of calibration equations evaluated through external validation (R^2_{EXT}).

	Traits[1]								
	L*	a*	b*	C*	h*	pH	PL	CL	WBSF
							%	%	N
Carcasses, N	1129	1133	1134	1133	1131	1127	1128	1134	1117
Laboratory measures:									
Mean	39.86	28.59	9.65	30.19	18.53	5.55	4.50	16.76	40.96
SD	3.46	1.74	1.66	2.15	2.04	0.05	1.19	3.45	10.43
Vis-NIRS predictions:									
Mean	39.87	28.60	9.63	30.18	18.50	5.56	4.47	16.80	40.89
SD	3.17	1.34	1.38	1.73	1.68	0.04	0.60	1.43	4.79
R^2_{EXT}[2]	0.84	0.55	0.63	0.58	0.64	0.30	0.31	0.16	0.16
Micro-NIRS predictions:									
Mean	39.89	28.62	9.70	30.22	18.56	5.55	4.50	16.67	40.89
SD	3.12	1.22	1.29	1.56	1.61	0.02	0.53	0.75	3.84
R^2_{EXT}[2]	0.80	0.52	0.61	0.55	0.63	0.22	0.27	0.19	0.19

[1] PL = purge losses, CL = cooking losses, WBSF = shear force
[2] R^2 of external validation

Table 2. Variance components and intraherd heritability of colour traits measured in the laboratory after meat aging and of their predictions by Vis-NIR and Micro-NIR spectra taken at abattoir 24 h after slaughtering.

	L*			a*			b*			C*			h*		
	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	Micro-NIRS
Phenotypic variance	11.64	9.96	9,74	3.12	1.83	1.52	2.79	1.88	1.67	4.71	3.01	2.46	4.17	2.81	2.59
Variance components[1]															
Additive genetic	0.23	0.32	0.28	0.09	0.04	0.04	0.10	0.02	0.04	0.09	0.03	0.03	0.10	0.03	0.09
Day of slaughter	0.18	0.15	0.15	0.25	0.31	0.22	0.23	0.26	0.16	0.25	0.30	0.21	0.21	0.24	0.18
Herd	0.06	0.06	0.05	0.11	0.11	0.10	0.08	0.08	0.07	0.10	0.10	0.10	0.06	0.06	0.07
Residual	0.54	0.46	0.52	0.55	0.53	0.64	0.59	0.64	0.73	0.56	0.58	0.65	0.62	0.67	0.67
Intraherd heritability h^2															
Estimate	0.30	0.41	0.35	0.14	0.08	0.07	0.14	0.04	0.05	0.14	0.04	0.05	0.14	0.05	0.11
SE	0.095	0.104	0.107	0.070	0.066	0.050	0.070	0.044	0.060	0.075	0.053	0.048	0.074	0.055	0.081

[1] ratio to phenotypic variance

Table 3. Variance components and intraherd heritability of meat quality traits measured in the laboratory after meat aging and of their predictions by Vis-NIR and Micro-NIR spectra taken at abattoir 24 h after slaughtering.

	pH			PL, %			CL, %		
	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	Micro-NIRS	Measured trait	Vis-NIRS	M
Phenotypic variance	0.30^3	0.13^3	0.06^3	1.39	0.36	0.28	11.78	2.07	0
Variance components[2]									
Additive genetic	0.08	0.08	0.06	0.10	0.17	0.10	0.10	0.03	0
Slaughter day	0.61	0.48	0.49	0.14	0.21	0.16	0.42	0.54	0
Herd	0.06	0.07	0.05	0.05	0.04	0.15	0.04	0.04	0
Residual	0.25	0.37	0.40	0.71	0.58	0.69	0.44	0.40	0
Intraherd heritability h^2									
Estimate	0.25	0.18	0.13	0.13	0.22	0.13	0.19	0.07	0
SE	0.087	0.077	0.087	0.072	0.103	0.070	0.085	0.057	0

[1] PL = purge losses, CL = cooking losses, WBSF = shear force
[2] ratio to phenotypic variance
[3] $\times 10^{-2}$

Table 4. Additive genetic (r_A) and residual (r_E) correlations between colour and meat quality traits measured in the laboratory after meat aging and their corresponding predictions obtained using Vis-NIR and Micro-NIR spectra taken at abattoir 24 h after slaughtering (SE in parentheses).

Traits[1]	Vis-NIRS		Micro-NIRS	
	r_A	r_E	r_A	r_E
L*	1.000 (0.001)	0.871 (0.016)	1.000 (0.001)	0.831 (0.022)
a*	0.958 (0.173)	0.671 (0.029)	0.783 (0.225)	0.646 (0.031)
b*	1.000 (0.001)	0.761 (0.021)	0.930 (0.189)	0.598 (0.025)
C*	1.000 (0.001)	0.703 (0.024)	0.771 (0.228)	0.687 (0.027)
h*	1.000 (0.001)	0.763 (0.057)	0.858 (0.134)	0.756 (0.026)
pH	0.701 (0.164)	0.358 (0.056)	0.448 (0.256)	0.262 (0.028)
PL, %	0.979 (0.085)	0.385 (0.054)	0.879 (0.162)	0.378 (0.045)
CL, %	0.703 (0.168)	0.120 (0.059)	0.248 (0.271)	0.265 (0.058)
WBSF, N	0.805 (0.187)	0.202 (0.055)	0.418 (0.316)	0.271 (0.070)

[1] PL = purge losses, CL = cooking losses, WBSF = shear force

Figure 1. Relationship between the coefficient of determination in external validation (R^2_{EXT}) of the meat quality prediction models based on spectra taken at the abattoir on intact muscle surface 24 h after slaughtering with (a) the Vis-NIR and (b) the Micro-NIR portable spectrometers and the decrease in phenotypic variance ($\Delta\sigma^2_p$) of predictions compared with the quality traits measured in the laboratory on meat samples aged for 7 daystraits.
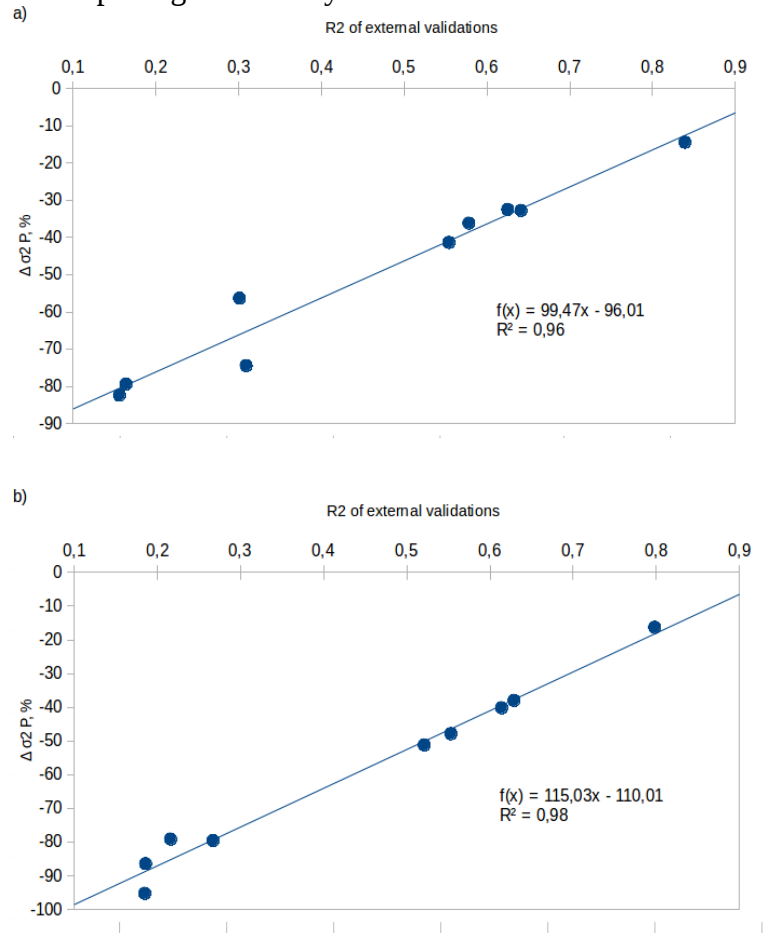
Figure 2. Relationship between the coefficient of determination in external validation (R^2_{EXT}) of the meat quality prediction models based on spectra taken at the abattoir on intact muscle surface 24 h after slaughtering with (a) the Vis-NIR and (b) the Micro-NIR portable spectrometers and the decrease in additive genetic variance ($\Delta\sigma^2_a$) of predictions compared with the quality traits measured in the laboratory on meat samples aged for 7 days.
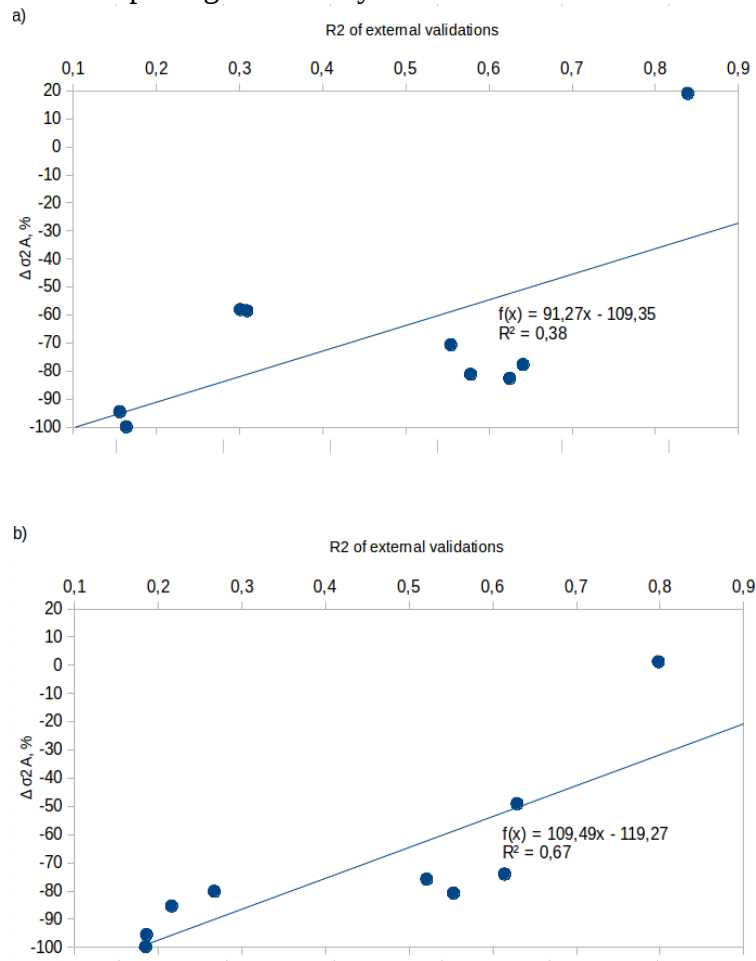
Figure 3. Relationship between the coefficient of determination in external validation (R^2_{EXT}) of the meat quality prediction models based on spectra taken at the abattoir on intact muscle surface 24 h after slaughtering with (a) the Vis-NIR and (b) the Micro-NIR portable spectrometers and the genetic correlation r_a) of predictions with the quality traits measured in the laboratory on meat samples aged for 7 days.
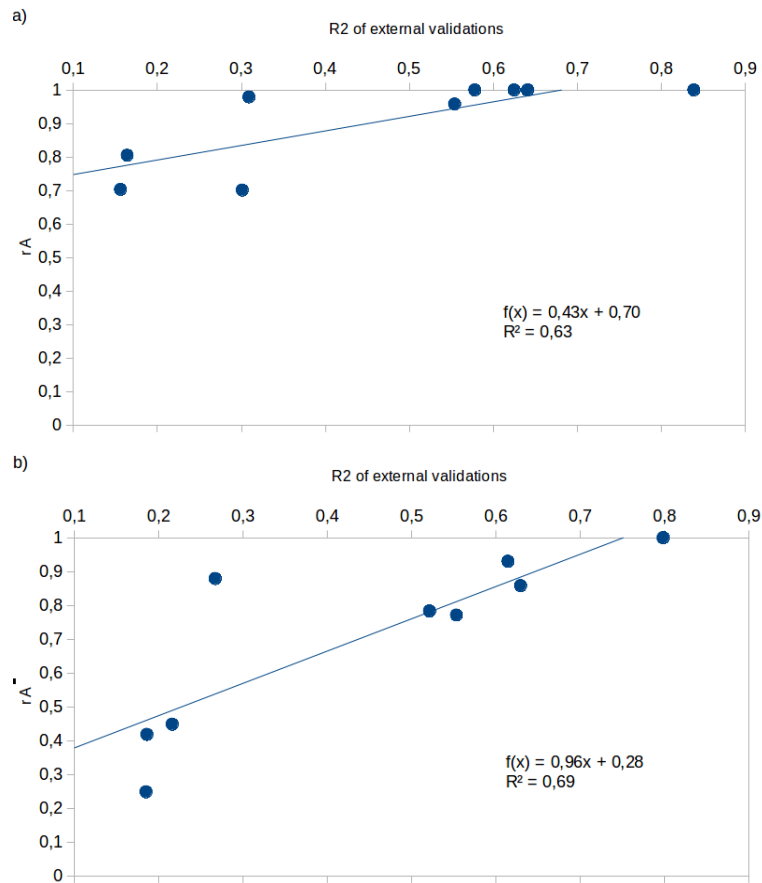
CHAPTER 5.

Genome-wide Association and Pathway Analysis of Carcass and Meat Quality Traits in Piemontese young bulls

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ABSTRACT

The improvement of carcass and meat quality traits represents a key concern in beef production. Therefore, the identification of genomic regions and biological pathways contributing to explain the variability for these traits is of great importance for selection purposes. In this study, genome wide-association and pathway-based analyses for carcass (age at slaughtering [AS], carcass weight [CW], carcass daily gain [CDG], conformation score and ribeye muscle area) and meat quality traits (pH, Warner-Bratzler shear force, purge loss, cooking loss and color parameters [lightness, redness, yellowness, chroma, hue]) were conducted using genotype data from the "GeneSeek Genomic Profiler Bovine LD" array in a cohort of 1,166 double-muscled Piemontese beef cattle. The GWAS analysis identified 37 significant SNP which were associated to 12 traits ($P<5 \times 10^{-5}$). In particular, 14 SNP associated with CW, CDG and AS were located at 38.57-38.94 Mb on BTA6 and mapped within 4 genes, i.e., Leucine Aminopeptidase 3 (*LAP3*), Family With Sequence Similarity 184 Member B (*FAM184B*), Non-SMC Condensin I Complex Subunit G (*NCAPG*) and Ligand Dependent Nuclear Receptor Corepressor Like (*LCORL*). A high linkage disequilibrium was detected in this region ($r^2=0.80$). For meat quality traits, most associations were 1 SNP-1 trait except for a signal on BTA25 (at ~11.96Mb) which was significant for 4 out of the 5 meat color parameters assessed. Gene-set enrichment analyses showed significant results (right-sided hypergeometric test, false discovery rate < 0.05) for 6 traits. In particular, several pathways related to transmembrane transport (i.e. oxygen, calcium, ion and cation) were overrepresented for meat color parameters. The obtained results might offer useful information for implementing genomic selection for beef production and quality in the Piemontese breed.

INTRODUCTION

Meat quality is a complex phenotype including several sensory attributes such as tenderness, juiciness, and color which represent major drivers of consumers' acceptance and product pricing (Grunert et al., 2004; McIlveen and Buchanan, 2001).

It depends on several factors related to both environmental conditions and animal genetic background (Warner et al., 2010). Although most of the meat quality traits proved to be heritable (Boukha et al., 2011; Johnston et al., 2003; Riley et al., 2003), difficulties and costs related to phenotypes collection make their improvement with traditional selection unfeasible, shifting the interest to genomic applications (Meuwissen et al., 2001). Genome-wide association studies (GWAS) allowed to identify single-nucleotide polymorphisms (SNP) associated with genes that influence meat quality traits in different conventional beef cattle breeds, improving the understanding of the trait biology and providing a list of positional candidate genes for quantitative trait loci (QTL). In addition, functional analyses coupled to GWAS showed that set of interacting genes and pathways are co-associated to carcass and meat quality traits. However, genomic selection and GWAS rely on the linkage disequilibrium (LD) between markers and QTL affecting a target trait, which can differ among various cattle populations and breeds.

The Piemontese breed is the most important Italian beef breed since it accounts for 330,000 heads, including 153,000 cows (Veterinary Information System 2017). The peculiarity of this breed is the double muscling conformation inducted by a specific mutation of myostatin (*MSTN*) gene located on *Bos taurus* autosome 2 (Grobet et al. 1998), which is almost fixed in this population. Piemontese animals display large muscular masses and low fat deposition, reduced weight of the skeleton, reduced feed intake and improved feed conversion (Fiems, 2012). Piemontese cattle are currently selected mainly for production traits (growth rate and muscularity) and direct and maternal calvine ease (Albera et al., 2004a). However, the recent acquisition of the EU Protected Geographical Indication (PGI) "Vitelloni Piemontesi della coscia" increased the interest in the

improvement of meat quality attributes of the Piemontese cattle.

So far in the Piemontese breed studies based on a candidate-gene approach have been carried out to identify putative markers affecting carcass and meat quality traits (Lisa et al., 2013; Ribeca et al., 2013; Ribeca et al., 2014), but a complete genome-wide investigation for the same traits has never been performed. Then, the aim of this study was to perform GWAS and pathway-based analyses on i) carcass traits (*i.e.* age at slaughtering [AS], carcass weight [CW], carcass daily gain [CDG], conformation score [EUS] and rib-eye muscle area [REMA]), and ii) meat quality traits (*i.e.* pH, Warner-Bratzler shear force [WBSF], purge loss [PL], cooking loss [CL] and color parameters [L*, a*, b*, C*, H*]) in Piemontese young bulls to increase the knowledge about the genomic regions and biological pathways controlling variation in these phenotypes.

MATERIAL AND METHODS

Animals and meat samples collection

This study was part of the Qualipiem project and involved 1,369 Piemontese young bulls. Animals were fattened in 115 farms representative of the beef production systems of the Piemonte region (north-west Italy) and slaughtered at the same commercial abattoir from April 2015 to February 2017. The beef farming systems, feeding regime, fattening conditions and slaughtering performances of young bulls were described in detail by Savoia et. al. (2018c). After slaughter, hot CW and carcass conformation according to the EU linear grading system (Commission of the European Communities 1982) were assessed. To obtain a more accurate differentiation, the six main grades (S, E, U, R, O, P from best to worst) were furtherly subdivided in + or − subclasses and then converted into numerical scores (EUS) ranging from 18 (S+ class) to 1 (P- class). Carcass daily gain (CDG) was computed as the ratio between CW and AS. Twenty-four hours after slaughter, individual samples of the *Longissimus thoracis* muscle were collected between the fifth and sixth thoracic vertebrae and vacuum-packaged. Samples were then immediately transferred to the

laboratory and stored at 4°C in a chilling room for 7 days *post-mortem* until assessment of beef quality traits.

Analysis of meat quality traits

At d7 after animal slaughtering, PL was determined according to the following procedure: the steaks were weighed initially in the bag (packaged weight, W1), weighed after bag removal (unpackaged weight, W3), and the bag was rinsed, dried, and weighed (bag weight, W2). PL (%) was then calculated as (W1-W2-W3)/(W1-W2)*100. The pH was measured using a portable Crison pH-meter equipped with a glass electrode suitable for meat penetration and an automatic temperature compensator. Color was measured on the freshly-cut surface of the steak after 1 h of blooming at 4°C using Minolta CR-331C colorimeter. CIELAB coordinates (CIE 1976), lightness (L*), redness (a*) and yellowness (b*) were recorded and hue angle (H*) and chroma (c*) were calculated as $h^* = \tan^{-1} (b^*/a^*)$ and $c^* = (a^{*2} + b^{*2})^{0.5}$. Measurements were taken at 3 random locations on the meat surface and averaged. The steak was then sealed in a polyethylene bag and cooked in a water bath pre-heated at 75°C, up to an internal temperature of 70°C to assess CL. After the temperature was reached, the steak was removed from the water bath and cooled for 30 min under tap water. Then, the steak was removed from the bag, blotted and reweighed (Honikel, 1998). Cooking loss (CL, %) was computed as the percentage weight difference between raw and cooked samples relative to the weight of raw meat samples. The same steak was also used for WBSF test. Six cylindrical cores, with the diameter of 1,27 cm, were taken parallel to muscle fibres and sheared perpendicular to the muscle fibres orientation with a V-shaped cutting Warner-Bratzler blade, fitted to an Instron Universal Machine model 5543. The WBSF was measured as the maximum force (Newtons) required to shear the cylindrical core at a crosshead speed of 200 mm*min^{-1} (AMSA 2015).

Genotype data

The 1,369 Piemontese young bulls were genotyped by using the array "GeneSeek Genomic

Profiler Bovine LD" (GGP Bovine LD) containing 30,111 SNP. Single nucleotide polymorphisms on the X chromosome were excluded from the analysis. Marker loci with missing data >5%, minor allele frequencies (MAF) <5%, call rate <95%, deviation from Hardy–Weinberg equilibrium (HWE) (P<0.001, Bonferroni corrected) were excluded. The final marker set included 23,173 SNP distributed on 29 Bos taurus autosomes (BTA). The SNP positions were based on the UMD3.1 assembly (ftp://ftp.cbcb.umd.edu/pub/data/assembly/Bos_taurus/Bos_taurus_UMD_3.1/). In total, 203 animals were excluded based on the following criteria: i) lack of concordance between SNP-based and pedigree-based ancestry; ii) the herd of belonging provided <3 animals; iii) the slaughter batch of belonging included <3 animals. The final number of animals included in the analyses was 1,166.

Genome-wide association analysis

Genome-wide association analyses were conducted using a single marker regression model with GenABEL R package and the GRAMMAR-GC (Genome wide Association using Mixed Model and Regression - Genomic Control) approach (Amin et al., 2007). Firstly, an additive polygenic model with a genomic relationship matrix was fitted:

$$y = X \beta + a + e \quad (1)$$

where y is the vector of observations for each trait; β is a vector with fixed effects of slaughter batch (n=106) and herd-date (n = 98); X is the incidence matrix that associates each observation to specific levels of the factors in β. The two random terms included in the model were animal and the residuals, which were assumed to be normally distributed as a $\sim$ N(0,Gσ^2_g) and e $\sim$ N(0,Iσ^2_e), where G is the genomic relationship matrix, I is an identity matrix, and σ^2_g and σ^2_e are the additive genomic and residual variances, respectively. The G matrix was constructed in the GenABEL R package, where for a given pair of individuals i and j, the identical by state coefficients ($f_{i,j}$) is calculated as:

$$f_{i,j} = \frac{1}{N}\sum_k \frac{(x_{i,k} - p_k) \times (x_{j,k} - p_k)}{p_k \times (1 - p_k)} \quad (2)$$

where N is the number of markers used, $x_{i,k}$ is the genotype of the ith individual at the kth SNP (coded as 0, ½ and 1), p_k is the frequency of the "+" allele and k= 1, …, N.

In a second step of GRAMMAR-GC, the residuals obtained in (1) are regressed on the SNP (single marker regression) to test for associations. Finally, the Genomic Control (GC) approach corrects for conservativeness of the GRAMMAR procedure and estimates the marker effects . A P-value threshold of 5×10^{-5} was adopted to determine significant associations (Burton et al., 2007). Manhattan plots were drawn using the R package qqman (Turner, 2014). The variance explained by each SNP was calculated as $2pqa^2$, where p is the frequency of one allele, q=1-p is the frequency of the second allele and a is the estimated additive genetic effect. Model (1) was also used to estimate variance components and the genomic heritability of the traits based on the genomic relationship matrix. Heritability was estimated as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

The r-squared statistic was chosen to predict the extent of LD using the R package LDheatmap (Shin et al., 2006).

Pathway analysis

Pathway analysis was performed to capture weaker but related single-variant signals which were missed by standard GWAS due to the stringent P-value threshold. It builds on the assumption that complex traits might be controlled by changes in biological pathways or cellular functions in which many highly coordinated genes might play a modest role.

Firstly, from the GWAS results we selected the "relevant" SNPs based on a nominal P-values < 0.05). Then, using the BiomaRt R package (Durinck et al., 2005; Durinck et al., 2009), these SNPs were assigned to a gene if they were located within a gene or at a distance <15 kb from

the coding region (Pickrell et al., 2010) based on the Ensembl *Bos taurus* UMD3.1 assembly (Venter et al., 2001). For each trait, functional enrichment analyses were carried on the significant genes using the Cytoscape plugin ClueGo (Bindea et al., 2009), to identify significantly overrepresented pathways and ontologies (right-sided hypergeometric enrichment test). All the SNP/genes in the chip (after quality filtering) were used as background. The Benjamini & Hochberg correction for multiple testing was used and the cut-off for significant enrichment was set at FDR < 0.05.

RESULTS

GWAS analysis

Descriptive statistics and genomic heritabilities for the carcass and meat quality traits are reported in Table 1. The average values and major phenotypic sources of variation were showed and discussed in a previous study (Savoia et al., 2018c).

As expected, the highest genomic heritabilities (>0.38) were detected for some carcass traits (CDG, REMA and CW), and for meat L*. The lowest heritabilities were found for meat pH and CL (<0.10), being intermediate (0.14 to 0.27) the values of the remaining traits.

The results of the GWAS analysis are summarized in Table 2. In total, we detected 37 significant SNP for 12 traits. No SNP passed the significance threshold for REMA and WBSF. The *P*-values ranged from 4.83E-05 to 2.44E-11. The traits showing the highest number of significant SNP were CDG (23) and CW (20).

Regarding carcass traits, we detected significant associations on BTA3 (at ~31.52 Mb) for CDG; on BTA6 for AS, CW, CDG (at ~38.46-40.56 Mb and 91.91 Mb) and EUS (at ~71.15 Mb); on BTA11 (at ~94.69 Mb) for EUS; and on BTA19 (at ~6.90 Mb) for CW.

For meat color parameters, we detected signals on BTA4 (at ~112.51 Mb), BTA23 (at ~3.91 Mb and ~7.25 Mb), BTA24 (at ~19.87 Mb) and on BTA25 (at ~11.96 Mb). In particular, this latter

corresponded to the marker BovineHD2500003345, which was associated to 4 out of the 5 color parameters assessed (*i.e.*, a*, b*, c*, H*). As regards the traits related to water holding capacity, 1 SNP located on BTA9 (at ~48.33 Mb) was significant for PL and 2 SNP respectively located on BTA6 (at ~29.23 Mb) and on BTA10 (at ~14.57 Mb) were significant for CL. Finally, 1 SNP located on BTA8 (at ~28.46 Mb) was associated to meat pH.

The highest signals were associated to CDG and corresponded to the markers ARS-BFGL-NGS-45457 (*P*=2.44E-11) and Hapmap26308-BTC-057761 (*P*=6.37E-09) which mapped on BTA6 at ~ 38.72 and 38.52 Mb, respectively. These SNP explained 9.61% (ARS-BFGL-NGS-45457) and 7.31% (Hapmap26308-BTC-057761) of additive genetic variance for CDG and were in strong linkage disequilibrium (LD; r^2=0.81). They were included in a window of 23 SNP located on BTA6 in the range ~38.46-40.56 Mb, which showed significant associations for CDG, CW and AS (Table 2; Figure 1). Several genes mapped in this region. In particular, 14 SNP located at ~38.57-38.94 Mb mapped within 4 genes: Leucine Aminopeptidase 3 (*LAP3*) (7 SNP), Family With Sequence Similarity 184 Member B (*FAM184B*) (2 SNP), Non-SMC Condensin I Complex Subunit G (*NCAPG*) (2 SNP) and Ligand Dependent Nuclear Receptor Corepressor Like (*LCORL*) (3 SNP) (Table 3). The marker BovineHD0600010666 was located within 2kb 5' UTR to *LAP3* and the markers Hapmap26308-BTC-057761 and BovineHD0600010673 corresponded to intron variants of *LAP3*. Additionally, 4 SNP, *i.e.* MS-rs110839532, MS-rs43702361, MS-rs109241256 and MS-rs41255599 were located within the 3' UTR of this gene. The markers BovineHD0600010685 and ARS-BFGL-NGS-45457 were intron variant of *FAM184B*. The markers MS-rs109570900 and MS-rs110251642 corresponded to missense mutation in *NCAPG*. Finally, 3 SNP, *i.e.*, BovineHD0600010755, Hapmap31285-BTC-041097 and Hapmap33628-BTC-041023 were intron variants of *LCORL* (Table 3). The average LD in this region was r^2=0.80 (Supplementary Table S3).

Pathway analyses

In total, 14,265 SNP (out of the 23,173 SNP in the chip) were located in annotated genes or within a 15 kb window surrounding the coding region. Based the on *Bos taurus* UMD3.1, the number of annotated genes used as background for the pathway analysis was 9,713.

For each trait, ~700 significant SNPs ($P<0.05$) were on average assigned to ~600 genes, which were mined using Cluego to identify biological pathways and cellular functions involved in the control of carcass and meat quality traits.

Significantly enriched GO terms and KEGG pathways (FDR<0.05) were found for 4 meat quality parameters, *i.e.*, a*, b*, C* and CL, and for 2 carcass traits, *i.e.*, CW and EUS (Figure 2 and Supplementary Table S2). In particular, for the meat color parameters we observed a high association between pathways and GO terms related to transmembrane transport activity. For instance, calcium channel activity was commonly enriched among a* (FDR=0.0137), b* (FDR=0.0214) and C* (FDR=0.03475); inorganic cation transmembrane transport was overrepresented for both a* (FDR=0.0470) and C* (FDR=0.04377); oxygen transport (FDR= 0.04747) and inorganic ion transmembrane transport (FDR=0.04578) were associated to C*. In addition, a set of genes pertaining to dopaminergic synapse was specifically enriched for a* (FDR=0.0421); ribonucleoside bisphosphate biosynthetic process, and purine nucleoside bisphosphate biosynthetic process were highly associated with b* (FDR=0.0195); circadian entrainment (FDR=0.04337) and long-term depression (FDR=0.04769) pathways were enriched for C*. Finally, regulation of synapse assembly was overrepresented for CL (FDR= 0.0274). Regarding carcass traits, a set of 5 genes involved in protein localization to synapse was enriched for CW (FDR=0.0292) while a set of 31 genes involved in response to organic cyclic compound was enriched for EUS (FDR= 0.0292).

DISCUSSION

This is the first study combining GWAS and biological pathway analysis for economically

important traits related to carcass and meat quality traits in the Piemontese breed.

The magnitude of genomic heritability for carcass traits was higher than the previous estimates based on pedigree analysis reported on the same traits in a field survey on this breed (Boukha et al., 2011; Cecchinato et al., 2011). The genomic estimates of this study were also generally higher than pedigree based estimates obtained on the same dataset, with the only exception of AS, pH, CL and WBSF (unpublished data). Overall, these results further support for the existence of exploitable genetic variation for carcass and meat quality traits of young bulls.

Gwas analysis

The results of GWAS study evidence that beside the role of *MSTN* mutation, which is fixed in the Piemontese population, other markers/genes contribute to explain the variability for carcass traits in this highly specialized beef breed.

We detected 14 SNP associated with CW, CDG and AS on BTA6 (Table 3) which mapped within 4 genes, *i.e., LAP3, FAM184B, NCAPG* and *LCORL*. LAP3 is involved in the control of oxytocin hydrolysis and it has been associated to milk protein and fat content in dairy cows (Zheng et al., 2011). Little information is available for FAM184B but it has been suggested to be associated with feed intake and gain in crossbred cattle (Snelling et al., 2011). NCAPG has been associated with fetal growth and carcass size in cattle (Eberlein et al., 2009; Setoguchi et al., 2009). In addition, the polymorphism in *NCAPG* shows significant associations with carcass weight, carcass yield estimate and *Longissimus* muscle area (Setoguchi et al., 2009; Hoshiba et al., 2013). LCORL was shown to control stature in cattle in association with NCAPG (Pryce et al., 2011). Significant SNP for bone weight were located in or near *LAP3, LCORL, FAM184B*, and *NCAPG* in Simmental cattle (Xia et al., 2017). In particular, the most significant SNP found by these latter authors (Hapmap26308-BTC-057761) was also found as the second strongest association with CDG (and with CW to a less extent) in the present study and with CW in Japanese Black cattle (Nishimura et al., 2012). Moreover, these genes have been considered as potential positional and functional

candidate genes for direct calving ease in Piemontese cattle (Bongiorni et al., 2012; Chillemi et al., 2018). In particular, the same SNP, i.e., Hapmap26308-BTC-057761 (intron variant of *LAP3*) was associated to CW and CDG in our study and to direct calving ease in the study of Bongiorni et al. (2012). In addition, the markers MS-rs109570900 and MS-rs110251642 (missense mutations in *NCAPG*) were associated to AS, CW and CDG (present study) and direct calving ease (Chillemi et al., 2018; Bongiorni et al., 2012). Calving performances are influenced by two components (Meijering, 1986): the ability of the dam to give births easily (maternal effect) and the ability of the calf to be born easily (direct or fetal effect). The direct effects are primarily connected to size of the calf which might explain the associations found in the present study with muscularity and growth parameters. This interpretation is confirmed by the study of Albera et al. (2004b) who estimated consistent positive genetic correlations between young bulls daily gain and direct calving difficulties in first and later parity Piemontese cows.

The marker BovineHD2500003345 (located at ~11,96 Mb on BTA25) which was associated to meat color parameters was an intron variant of shisa family member 9 (*SHISA9*) which maps in QTL associated to traits of economic importance in bovine (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index) such as body weight but also clinical mastitis, dystocia (maternal) and milk protein percentage and yield. Moreover, the marker BovineHD2300001826, which was associated to a*, corresponded to a cds-synonymous mutation in bromodomain containing 2 (*BRD2*). This gene is a nuclear serine/threonine kinase involved in transcriptional regulation and it has been suggested as candidate gene for to meat quality parameters, including meat color (a*), in pigs (Lee et al., 2018).

Variation in calpain 1 (*CAPN1*) and calpastatin (*CAST*), which are involved in an important proteolytic system, has been associated to beef tenderness in different breeds (Page et al., 2004; Casas et al., 2006; Tizioto et al., 2013; Ramayo-Caldas et al., 2016). Despite markers located within these genes were included in the chip (UA-IFASA-1370, intron variant *CAPN1*;

BovineHD0700028726, BovineHD0700028737 and ARS-USMARC-116 intron variants *CAST*; BovineHD0700028758, nearGene-5 *CAST*; ARS-USMARC-670, cds-synon *CAST*), no significant association was detected with WBSF in this study. This is in line with previous results based on a candidate gene approach which did not report significant association for markers located in *CAPN1* and *CAST* with SF in Piemontese cattle (Ribeca et al., 2013). A possible explanation might be that, in double-muscled breeds, *MSTN* mutation might play the major role in the regulation of meat tenderness while other genes providing only a marginal contribution. Indeed, in agreement with its function as a regulator of muscle cell growth and differentiation, *MSTN* was identified among the top transcription factors for meat quality traits including tenderness in French beef cattle breeds (Ramayo-Caldas et al., 2016). However, it is worth mentioning that other authors did not detect any significant SNP on *CAST* or *CAPN1* associated with WBSF in Simmental cattle as well (Xia et al., 2016).

Pathway analysis

The quality of meat is dependent on biochemical and biophysical changes occurring post-mortem in muscle. The results of our pathway analysis showed that calcium, ion and cation transport pathways as well as oxygen transport were enriched for meat color parameters. Calcium is essential for muscle contraction by acting as a catalyst of enzymatic proteolytic activity and metabolic pathways related to calcium transport have been associated to meat quality, in particular tenderness (Ramayo-Caldas et al., 2016; Fonseca et al., 2017). Moreover, seasonal variation in L* values (more pale muscle in the summer) have been attributed to changes in the Ca^{2+} release channels (Kuchenmeister et al., 2000). Several potassium channel and solute carriers were also included in the enriched pathways. Indeed, K^+ is necessary for muscle contraction and nerve impulses, and together with sodium, it contributes in the maintenance of fluids balance in the cells (Knochel & Schlein, 1972). Moreover, K^+ content has been reported to influence meat quality traits, i.e. tenderness (Tizioto et al., 2014). Our results seemed to suggest that K^+ transport might also have

a role in the control of beef color. The enrichment of oxygen transport observed for C* might be related to post-mortem aging. Increased aging was reported to improve blooming by decreasing the competition for oxygen between mitochondria and myoglobin and improving myoglobin oxygenation (Mac Dougall, 1982). Aging can also influence cellular mechanisms (such as oxygen scavenging and reducing enzymes) which are critical to meat color stability (King et al., 2012; Nair et al., 2018). It is worth mentioning that a greater heme/iron content was reported in meat from Piemontese breed respect to other European beef cattle breeds (Chambaz et al. 2001); in addition, variation in genes involved in the heme metabolism has been detected between Piemontese and Marchigiana breeds (Sorbolini et al., 2015). Finally, a close link between purine metabolism in skeletal muscle and its physical and chemical properties, including color, has been recently highlighted (Zheng et al., 2018), which might explain overrepresentation of purine nucleoside bisphosphate biosynthetic process for b*.

Within the response to organic cyclic compound biological process, which was enriched for EUS, several genes involved in the gamma-aminobutyric acid (GABA)ergic signaling were included such as some GABA receptors, namely *GABRB1*, *GABRB3* and *GABRG2*, as well as down-stream G-protein, i.e. G protein subunit gamma 2 (*GNG2)* and protein kinase C, i.e. protein kinase C Gamma (*PRKCG)*. GABA is the main inhibitory neurotransmitter in the mammalian central nervous systems. It is synthesized from glutamate by the enzyme glutamic acid decarboxylase and has been reported to play a role in the control of feeding behavior in ruminant animals (Seoane et al., 1984). Recently, GABAergic synapse pathway has been also associated with live weight in Simmental cattle (Fan et al., 2015).

More difficult to interpret was the enrichment of regulation of synapse-related pathways for CL and CW. Notably however, among the genes associated to CL, Wnt family member 5A (*WNT5A*) and Wnt family member 7A (*WNT7A*) were included; these genes have been related to meat quality, muscle fiber types and post-mortem energy metabolism in pigs (Men et al., 2017).

Moreover, Wnt signaling has been reported to affect the intramuscular fat content (Jeong et al., 2013).

CONCLUSIONS

In summary, results of this study allowed to identify genomic regions and biological pathways controlling carcass and meat quality traits in the Piemontese breed. In particular, we proved that beside the major role of MSTN, other genes contribute to explain the variability in carcass and growth characteristics. Of interest is the association of pathways related to transporter activity (oxygen, calcium, ion and cation) with meat color parameters. The obtained results might therefore provide useful information to be exploited for implementing selection programs aimed at the improvement of meat production and quality.

TABLES AND FIGURES

Table 1. Descriptive statistics for carcass and meat quality traits in Piemontese cattle (n = 1,166).

Trait[1]	Mean	SD	h^2_g	#SNP
Carcass traits				
AS, d	539	61.87	0.151	3
CW, kg	438.03	45.40	0.443	20
CDG, kg/d	0.82	0.11	0.517	23
EUS	14.69	1.54	0.214	2
REMA, cm²	92.22	14.21	0.486	0
Meat quality traits				
pH	5.55	0.06	0.072	1
Water holding capacity				
PL, %	4.51	1.19	0.139	1
CL, %	16.76	3.43	0.081	2
Color parameters				
L*	39.91	3.52	0.386	1
a*	28.59	1.80	0.197	2
b*	9.64	1.70	0.247	2
c*	30.19	2.21	0.212	1
H*	18.48	2.14	0.266	2
WBSF,N	41.19	11.56	0.221	0

[1]AS: age at slaughtering; CW: carcass weight; CDG: carcass daily gain, carcass; carcass conformation according to the EU linear grading system (Commission of the European Communities 1982) were assessed. To obtain a more accurate differentiation, the six main grades (S, E, U, R, O, P from best to worst) were furtherly subdivided in + or − subclasses and then converted into numerical scores (EUS) ranging from 18 (S+ class) to 1 (P- class); LT: area *longissimus toracis*; PL: purge loss; CL: cooking loss; WBSF: Warner-Bratzler shear force
SD: standard deviation; h^2_g: genomic heritability
[2]#SNP: number of significant SNP (5×10^{-5}) for each trait

Table 2. Results of genome wide association analysis for carcass and meat quality traits in Piemontese beef cattle (n=1,166).

BTA[1]	#SNP	P-value (range)	Top SNP	Top SNP location, bp	Top SNP MAF	Trait[2]
3	1	3.75E-05	ARS-BFGL-NGS-76281	31524593	0.39	CDG
4	1	3.34E-05	BovineHD0400032408	112507562	0.46	H*
6a	1	4.04E-05	BovineHD0600008146	29225507		CL
6b	23	2.44E-11, 4.55E-05	ARS-BFGL-NGS-45457	38715250	0.43	**CDG**, CW, AS
6c	1	9.69E-06	BTA-76623-no-rs	71154473	0.12	EUS
6d	1	1.78E-05	Hapmap49816-BTA-98191	91906227	0.18	AS
8	1	2.02E-05	ARS-BFGL-NGS-114722	28464160	0.19	pH
9	1	1.54E-05	BovineHD0900013319	48330996	0.43	PL
10	1	4.15E-05	ARS-BFGL-NGS-70946	14574453	0.42	CL
11	1	4.83E-05	ARS-BFGL-NGS-116123	94686959	0.32	EUS
19	1	3.07E-05	ARS-BFGL-NGS-4893	6895198	0.43	CW
23	1	4.47E-05	BovineHD2300000877	3907142	0.36	a*
23	1	3.56E-05	BovineHD2300001826	7245409	0.37	L*
24	1	4.58E-05	BovineHD2400005258	19872257	0.38	b*
25	1	7.79E-06, 3.53E-05	BovineHD2500003345	11960157	0.27	**a***,b*,c*,H*

[1]BTA= *Bos taurus* autosome chromosome; #SNP = number of the single nucleotide polymorphisms significantly associated to the trait; Interval: The region on the chromosome spanned among the significant SNP(s) (in Mb); *P*-value (range)= The *P*-value of the highest significant SNP adjusted for genomic control and the range of the *P*-values when multiple SNP were significantly associated to one trait; Top SNP location (bp)= position of the highest significant SNP on the chromosome in base pairs on UMD3.1 (http://www.ensembl.org/index.html); Top SNP MAF= minor allele frequency of the top SNP.

[2] CDG: carcass daily gain; CL: cooking loss; CW: carcass weight; AS: age at slaughtering; PL: purge loss; carcass conformation according to the EU linear grading system (EUS).

The trait with the highest *P*-value in each genomic region is bolded.

**Table 3.** Significant SNP mapping in the region 38.57-38.94 Mb on BTA6.

SNP	CHR	BP	P	MAF	Gene[1]	Variant effect[2]
BovineHD0600010666	6	38574125	1.73E-05	0.48	*LAP3*	nearGene-5'
Hapmap26308-BTC-057761	6	38576012	4.81E-07	0.38	*LAP3*	intron
BovineHD0600010673	6	38590515	3.25E-05	0.48	*LAP3*	intron
MS-rs110839532	6	38599667	3.25E-05	0.48	*LAP3*	3'UTR
MS-rs43702361	6	38599672	1.32E-05	0.48	*LAP3*	3'UTR
MS-rs109241256	6	38599864	2.79E-05	0.48	*LAP3*	3'UTR
MS-rs41255599	6	38599993	3.25E-05	0.48	*LAP3*	3'UTR
BovineHD0600010685	6	38616248	3.25E-05	0.48	*FAM184B*	intron
ARS-BFGL-NGS-45457	6	38715250	1.86E-06	0.43	*FAM184B*	intron
MS-rs109570900	6	38777311	3.35E-05	0.46	*NCAPG*	missense
MS-rs110251642	6	38808241	5.98E-06	0.48	*NCAPG*	missense
BovineHD0600010755	6	38866381	5.33E-08	0.48	*LCORL*	intron
Hapmap31285-BTC-041097	6	38869785	5.98E-06	0.48	*LCORL*	intron
Hapmap33628-BTC-041023	6	38939012	1.23E-08	0.46	*LCORL*	intron

MAF: minor allele frequency; CHR: chromosome; BP: SNP location in bp
[1]Gene: gene in which SNP is located; *LAP3*: Leucine Aminopeptidase 3; *FAM184B*: Family With Sequence Similarity 184 Member B; *NCAPG* : Non-SMC Condensin I Complex Subunit G; *LCORL:* Ligand Dependent Nuclear Receptor Corepressor Like
[2]Variant effect: SNP effect on the gene.

Figure 1. Manhattan plots for the genome-wide association results of age at slaughtering, carcass weight and average daily gain in carcass on *Bos taurus* autosome 6 (BTA6). a) AS: age at slaughtering; b) CW: carcass weight; c) CDG: carcass daily gain. The red horizontal lines indicate a $-\log10$ (*P*-values) of 4.30 (corresponding to *P*-value $= 5 \times 10^{-5}$). The region 6b of BTA6 is highlighted.
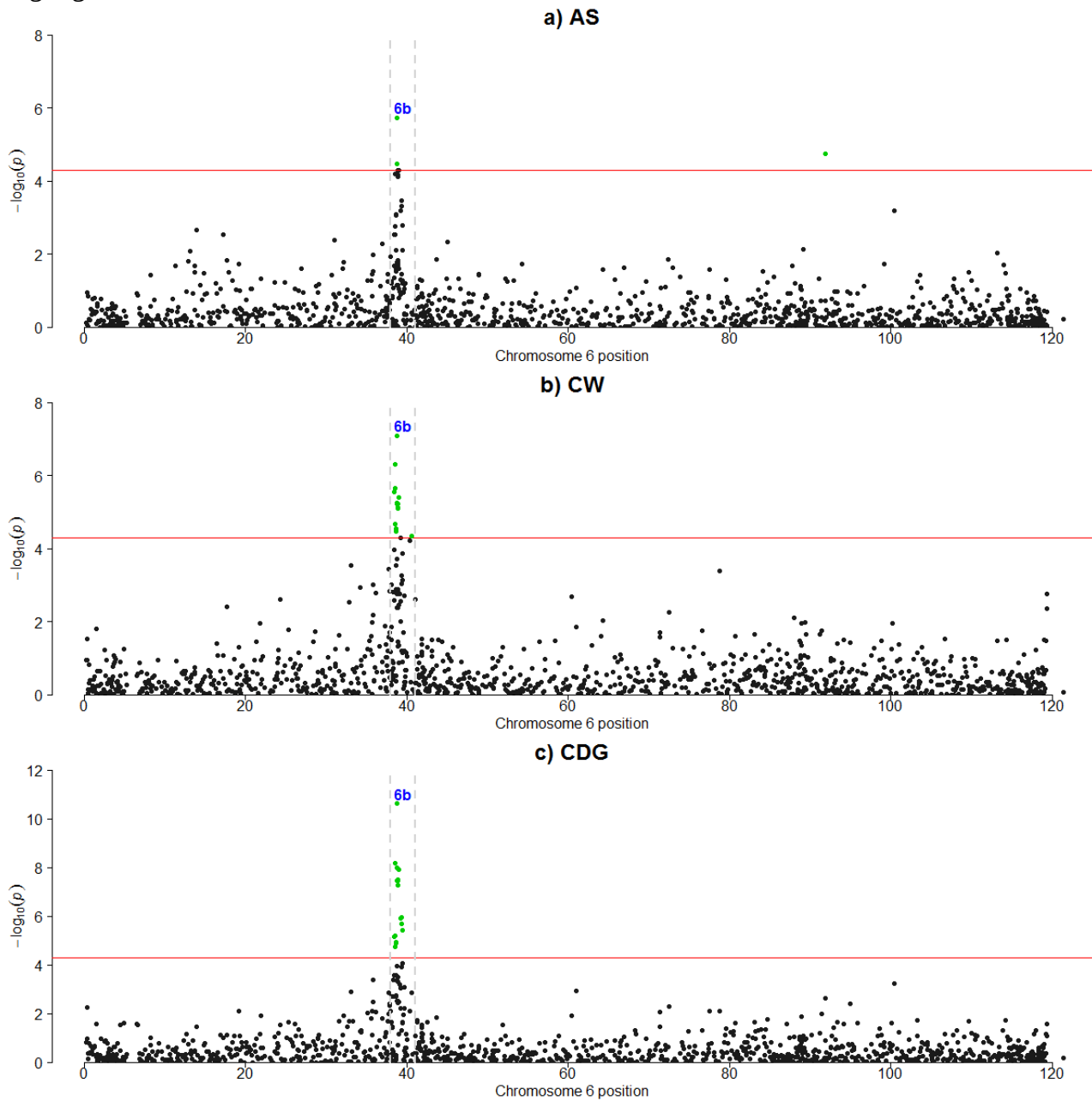
Figure 2. Significantly enriched GO terms and KEGG pathways for carcass and meat quality traits. Only the significant terms are displayed (FDR<0.05). %AG=percentage of genes associated to the significant pathways respect to the total number of genes in the pathway; CW: carcass weight; CL: cooking loss; carcass conformation according to the EU linear grading system (Commission of the European Communities 1982) were assessed. The size of the circles corresponds to the number of significant genes for each term and the colour of the circle corresponds to the trait of interest.
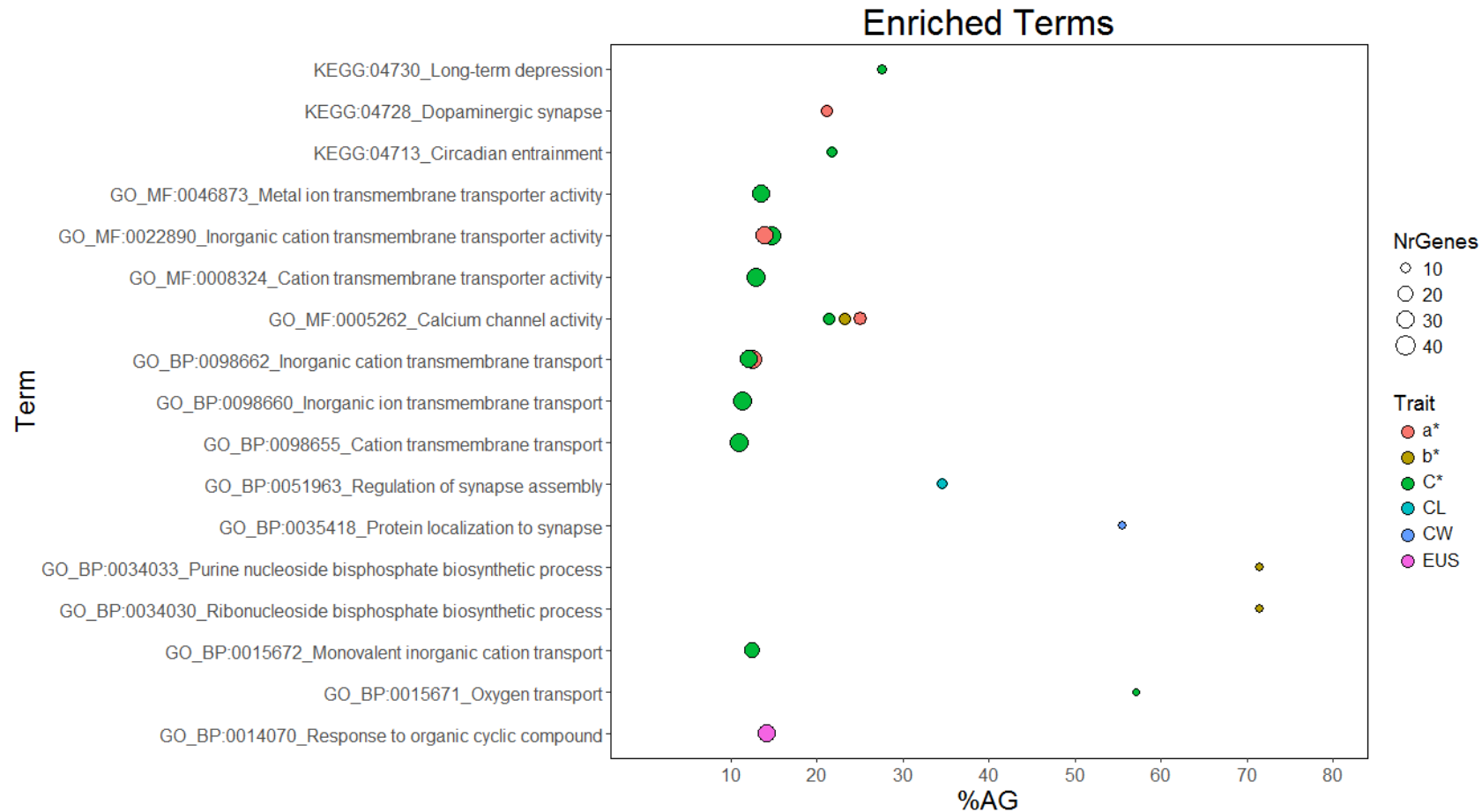
CHAPTER 6.

Genomic breeding value estimation for meat quality traits in Piemontese young bulls

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ABSTRACT

The aim of this study was to estimate genetic and genomic parameters and to assess the accuracy of genomic predictions for pH, water losses, colour parameters and shear force traits evaluated on *Longissimus Thoracis* muscle in Piemontese beef cattle. Phenotypic data used were from 1,327 Piemontese young bulls, progeny of 204 A.I. purebred sires and 1,286 dams, all registered in the Italian Piemontese Herd Book. Animals were fattened in intensive conditions and slaughtered before 2 years of age. All animals were genotyped using the array "GeneSeek Genomic Profiler Bovine LD" (GGP Bovine LD) containing 30,111 SNP. The estimates of genetic and genomic parameters and the accuracy of direct genomic breeding values (DGV) were assessed by performing a 15-fold cross-validation scheme and modelling a SNP-BLUP models. Phenotypes adjusted for environmental effects were used as response variables and polygenic effect was fitted in the model to account for pedigree relationships.

The amount of variance explained by markers and pedigree relationships confirmed that genetic variability exploitable for the genetic improvement of these traits exist. The accuracies of genomic predictions were moderate and varied by trait, ranging from 0.216 (pH) to 0.380 (shear force). Except for Lightness, DGV underestimated the pre-corrected phenotypes of animals in validation populations.

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.

Keywords*: heritability, meat color traits, tenderness, cooking losses, Piemontese*

INTRODUCTION

Meat quality has always been important for the consumer, and it is especially a critical issue for the meat industry in the 21st century (Joo et al., 2013). As consumer's demand for high quality meat is increasing in most countries, the meat industry should consistently produce and supply meat that satisfies their expectations.

Although tenderness is the primary determinant of satisfaction among beef consumers (Mullen et al., 2006), customers have to base their quality evaluation at the time of purchase mostly on the appearance (Grunert et al., 2004), as the final product is marketed mostly unbranded and unlabelled. As a consequence, colour traits and water losses of fresh meat play an important role in determining consumers choice. Hence, the improvement of quality traits related to physical properties of meat could be of considerable economic importance.

However, the establishment of a selection procedure concerning meat quality traits by traditional breeding value estimation relies on the availability of a large number of phenotypes collected on the progeny of candidates to selection. Nowadays, this is not routinely feasible as it requires the collection of meat samples at slaughterhouse causing a depreciation of carcasses and laboratory instrumental analyses which are destructive, expensive and time consuming.

In such a complex situation genomic selection (GS) is an appealing alternative for the genetic improvement of meat quality. GS refers to selection decisions that are based on breeding values predicted using genomewide marker data such as single nucleotide polymorphism (SNP) (Meuwissen et al., 2001). The theoretical basis of GS is that the genetic variance of every quantitative trait locus (QTL) for a trait can be captured by SNP markers due to linkage disequilibrium (LD) between QTLs and markers. The estimation of SNP effects is performed in a training population that has been phenotyped for the trait and genotyped for the markers. All markers effects are simultaneously estimated and genomic breeding values of selection candidates can then be predicted from genotype data only. GS allows a reduction of generation interval in

selected populations and, when the genotyping cost is low, a large number of candidates can be screened, increasing also the selection intensity. The accuracy of prediction, however, depends on the genetic basis of the trait (namely its heritability), the extent of the LD between SNPs and QTLs and the size of the training population (Hayes et al., 2009; VanRaden et al., 2009). Moreover, also the genetic relationship or relatedness of selection candidates with the training population has a great impact on the accuracy of predicting genomic breeding values (Chen et al., 2015).

A number of statistical method for the prediction of genomic breeding values have been developed and compared (Habier et al., 2011). Differences across methods in the accuracies of genomic predictions have been obtained depending on the genetic architecture of the investigated traits (Legarra et al., 2011; Mehrban et al., 2017). In general, all the studies reported an advantage of genomic breeding values over parental averages in terms of reliability (Saatchi et al., 2012). Genomic selection can yield a greater genetic gain particularly for traits which are difficult to select in traditional way, as the traits related to meat quality are (Bolormaa et al., 2013).

Concerning beef cattle, several investigations were carried out on the prediction accuracy of genomic breeding values for carcass traits (Fernandes Júnior et al., 2016; Mehrban et al., 2017; Saatchi et al., 2011). Only very few studies assessed the prediction accuracy of genomic breeding values for meat quality traits, but they were limited to tenderness (Bolormaa et al., 2013; Rolf et al., 2015) or specific meat chemical properties, as lipid content (Chiaia et al., 2017). Currently literature lacks of a comprehensive investigation of prediction accuracy of genomic breeding value for meat quality traits. The analysis of the performance of genomic predictions of meat quality traits can be of particular interest in the frame of cattle breeds showing muscular hypertrophy (double muscling) (King and Menissier, 1982), due to their peculiar characteristics. Indeed, hypertrofic breeds are well known for their ability to produce heavily muscled and lean carcasses (Casas et al., 1998), improving feed efficiency and reducing fat deposition (Fiems, 2012) in comparison with conventional beef breeds. Beside differences in carcass composition, double muscled cattle also

produce meat with higher water and protein contents, low levels of intramuscular fat (Barge et al., 1993) and a large reduction in muscle collagen, responsible for the increased tenderness (Destefanis et al., 1994). Currently, studies about the application of genomic prediction technology in double muscling breeds could not be found neither for production nor for meat quality traits.

The Piemontese (Piedmontese) breed suits for this purpose, as characterised by double muscling inducted by a specific mutation of myostatin gene (mh) located on Chromosome 2 (Grobet et al. 1998), which is almost fixed in this population. Moreover, a recent study by Savoia et al. (2018a), highlighted the existence of genetic variability for meat quality traits in the Piemontese breed, which is exploitable for genomic investigations.

Therefore, the aim of this study was to estimates genetic and genomic parameters and to assess the accuracy of prediction of direct genomic breeding values for meat quality traits in the Piemontese breed.

MATERIAL AND METHODS

Animals and beef samples collection

The study was part of the "Qualipiem" project and involved 1,327 Piemontese young bulls slaughtered at the same commercial abattoir from April 2015 to February 2017. Young bulls were progeny of 204 A.I. purebred sires and 1,286 dams, all registered in the Italian Piemontese Herd Book. Animals were fattened in 115 farms representative of the beef production systems of the Piemonte region (north-west Italy). The beef farming systems, feeding regime, fattening conditions and slaughtering performances of young bulls were described in detail by Savoia et. al. (2018c). The average carcass weight of the sampled Piemontese young bulls was 438.1 (±43.6) kg, while the average age at slaughter was 540.9 (±63.2) days, giving an average daily carcass gain of 0.818 (±0.107) kg/d. Average carcass conformation score (SEUROP systems with each category divided in 3 subclasses, 1–18 point scale) was 14.66, corresponding to an average evaluation approaching

"E+" in the EU linear grading system and average rib eye area measured at the 5th rib was 92 cm^2 (±14.3).

Analysis of meat quality traits

Twenty-four hours after slaughter, individual samples (4.0 cm thick) of the *Longissimus thoracis* (LT) muscle were collected between the 5th and 6th rib, then were individually vacuum packed and transferred under refrigerated conditions to the laboratory, where they were stored in a chilling room at 4°C for 7 days, after which meat quality traits were measured.

Assessment of meat quality included muscle pH, lightness (L*), redness (a*), yellowness (b*), hue angle (h*), Chroma (C*), purge losses (PL, %), cooking losses (CL, %), and Warner Bratzler shear force (WBSF, N), all measured at 7 d after slaughter. Details on procedures used to assess meat quality traits can be found in Savoia et al. (2018c).

For each trait the observations falling outside the range of 3 standard deviations from the mean were removed.

Genotype data

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 excluding SNP markers with unknown genomic position, located on sex chromosomes, monomorphic, markers with minor allele frequency (MAF) < 0.05 and call rate < 90%. Animals with a call rate less than 90% or with an inconsistency between pedigree and genomic relationships were also excluded from further analyses. After quality control, there were 1,166 animals and 23,400 SNPs available for the analyses.

Genomic and pedigree-based relationships

The degree of relationship between individuals in the reference population is likely to affect the accuracy of genomic predictions (Lee et al., 2017). Moreover, due to incomplete linkage disequilibrium of SNP markers to genes or causal mutations responsible for genetic inheritance of

quantitative traits, a residual polygenic effect is normally fitted in the genomic model to account for the genetic variance not explained by markers (Liu et al., 2016). Without taking into account pedigree information, a BLUP with genomic relationship matrix (GBLUP) or single nucleotide polymorphism BLUP (SNP-BLUP) model, were proven to give equivalent predictions for genotyped animals (Liu et al., 2016). For these reasons, genomic and pedigree-based relationships between the 1,166 Piemontese young bulls were investigated before the computation of direct genomic breeding values.

Genomic relationship matrix (GRM) was created for the 1,166 genotyped young bulls with R package 'snpReady' (Granato and Fristche-Neto, 2018) following the method proposed by VanRaden (2008) for additive genomic relationship. With p containing allele frequencies and with Z containing values of $0 - 2p$ for homozygotes, $1 - 2p$ for heterozygotes, or $2 - 2p$ for opposite homozygotes of each animal (row) and each marker (column), GRM was computed as GRM = ZZ' / $\Sigma 2p(1 - p)$.

From the pedigree registered in the Italian Piemontese Herd Book, the additive relationship matrix was built with R package 'pedigree' (Coster, 2015). Pedigree-based relationships matrix included 13,094 animals. Only the diagonal and off-diagonal elements belonging to the 1,166 genotyped young bulls were used for the comparison between the relationship matrices.

Variance components, heritability, SNPs effects and direct genomic breeding value

Phenotypes were pre-corrected for all the non-genetic effects prior to implement estimation of variance components and SNPs effects. This pre-correction step was necessary to allow the subsequent partitioning of animals into training and validation populations without the need to correct the data within each subpopulation for non-genetic effects. The model used to pre-correct the phenotypes included the fixed effects of parity of the dam (4 classes: 1st, 2nd, 3rd-8th, >8) and birth season (4 classes: January-March, April-June, July-September, October-December) The random effects of the day of slaughter (117 levels) and fattening farm (98 levels) were also inclued.

Both random effects were assumed to be normally and independently distributed $\sim$ N (0, σ^2). A minimum cell size of 3 observations was required for both the day at slaughter and the fattening farm effects.

For each trait, the entire data-set was randomly split into training population (80% of animals) and validation population (20% of animals), ensuring that animals from the same farm were included in the same population. Due to the contemporaneity of the sampled animals, no rule concerning the age or the relationships of animals was defined to build the two populations.

Pre-corrected phenotypes and the genotypes of the training populations were then used for the estimation process. The described procedure was replicated 15 times (fifteenfold cross-validation).

The estimation of variance components and SNP effects was performed simultaneously using Bayesian approach and Markov-chain Monte Carlo (MCMC) with the GS3 software (Legarra et al., 2016). A single chain of 2,000,000 iterations, with a burn-in period of 400,000 iterations was used for Gibbs sampling. Samples were saved every 200 iterations.

The general model adopted can be expressed as follows:

$$y = \mu + Xg + Zv + e$$

where y is the vector of pre-corrected phenotypes, μ is the overall mean; X is the matrix (n × p) allocating records to p SNP effects with element X_{ij} =0,1,2 if the genotype of animal i at SNP j is AA, AB, or BB, respectively (A and B assigned according to Illumina classification); g is thevector of random SNP effects; v is the vector of the random additive genetic animal effects; e is the vector of random residual effects. Prior distributions for the SNP effects were assumed to be normal, g ~ N (0,I σ^2_g) with σ^2_g representing the variance explained by markers. Animal additive genetic effects were assumed to be normally distributed, v ~ N(0 Aσ^2_a) where A is the pedigree-based numerator relationship matrix (13,094 animals) and σ^2_a is the additive pedigree-based variance. Residuals were assumed to follow a normal distribution, e ~ N(0,I σ^2_e). Flat priors were used for the a priori distributions of variance components. The posterior mean was used as a point estimate of variance

components.

The total genetic variance due to markers was calculated as $\sigma^2_{SNP} = 2\,\sigma^2_g \Sigma\, p_j q_j$, where p_j and q_j are the allele frequencies for the jth SNP. Using the estimates of σ^2_{SNP}, σ^2_a and σ^2_e genomic, pedigree-based and total heritability were calculated as $h^2_{SNP}=\sigma^2_{SNP}/\sigma^2_P$, $h^2_{Pedig}=\sigma^2_a/\sigma^2_P$ and $h^2=(\sigma^2_{SNP}+\sigma^2_a)/\sigma^2_P$, where σ^2_P is the phenotypic variance.

Using the estimated SNP effects, direct genomic breeding values (DGV) were calculated for the young bulls in the validation populations as DGV=Xu where X is a matrix of the young bulls' genotypes, and u is the vector of predicted SNP effects. To evaluate the prediction ability of DGV, 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. The slope of linear regression of the pre-corrected phenotypes on DGV was considered to express the magnitude of the bias of the DGV relative to the phenotypes. Results were reported as the average of the values obtained in the 15 folders, with the relative standard deviation as a measure of the uncertainty of the estimates.

RESULTS

Meat quality traits

Descriptive statistics of meat quality traits for Piemontese young bulls are shown in Table 1. We illustrated these quality traits of Piemontese beef in a previous survey (Savoia et al., 2018c). Briefly, pH displays a very limited variability with a mean of 5.56 and the largest values do not exceed 5.87. Concerning water holding capacity, CL (%) are greater than PL (%) with mean values of 16.76 and 4.56 respectively. PL (%) is also the most variable among meat quality traits with a CV of 28.91%. Colour parameters exhibit moderate variability, which ranged from 6.33% for a* to 17.66% for b*. The WBSF value averaged 41.44 N and it was characterised by high variability (CV of 26.88%).

Genomic and pedigree-based relationships

The genomic relationship among Piemontese young bulls ranged from -0.081 to 0.499 with a mean value of -0.001 (0.037). The average value of the off-diagonal elements of the pedigree-based matrix was 0.036 (0.035), varying from 0.000 to 0.570. The average value of the diagonal elements of the genomic relationship matrix was 1.00 (±0.022) and ranged from 0.936 to 1.131, lower than the average value of the diagonal elements of the pedigree-based relationship matrix of 1.013 (±0.013).

The correlation between the off-diagonal elements of the two matrices was 0.64. The regression of the genomic relationship on the pedigree-based relationship coefficients had an intercept value of -0.025 and a regression coefficient of 0.67.

Variance components, heritability and direct genomic breeding values

Genomic, pedigree-based and residual variance estimates are reported in Table 3. For all meat quality traits the residual variance was the largest between the three different components, even if both the genetic effects always explained a not negligible amount of variance. For pH and CL (%), the variance explained by polygenic additive effect was slightly greater than the variance explained by SNPs. On the contrary, for PL (%), WBSF and all colour parameters the variance explained by SNPs was from 1 to 4 times greater than the variance explained by pedigree relationships.

The amount of variance explained by markers and by pedigree relationships was not negligible for all the traits, producing considerable estimates of total heritability (Table 4). These ranged from 0.20 (±0.03) for water holding capacity traits (PL and CL) to 0.41 (±0.04) for L*.

The accuracy of genomic predictions and the regression coefficient of the pre-corrected phenotypes on direct genomic breeding values are illustrated in Table 5. The accuracy of DGV was 0.23 for pH, 0.305 and 0.216 for PL (%) and CL (%) respectively. Colour parameters showed similar accuracies, varying from 0.277 for C* to 0.357 for b*. The highest accuracy was reported for WBSF with a value of 0.380.

Except for Lightness, which reported the expected value of 1.00, all the others traits investigated showed coefficient of regression larger than 1.00. "b" values varied then from 1.25 for pH to 1.73 for b*, highlighting that DGV underestimated the pre-corrected phenotypes of animals in validation populations.

DISCUSSION

The characteristics of meat quality traits for Piemontese young bulls were discussed in detail in our previous survey (Savoia et al., 2018c).

Focussing the discussion on genomic data, the most critical issues of the present investigation could be represented by the low markers density of the array used, containing 30,111 SNPs.

It is known that genomic selection, as described by Meuwissen et al. (2001), is aimed at exploring linkage disequilibrium between QTL and high-density markers across the genome for breeding value estimation. However, several studies (Ogawa et al., 2014; Ogawa et al., 2016; Pryce et al., 2012) demonstrated that also with low-density markers arrays satisfying results can be achieved. Ogawa et al. (2014), investigating the carcass weight in the Japanese Black beef cattle, highlighted that 90% of the genetic variance estimated using 40,000 SNPs was explained using only 4,000–6,000 SNPs. Moreover, Pryce et al. (2012) investigating residual feed intake and body weight gain in growing heifers found little advantage in the accuracy of DGV using the HD-SNP panel over the LD-SNP panel. In the latter study a greater effect on the accuracy of genomic predictions was exerted by the degree of relationship between individuals in the training population. Indeed, as outlined by Pszczola et al. (2012), the design of the reference population with respect to its family structure may influence the accuracy of genomic selection. Then, the relationships between the sampled Piemontese young bulls were investigated.

Genomic and pedigree-based relationships

The pedigree-based relationship matrix estimates fractions of alleles expected to be identical

by descent (VanRaden et al., 2011). It assumes an infinite number of loci and its coefficients represent the expected average relationships between relatives. Otherwise the coefficients of the genomic relationship matrix, using markers information represent the observed relationships, accounting then for Mendelian sampling (Visscher et al., 2006) and unknown or far relationships. Ideally, genomic relationships should be estimated using the allele frequencies from the unselected base population (Forni et al., 2011). However, these information are rarely available and approximations need to be used, although the allele frequencies calculated in earlier or later base population can lead to a greater or fewer relationships and to more or less inbreeding (VanRaden, 2007).

The relationship coefficient obtained in our investigation were in agreement with the expected values. The average value of the diagonal elements of the genomic matrix was 1.000 as expected, as well as the average value of the off-diagonal elements which was close to 0. Indeed, the contribution of SNPs to off-diagonal elements of the genomic matrix was proportional to the allelic frequencies, and they roughly cancelled out (Chen et al., 2015). The average values of diagonal and off-diagonal elements of genomic matrix were lower than the average values obtained from pedigree as the pedigree-based matrix does not allow negative values, observed instead in the genomic matrix. In general, the coefficients of the genomic relationship matrix had greater variability than the corresponding elements of the pedigree-based matrix because individuals equally related in the pedigree could have more or less alleles in common than expected (Forni et al., 2011). Bolormaa et al. (2013) investigating genomic relationships between and within 9 beef cattle populations of 3 breeds types, using a genomic relationships matrix build by the method of Yang et al. (2010), found average diagonal elements within population ranging from 0.94 to 1.34 while average off-diagonal elements ranged from 0.04 to 0.68.

As illustrated in Figure 1, except for the little groups of half-sibs, genomic relationships between animals are low. The correlation between marker and pedigree additive relationship

coefficient was high and similar to 0.69 found by Hayes and Goddard (2014) in the Angus breed. In agreement with the results obtained by VanRaden et al. (2011), which investigated the genomic and pedigree relationships among Holstein, Jersey and Brown Swiss breeds, in our study the coefficient of regression of genomic relationships on pedigree relationships was less than the expected value 1.00 while the intercept was slightly less than the expected value of 0.

Variance components

The SNP effects explained a considerable amount of variance in all the meat quality traits investigated, showing that a linkage disequilibrium bewteen SNPs and QTL existed. However, the polygenic additive effect included in the model explained a never negligible amount of variance for all the traits investigated in this study. These results confirmed that the linkage disequilibrium between SNPs and QTLs is incomplete and that taking into account pedigree information, when available, is needed in the prediction of genomic breeding values. Although this is always considered a good practice (Liu et al., 2016), it is even more so when arrays with low density of markers are used for genotyping.

Hayes and Goddard (2014) indeed, had demonstrated with simulated data that the higher is the number of genomic markers used, the higher is the level of accuracy in estimating the true genetic variance of a trait.

Although both the genetic effects were able to detect differences between animals, the residual variances were the largest components in all the traits. Comparing this results with those previously obtained by us estimating traditional genetic parameters for meat quality traits (Savoia et al, 2018a) in the same dataset, a reduction in the residual variance components was highlighted for all the traits. This could be explained for two reasons. Firstly, marker effects fitted in the model could have captured Mendelian sampling not detected by traditional pedigree relationships with a consequent reduction of residual variances. In support of this, for all colour traits and PL (%), the amount of variance explained by markers added to that explained by polygenic additive effect is

higher than the additive variance component of the previous study.

Secondly, the estimation of variances components pertaining to fattening farm and slaughter batch for the pre-correction of the phenotypes before the estimate of genetic variance components performed in this study, could have removed more variability from pre-corrected phenotypes, resulting in lower residual variance components. It is worth to note that in the previous study pH, CL (%) and WBSF were the traits most affected by slaughter batch effect (which explained from 40% to 62% of total variance) and were exactly the same traits for which the additive genetic variance components were higher than latest variances components pertaining to marker and polygenic effects. In the case of traits strongly affected by environmental effects, pre-correction of phenotypes could be discouraged to avoid biased estimates. However, in our study no alternative existed to estimate the accuracy of genomic breeding values.

Heritabilities

As a consequence of the previous results concerning variance components, for all the investigated traits genomic enhanced heritabilities were not negligible. Pegolo et al. (2018), combining GWAS and biological pathway analysis on the same data-set, estimated markers-based heritabilities without taking into account a polygenic effect based on pedigree relationships. Our genomic enhanced heritabilities were similar (yellowness) or greater (all the other traits) than those found by Pegolo et al. (2018) highlighting the added value of using pedigree-based relationships.

Also comparing our estimates with the traditionals intra-herd heritabilities estimated in our previous study (Savoia et al., 2018a) emerged that latest genomic enhanced heritabilities were higher for all the traits except pH and WBSF. For these last two traits the gap between the latest and the traditional estimates was however low, around only 4% for both traits.

The comparison of our results with others is difficult because the scientific literature lacks of study investigating genomic enhanced heritabilities for the meat quality traits analysed in the present study. However, these results further confirm the existence of genetic variability exploitable

for a durable improvement of meat quality traits in Piemontese breed.

Accuracy of prediction of direct genomic breeding values

Scientific literature seems to lack of a comprehensive investigation concerning the accuracy of prediction of meat quality traits using genomic information in cattle.

Most of the studies focussed on estimating genomic breeding values for meat quality traits limited their investigation to marbling score and fat thickness (Boddhireddy et al., 2014; Fernandes Júnior et al., 2016; Mehrban et al., 2017; Saatchi et al., 2011), traits not considered in our analysis due to the leanness of Piemontese meat. However, the studies by Fernandes Júnior et al. (2016) on Nellore cattle and Mehrban et al. (2017) on Hanwoo beef cattle found accuracies of genomic predictions near to 0.25 for each trait, concluding in both the investigations that applying genomic selection to improve these traits was feasible.

Very few studies investigated the accuracies of genomic predictions for traits related to physical properties of meat, and they focussed only on tenderness (Bolormaa et al., 2013; Miller et al., 2014, Rolf et al., 2015). A great variability in the values of the prediction accuracy was found depending on the arrays adopted and the population used for validation. Miller et al. (2014) developing a multi-breed approach for Canadian beef cattle, using HD array and deregressed EBVs as phenotypes, founded that accuracies ranged from 0.10 to 0.50 with an average value of 0.28. Bolormaa et al. (2013) predicting genomic breeding values in 9 different populations of 3 breed types, using phenotypes and HD genotyping, found an average accuracy of 0.25. Higher accuracies with values greater than 0.54 were found by Rolf et al. (2015) using, however, a custom Illumina GoldenGate assay for the generation of genotypes for 96 putative SNPs located within 186 kb of *calpastatin* and *calpain-1* genes in addition to the markers of the Illumina BovineSNP50 BeadArray. Comparing to afore mentioned studies we obtained an accuracy for tenderness which was higher than those reported by Bolormaa et al. (2013) and Miller et al. (2014), despite the lower density of markers at our disposal. In our investigation the tenderness reached the highest accuracy

of prediction between the analysed traits. This could be however related to the presence of several markers in LD with the inhibitor of calpain, the calpastatin and the calpain I genes (Gao et al., 2007).

Also pH, water losses traits and colour parameters showed considerable accuracies in our investigation, suggesting that genetic improvement of these traits adopting genomic selection could be feasible.

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 (Figure 2). This tendency is supported by the findings of Bolormaa et al. (2013), Fernandes Júnior et al. (2016) and Luan et al. (2009) which all highlighted a strong relation between accuracies of genomic breeding values and heritabilities. As outlined by Luan et al. (2009), to achieve better accuracies for the traits characterised by low heritabilities, probably more records in the training population could be needed.

However, the availability of phenotypes for traits that are difficult or expansive to measure, as meat quality traits are, strongly limited the constitution of sufficiently large training populations and, consequently, rarely genomic selection was applied for the genetic improvement of similar traits in beef cattle (Fernandes Júnior et al., 2016). To collect of a large amount of phenotypes a valid alternative to the traditional laboratory analysis can be represented by the Vis-NIR spectroscopy. Indeed, our recent investigation (Savoia et al., 2018b) showed the very good ability of portable spectrometers used at abattoir to capture the major source of variation of meat colour traits and purge losses and the acceptable ability for pH, cooking losses and shear force.

Further specific researches are needed to investigate if phenotypes predicted by Vis-NIR spectral data can be used to perform a reliable calibration for an accurate prediction of genomic breeding values for selection candidates.

CONCLUSIONS

To date, the present research represented the first attempt to implement genomic prediction for quality traits related to physical characteristics of meat in a specialised beef breed. The amount of variance explained by markers and pedigree relationships confirmed that genetic variability exploitable for the genetic improvement of these traits exists. Moreover, 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.

TABLES AND FIGURES

Table 1. Descriptive statistics[1] of meat quality traits for Piemontese young bulls.

Trait[2]	Sample size	Mean	sd	cv
Beef quality traits				
pH	1,157	5.56	0.06	1.04
Water holding capacity				
PL, %	1,155	4.56	1.32	28.91
CL, %	1,166	16.76	3.43	20.46
Colour parameters				
L*	1,156	39.98	3.64	9.10
a*	1,156	28.59	1.81	6.33
b*	1,159	9.64	1.70	17.66
C*	1,157	30.19	2.22	7.34
h*	1,155	18.50	2.17	11.74
WBSF, N	1,166	41.44	11.14	26.88

[1] sd: standard deviation; cv: coefficient of variation.

[2] PL: purge losses; CL: cooking losses; WBSF: Warner-Bratzler shear force.

Table 2. Descriptive statistics of diagonal and off-diagonal elements of the Genomic Relationship Matrix (GRM) and of Pedigree Relationship Matrix (PRM) belonging to the 1,166 genotyped young bulls.

	Mean	sd	min	max
GRM[1]				
diagonal	1.000	0.022	0.936	1.131
off-diagonal	-0.001	0.037	-0.083	0.508
PED				
diagonal	1.013	0.013	1.000	1.139
off-diagonal	0.036	0.035	0.000	0.570

[1] GRM built following the method proposed by VanRaden (2008).

Table 3. Genomic (σ^2_{SNP}), pedigree-based (σ^2_{Pedig}), and residual (σ^2_e) variances estimates by pre-corrected phenotypes and medium density SNP panel for meat quality traits of Piemontese young bulls.

Trait[1]	σ^2_{SNP}		σ^2_{Pedig}		σ^2_e	
	Mean	sd	Mean	sd	Mean	sd
Beef quality traits						
pH	0.00010	0.00006	0.00013	0.00008	0.00064	0.00008
Water holding capacity						
PL, %	0.138	0.074	0.065	0.060	0.802	0.081
CL, %	0.510	0.327	0.598	0.437	4.524	0.437
Colour parameters						
L*	2.679	0.766	0.512	0.465	4.510	0.678
a*	0.272	0.138	0.133	0.111	1.296	0.147
b*	0.267	0.134	0.127	0.108	1.231	0.141
C*	0.444	0.219	0.219	0.179	1.941	0.227
h*	0.524	0.233	0.221	0.190	1.831	0.238
WBSF, N	0.100	0.044	0.056	0.044	0.384	0.045

[1]PL: purge losses; CL: cooking losses; WBSF: Warner-Bratzler shear force.
Mean: average of the values obtained in the 15 folders; sd: standard deviation of the values obtained in the 15 folders.

Table 4. Genomic (h^2_{SNP}), pedigree-based (h^2_{Pedig}) and total (h^2) heritability estimates by pre-corrected phenotypes and medium density SNP panel for meat quality traits of Piemontese young bulls for meat quality traits of Piemontese young bulls.

Trait[1]	h^2_{SNP}		h^2_{Pedig}		h^2	
	Mean	SD	Mean	SD	Mean	SD
Beef quality traits						
pH	0.12	0.02	0.15	0.03	0.27	0.03
Water holding capacity						
PL, %	0.14	0.03	0.06	0.01	0.20	0.03
CL, %	0.09	0.02	0.11	0.02	0.20	0.03
Colour parameters						
L*	0.35	0.04	0.07	0.01	0.41	0.04
a*	0.16	0.02	0.08	0.03	0.24	0.03
b*	0.16	0.02	0.08	0.02	0.24	0.03
C*	0.17	0.03	0.08	0.02	0.25	0.04
h*	0.20	0.03	0.09	0.02	0.29	0.04
WBSF, N	0.18	0.03	0.10	0.02	0.29	0.04

[1]PL: purge losses; CL: cooking losses; WBSF: Warner-Bratzler shear force.
Mean: average of the values obtained in the 15 folders; sd: standard deviation of the values obtained in the 15 folders.

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
Beef quality traits				
pH	915	242	0.231	1.25
Water holding capacity				
PL, %	905	249	0.305	1.48
CL, %	919	247	0.216	1.54
Colour parameters				
L*	910	246	0.324	1.00
a*	910	246	0.290	1.34
b*	909	250	0.357	1.73
C*	908	249	0.277	1.40
h*	908	247	0.290	1.26
WBSF, N	897	249	0.380	1.65

[1]PL: purge losses; CL: cooking losses; WBSF: Warner-Bratzler shear force.

Figure 1. Genomic relationships among Piemontese young bulls in the study, derived from the 24,000 SNP genotypes for each young bulls. Each individual square is the proportion of the genome that an animal shares with another animal. Young bulls are ordered across the rows and across the columns by sire. The intensity of red indicates the degree of relationship: the darker the square, the closer the relationship.
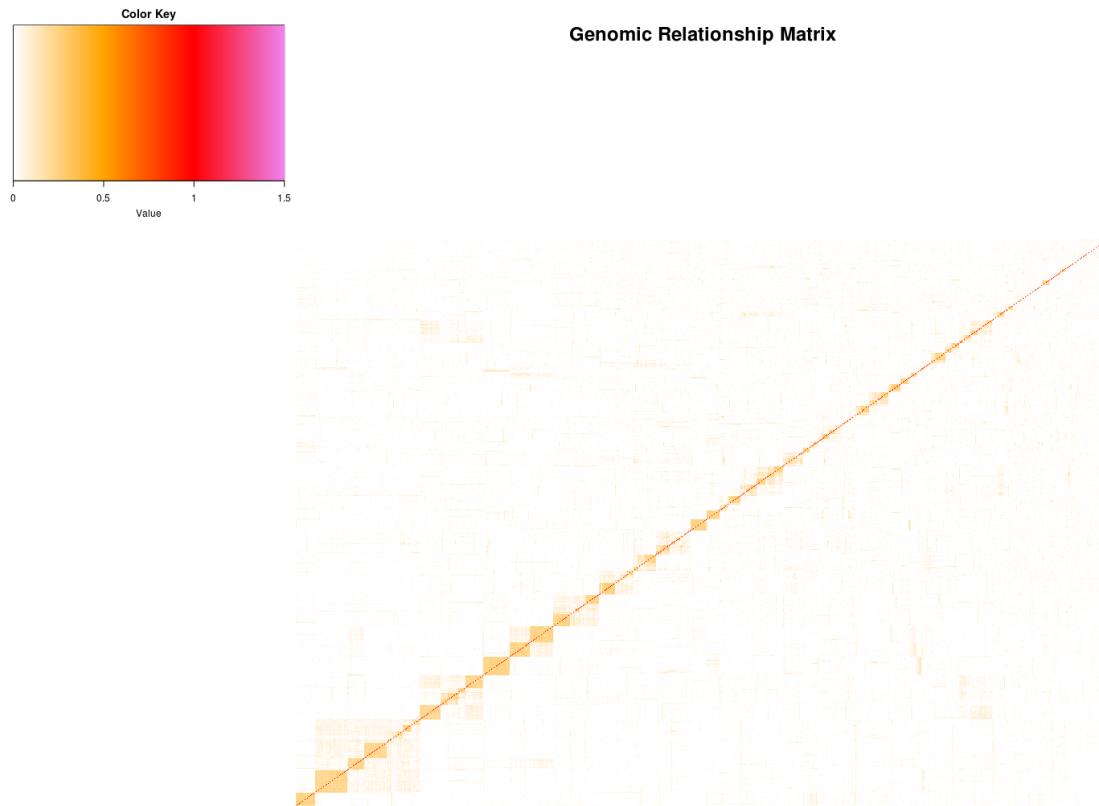
Figure 2. Relationships between heritability of meat quality traits (pH, purge loss, cooking loss, lightness, redness, yellowness, hue angle, saturation index, shear force) and the coefficient of correlation (r) between direct genomic breeding values and pre-corrected phenotypes in the population of validation.
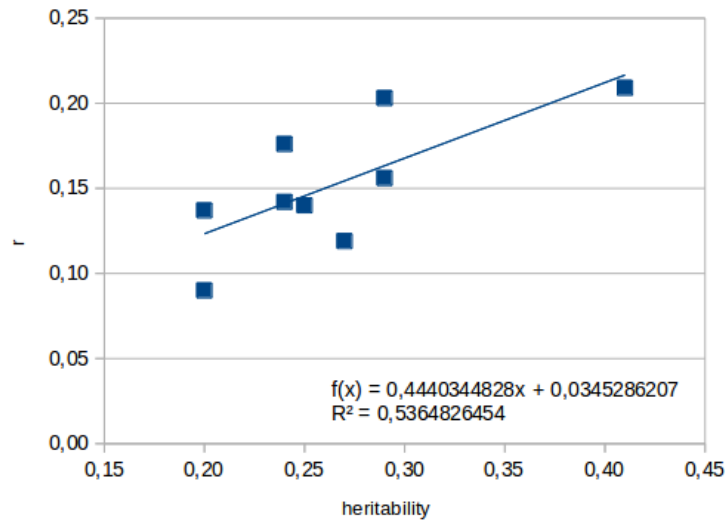
GENERAL DISCUSSION AND CONCLUSIONS

This thesis provided a comprehensive investigation of meat quality traits in the Piemontese breed with focus on the possibilities for their genetic improvement using innovative tools such as visible near-infrared spectroscopy and genomic information.

First of all, an investigation assessing the effects of production system on carcass and meat quality trait was carried out. The beef production systems in the rearing of the Piemontese breed were described in detail, identifying six main typologies according to specific management strategies. The coexistence of traditional systems alongside more advanced systems using modern technologies was highlighted. Carcass traits resulted strongly affected by production system, with traditional management conditions having lower production efficiency. However, production system exerted only a very small effect on meat quality, limited to colour traits, suggesting that future improvement should look, in particular, at genetics.

As expected, carcass and meat quality traits showed moderate heritabilities, confirming that genetic variability among animals exists and it's theoretically exploitable for a durable improvement of meat quality. However, due to the costs and the complexity of meat quality traits' collection, a selection of these traits based on traditional phenotyping of slaughter animals appears unaffordable for the beef industry. Anyhow, the assessment of the genetic relations between the carcass and meat quality traits highlighted that the current selection of Piemontese breeds, focusing on the improvement of growth rate and muscularity, could cause an indirect modification of some meat characteristics. Correlated response to selection will be in a positive direction for colour traits while an unfavourable response is expected for tenderness.

Visible near-infrared spectroscopy proved to be a valid alternative for the phenotyping of some of the meat quality traits. Portable and hand-held spectrometers have been tested at the abattoir with satisfactory results for predicting colour traits and purge losses, but with less reliable results for predicting meat pH, cooking losses and tenderness. The latter traits were, however,

highly affected by several causes of variation (sampling, chilling, ageing, transportation, sample processing, instrument calibration) which reduced the prediction performance of spectroscopy. The inability of the visible near-infrared *spectra* taken after slaughtering to predict the fate of meat samples till analyses can be considered a pro if the aim of prediction is to represent the animal "native" conditions, like in the case of their use for the genetic improvements of beef populations.

The phenotypes of meat quality traits predicted by visible near-infrared *spectra* showed a reduction of additive genetic variance and heritability compared to the corresponding measured traits. However, the magnitude of the estimated genetic parameters showed that the predictions of colour traits, pH and purge losses can be used as indicator traits of the corresponding measurements for selection purposes. For cooking losses the results were more controversial, while estimates for tenderness predictions were not reliable, suggesting to exploit genomic data for their improvement.

Before evaluating the possibility of using genomic selection in order to improve meat quality traits in the Piemontese breed, a first study combining GWAS and biological pathway analysis was performed to investigate the genetic architecture of the traits. Some genomic regions and biological pathways involved in the expression of carcass and meat quality traits were identified. Regarding meat quality, the association of pathways related to transporter activity (oxygen, calcium, ion and cation) with meat color parameters was proved.

A second study was carried out to investigate the genetic variance of meat quality traits estimated with a model combining SNP markers and pedigree relationship information. Results indicated that SNP marker effects were able to explain a considerable amount of variance, but also that the linkage disequilibrium between SNPs and QTLs was incomplete, as proven by the not negligible variance explained by the polygenic additive genetic effect. In the same study, direct genomic breeding values were predicted and their accuracies assessed, showing that the implementation of genomic selection to improve the quality of meat is feasible.

Overall, the results of this thesis indicate that the genetic improvement of complex traits like

those related to the quality of meat, which are difficult to select with traditional methodologies, could take advantage from the application of new technologies, such as visible near-infrared sprectroscopy and genomics.

REFERENCES

Aass, L. 1996. Variation in carcass and meat quality traits and their relations to growth in dual purpose cattle. *Livestock Production Science*, 46:1-12.

Adzitey, F. 2011. Effect of pre-slaughter animal handling on carcass and meat quality. *International Food Research Journal*, 18:485-491.

Albera, A. 2015. Relazione tecnica sul programma di selezione della razza Piemontese. Carrù (CN): ANABoRaPi. www.anaborapi.it.

Albera, A., P. Carnier, and A.F. Groen. 2004a. Definition of a breeding goal for the Piemontese breed: economic and biological values and their sensitivity to production circumstances. *Livestock Production Science*, 89:66-77.

Albera, A, A.F. Groen, and P. Carnier. 2004b. Genetic relationships between calving performance and beef production traits in Piemontese cattle. *Journal of Animal Science*, 82:3440-3446.

Albera, A., R. Mantovani, G. Bittante, A.F. Groen and P. Carnier. 2001. Genetic parameters for daily live-weight gain, live fleshiness and bone thinness in station-tested Piemontese young bulls. *Journal of Animal Science*, 72: 449-456.

Alomar, D., C. Gallo, M. Castaneda, R. Fuchslocher. 2003. Chemical and discriminant analysis of bovine meat by near infrared reflectance spectroscopy (NIRS). *Meat Science*, 63: 441–450.

A.M.S.A. 2001. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. Champain, Illinois: American Meat Science Association.

A.M.S.A. 2015. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. Champain, Illinois: American Meat Science Association.

Amin, N., C.M. van Duijn, and Y.S. Aulchenko. 2007. A Genomic Background Based Method for Association Analysis in Related Individuals. P. Heutink, editor. *PLoS One*. 2:e1274.

ANABORAPI 2017. Relazione tecnica e Statistiche [Technical report and statistics]. Carrù (IT): Anaborapi. Available from: www.anaborapi.it.

Andersen, B.B., A. De Baerdemaeker, G. Bittante, B. Bonaiti, J.J. Colleau, E. Fimland, J. Jansen, W.H.E. Lewis, R.D. Politiek, G.Seeland, T.J. Teehan and F. Werkmeister. 1981. Performance testing of bulls in AI: report of a working group of the commission on cattle production. *Livestock Production Science,* 8:101-119.

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.

Arthur, P.F. 1995. Double muscling in cattle: A review. *Australian Journal of Agricultural Research*, 46:1493–1515.

Asem-Hiablie, S., C.A. Rotz, J.D. Sandlin, M.R. Sandlin, and R.C. Stout. 2017. Management characteristics of beef cattle production in Hawaii. *The Professional Animal Scientist*, 34:167–176.

Asem-Hiablie, S., C.A. Rotz, R.C. Stout, and K. Fisher. 2017. Management characteristics of beef cattle production in the western United States. *The Professional Animal Scientist*, 33:461–471.

Avilés, C., A.L. Martínez, V. Domenech, and F. Peña. 2015. Effect of feeding system and breed on growth performance, and carcass and meat quality traits in two continental beef breeds. *Meat Science,* 107:94-103.

Barendse, W. 2011. Haplotype Analysis Improved Evidence for Candidate Genes for Intramuscular Fat Percentage from a Genome Wide Association Study of Cattle. M. R. Schneider, editor. *PLoS One*. 6:e29601.

Barge, M.T., A. Brugiapaglia, G. Destefanis, and P. Mazzocco. 1993. The influence of muscle type, ethnic group, muscular hypertrophy on the composition of beef meat. Proceedings of the 39nd international congress of meat science and technology (File S5P02.WP); August 1-6; Calgary, Canada.

Bellinge, R.H.S., D.A. Liberles, S.P.A. Iaschi, P.A. O'Brien, and G.K. Tay. 2005. Myostatin and its implications on animal breeding: a review. *Animal Genetics*, 36:1-6.

Bergamaschi, M., A. Cecchinato and G. Bittante. 2018. Milk and cheese authentication using FTIR, NIR spectra, fatty acid, and volatile organic compounds. 69th Annual Meeting of the European Federation of Animal Science. Dubrovnik. Croatia. 27-31 August 2018.

Bindea, G., B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.H. Fridman, F. Pagès, Z. Trajanoski, and J. Galon. 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*, 25:1091–1093.

Bittante, G., A. Cecchinato, R. Dal Zotto, M. De Marchi, and M. Penasa. 2011. Adjusting for age can lead to biased genetic evaluation for body weight in cattle. *Livestock Production Science,* 140:1-7.

Bittante, G., A. Cecchinato, F. Tagliapietra, R. Verdiglione, A. Simonetto, and S. Schiavon. 2018. Crossbred young bulls and heifers sired by double-muscled Piemontese or Belgian Blue bulls exhibit different effects of sexual dimorphism on fattening performance and muscularity but not on meat quality traits. *Meat Science*, 137:24-33.

Bittante, G., and C. Cipolat-Gotet. 2018. Direct and indirect predictions of enteric methane daily production, yield, and intensity per unit of milk and cheese, from fatty acids and milk Fourier-transform infrared spectra. *Journal of Dairy Science*, 101:7219-7235.

Bittante, G., A. Ferragina, C. Cipolat-Gotet, and A. Cecchinato. 2014. Comparison between genetic parameters of cheese yield and nutrient recovery or whey loss traits measured from individual model cheese-making methods or predicted from unprocessed bovine milk samples using Fourier-transform infrared spectroscopy. *Journal of Dairy Science*, 97:6560-72.

Boccard, R., L. Buchter, E. Casteels, E. Cosentino, E. Dransfield, D.E. Hood, R.L. Joseph, D.B. MacDougall, D.N. Rhodes, L. Schon, B.J. Tinbergen, and K. Touraille. 1981. Procedures for measuring meat quality characteristics in beef production experiments, report of a working group in the Commission of the European Communities (CEC) beef production research programme. *Livestock Production Science*, 8:385-397.

Boddhireddy, P., M.J. Kelly, S. Northcutt, K.C. Prayaga, J. Rumph, and S. DeNise. 2014. Genomic predictions in Angus cattle: comparisons of sample size, response variables, and clustering methods for cross-validation. *Journal of Animal Science*, 92:485–497.

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.

Bonfatti, V., A. Albera, and P .Carnier. 2013. Genetic associations between daily BW gain and live fleshiness of station-tested young bulls and carcass and meat quality traits of commercial

intact males in Piemontese cattle. *Journal of Animal Science*, 91:2057-2066.

Bonfatti, V., D. Vicario, A. Lugo, and P. Carnier. 2017. Genetic parameters of measures and population-wide infrared predictions of 92 traits describing the fine composition and technological properties of milk in Italian Simmental cattle. *Journal of Dairy Science,* 100:5526-40.

Bongiorni, S., G. Mancini, G. Chillemi, L. Pariset, and A. Valentini. 2012. Identification of a Short Region on Chromosome 6 Affecting Direct Calving Ease in Piedmontese Cattle Breed. *PLoS One*. 7:6–12.

Bonny, S.P.F., R.A. O'Reilly, D.W. Pethick, G.E. Gardner, J.F. Hocquette, and L. Pannier. 2018. Update of Meat Standards Australia and the cuts based grading scheme for beef and sheepmeat. *Journal of Integrative Agriculture*, 17, 1641–1654.

Boucqué, Ch.V., L.O. Fiems, B.G. Cottyn, and F.X. Buysse. 1984. Besoin en protéines de taureaux culards au cours de la période de finition [Protein needs of hypertrophic bulls during finishing period]. *Revue de Agriculture*, 37:661–670.

Boukha, A., V. Bonfatti, A. Cecchinato, A. Albera, L. Gallo, P. Carnier, and G. Bittante. 2011. Genetic parameters of carcass and meat quality traits of double muscled Piemontese cattle. *Meat Science,* 89:84-90.

Bovenhuis, H., S. van Engelen, and M.H.P.W. Visker. 2018. Letter to the Editor: A response to Huhtanen and Hristov. 2018. *Journal of Dairy Science,* 101:9621–9622.

Brad Kim, Y.H., H.A. Channon, D.N. D'Souza, and D. Hopkins. 2016. Production of high-quality meat. in Przybylski, W., and D. Hopkins (eds, 2016). Meat quality genetic and environmental factors, CRC Press, Taylor & Francis Group. Boca Raton, Florida, USA.

Brugiapaglia, A., and G. Destefanis. 2012. Influence of the housing system on meat quality of double muscled Piemontese young bulls. *Livestock Science*, 147:73-78.

Brugiapaglia A., C. Lussiana, and G. Destefanis. 2014. Fatty acid profile and cholesterol content of beef at retail of Piemontese, Limousin and Friesian breeds. *Meat Science,* 96:568-573.

Burrow, H.M., S.S. Moore, D.J. Johnston, W. Barendse, and B.M. Bindon. 2001. Quantitative and molecular genetic influences on properties of beef: A review. *Australian Journal of Experimental Agriculture,* 41:893–919.

Burton, P. R., et al. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature,* 447:661–678.

Carnier, P., A. Albera, R. Dal Zotto, A.F. Groen, M. Bona, and G. Bittante. 2000. Genetic parameters for direct and maternal calving ability over parities in Piedmontese cattle. *Journal of Animal Science,* 78:2532–2539.

Casas E., J.W. Keele, S.D. Shackelford, M. Koohmaraje, T.S. Sonstegard, T.P.L. Smith, S.M. Kappes, and R.T. Stone. 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. *Journal of Animal Science,* 76:468-473.

Casas, E., S.N. White, T.L. Wheeler, S.D. Shackelford, M. Koohmaraie, D.G. Riley, C.C. Chase, D.D. Johnson, and T.P.L. Smith. 2006. Effects of calpastatin and micro-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science,* 84:520–525.

Cavalcante, D.H., JE.G. Campelo, N.P.S. Santos, R.R. Ferreira, W.J.L. Fonseca, and A.F. Evangelista. 2018. Characterisation of beef cattle farming and the perspective for implementation of traceability. *Acta Scientiarium*, *Animal Science* [online], (40), e36282.

Cecchinato, A., M. De Marchi, L. Gallo, G. Bittante, and P. Carnier. 2009. Mid-infrared

spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. *Journal of Dairy Science,* 92:5304-13.

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.

Cecchinato, A., M. De Marchi, M. Penasa, J. Casellas, S. Schiavon, and G. Bittante. 2012. Genetic analysis of beef fatty acid composition predicted by near-infrared spectroscopy. *Journal of Animal Science,* 90:429-438.

Cerdeño, A., C. Vieira, E. Serrano, P. Lavín, and A.R. Mantecón. 2006. Effects of feeding strategy during a short finishing period on performance, carcass and meat quality in previously-grazed young bulls. *Meat Science,* 72:719-726.

Chambaz, A., M. Kreuzer, M.R.L. Scheeder, and P.A. Dufey. 2011. Characteristics of steers of six beef breeds fattened from eight months of age and slaughtered at a target level of intramuscular fat: II, Meat quality. *Archiv für Tierzucht (Archives Animal Breeding),* 44:473–488.

Chen L., M. Vinsky and C. Li. 2015. Accuracy of predicting genomic breeding values for carcass merit traits in Angus and Charolais beef cattle. *Animal Genetics,* 46:55–59.

Chiaia H., E. Peripoli, R. Silva, C. Aboujaoude et al. 2017. Genomic prediction for beef fatty acid profile in Nellore cattle. *Meat Science,* 128:60-67.

Chillemi, G., S. Bongiorni, S. Gioiosa, T. Flati, T. Castrignanò, M. Milanesi, P. Ajmone Marsan, and A. Valentini. 2018. Missense mutations of NCPAG gene affect calving ease in Piedmontese cattle: preliminary evidences. *Italian Journal of Animal Science,* 17:301–305.

CIE. 1976. Commission Internationale de l'Éclairage. Recommendations on uniform colour spaces-colour difference equations, psychometric colour terms. Supplement no. 2 to CIE publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1.3.) Commission Internationale de l'Éclairage Paris, France.

Commission of the European Communities. 1982. Commission of the European Communities (Beef Carcass Classification) Regulations. Council Regulations 1358/80, 1208/81, 1202/82. Commission Regulations 2930/81, 563/82, 1557/82. Brussels: Commission of the European Communities.

Coster, A. 2015. Pedigree: Package to deal with pedigree data. R package version 1.4. http://CRAN.R-project.org/packages=pedigree.

Cozzi, G., A. Mazzenga, B. Contiero, and G. Burato. 2008. Use of maize silage in beef cattle feeding during the finishing period. *Italian Journal of Animal Science,* 7:39-52.

Craigie, C., D. Ross, C. Maltin, R. Purchas, S. Morris, and R. Roehe. 2010. The relationship between beef quality and carcass quality attributes measured under commercial conditions. Conference: British Society of Animal Science, Queens University Belfast.

Crews, D.H., M. Lowerison, N. Caron, and R.A. Kemp. 2004. Genetic parameters among growth and carcass traits of Canadian Charolais cattle. *Canadian Journal of Animal Science,* 84:589–597.

Dannenberger, D., K. Nuernberg, G. Nuernberg, and K. Ender. 2006. Carcass and meat quality of pasture *vs* concentrate fed German Simmental and German Holstein bulls. *Archiv für Tierzucht (Archives Animal Breeding),* 49:315-328.

Daza, A., A.I. Rey, C. Lopez-Carrasco, and C.J. Lopez-Bote. 2014. Influence of feeding system on

growth performance, carcass characteristics and meat and fat quality of Avileña-Negra Ibérica calves' breed. *Spanish Journal of Agricultural Research*, 12:409-418.

De Campeneere, S., L.O. Fiems, B.G. Cottyn, and C.V. Boucqué. 1999. Phase feeding to optimize performance and quality of Belgian Blue double-muscled bulls. J*ournal of Animal Science,* 69:275–285.

De Marchi, M. 2013. On-line prediction of beef quality traits using near infrared spectroscopy. *Meat Science*, 94:455-460.

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.

Destefanis G., and M.T. Barge. 1988. Valutazione sensoriale della qualità della carne in vitelloni appartenenti a diversi tipi genetici. *Zootecnica e Nutrizione Animale*, 15:407-416.

Destefanis, G., M.T. Barge, and A. Brugiapaglia. 1994. pH, colour and water holding capacity in muscles of young bulls differing for ethnic group. Proceedings of the 40th international congress of meat science and technology; August 28–September 2; The Hague, Netherlands.

Destefanis, G., Barge, M.T., Brugiapaglia, A., & Tassone, S. 2000. The use of principal component analysis (PCA) to characterize beef. *Meat Science*, 56:255-259.

Destefanis, G., A. Brugiapaglia, M.T. Barge, and C. Lazzaroni. 2003. Effect of castration on meat quality in Piemontese cattle. *Meat Science*, 64:215-218.

Destefanis, G., L. DiStasio, A. Brugiapaglia, S. Barbera, S. Tassone, M. Bassanino, G. Franco, and A. Bosticco. 2005. Patrimonio zootecnico del Piemonte, la razza bovina Piemontese [Heritage livestock of Piemonte, Piemontese breed]. Torino (IT): agricoltura&ricerca.

Chapter 1, La razza bovina Piemontese, la filiera produttiva [Piemontese breed, the production chain]; p:19-30.

Do, C., B. Park, S. Kim, T. Choi, B. Yang, S. Park, and H. Song. 2016. Genetic parameters estimates of carcass traits under national scale breeding scheme for beef cattle. *Asian-Australasian Journal of Animal Sciences*, 29:1083-1094.

Dunne, P.G., F.P O'Mara, F.J. Monahan,P. French, and A.P. Moloney. 2005. Colour of muscle from 18-month-old steers given long term daily exercise. *Meat Science,* 71:212-229.

Durinck, S., Y. Moreau, A. Kasprzyk, S. Davis, B. De Moor, A. Brazma, and W. Huber. 2005. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. *Bioinformatics,* 21:3439–40.

Durinck, S., P.T. Spellman, E. Birney, and W. Huber. 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nature Protocols*, 4:1184–1191.

Eberlein, A., A. Takasuga, K. Setoguchi, R. Pfuhl, K. Flisikowski, R. Fries, N. Klopp, R. Furbass, R. Weikard, and C. Kuhn. 2009. Dissection of Genetic Factors Modulating Fetal Growth in Cattle Indicates a Substantial Role of the Non-SMC Condensin I Complex, Subunit G (NCAPG) Gene. *Genetics,* 183:951–964.

Eichinger, H., and G. Beck. 1992. Possibilities for improving breeding value estimation of meat quality in cattle by using the near-infrared measurement technique. *Archiv für Tierzucht (Archives Animal Breeding)*, 35:41–50.

Ellies-Oury, M.P., R. Dumont, G. Perrier, M. Roux, D. Micol, and B. Picard. 2017. Effect of age and carcass weight on quality traits of m. rectus abdominis from Charolais heifers. *Animal*,

11:720–727.

ElMasry, G., D.W. Sun, and P. Allen. 2012. Near-infrared hyperspectral imaging for predicting colour, pH and tenderness of fresh beef. *Journal of Food Engineering,* 110:127-140.

Eskildsen, C E., M.A. Rasmussen, S.B. Engelsen, L.B. Larsen, N.A. Poulsen, and T. Skov. 2014. Quantification of individual fatty acids in bovine milk by infrared spectroscopy and chemometrics: Understanding predictions of highly collinear reference variables. *Journal of Dairy Science,* 97:7940-51.

European Commission, Directorate-General for Agriculture and Rural Development. 2011. EU beef farms report 2010 based on FADN data.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Longman, Harlow, England.

Fan, H., Y. Wu, X. Zhou, J. Xia, W. Zhang, Y. Song, F. Liu, Y. Chen, L. Zhang, X. Gao, H. Gao, and J. Li. 2015. Pathway-Based Genome-Wide Association Studies for Two Meat Production Traits in Simmental Cattle. *Scientific Reports*, 5:18389.

Farmer, L.J., and D.T. Farrell. 2018. Review: Beef-eating quality: a European journey. *Animal,* 12:2424-2433.

Fernandes Júnior, G.A., G.J.M. Rosa, B.D. Valente, R. Carvalheiro, F. Baldi, D.A. Garcia, D.G.M. Gordo, R. Espigolan, L. Takada, R.L. Tonussi, et al. 2016. Genomic prediction of breeding values for carcass traits in Nellore cattle. *Genetic Selection Evolution*, 48:1–8.

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.

Fiems, L.O. 2012. Double Muscling in Cattle: Genes, Husbandry, Carcasses and Meat. *Animal*, 2:472-506.

Fiems, L.O., S. De Campeneere, W. Van Caelenbergh, J.L. De Boever, and J.M. Vanacker. 2003. Carcass and meat quality in double-muscled Belgian Blue bulls and cows. *Meat Science*, 63:345–352.

Fonseca, L.F.S., D.F.J. Gimenez, D.B. Dos Santos Silva, R. Barthelson, F. Baldi, J.A. Ferro, and L.G. Albuquerque. 2017. Differences in global gene expression in muscle tissue of Nellore cattle with divergent meat tenderness. *BMC Genomics*, 18:945.

Forni, S., I. Aguilar and I. Misztal. 2011. Different genomic relationship matrices for single-step analysis using phenotypic, pedigree and genomic informations. *Genetic Selection Evolution*, 43:1.

French, P., E.G. O'Riordan, F.J. Monahan, P.J. Caffrey, M.T. Mooney, D.J. Troy, and A.P. Moloney. 2001. The eating quality of meat from steers fed grass and/or concentrate. *Meat Science*, 57:379-386.

Gagaoua, M., V. Monteils, S. Couvreur, and B. Picard. 2017. Identification of Biomarkers Associated with the Rearing Practices, Carcass Characteristics, and Beef Quality: An Integrative Approach. *Journal of Agricultural and Food Chemistry*, 65):8264−8278.

Gagaoua, M., B. Picard, J. Soulat, and V. Monteils. 2018. Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. *Livestock Science*, 214:245–258.

Gao Y., R. Zhang, X. Hu, and N. Li. 2007. Application of genomic technologies to the improvement

of meat quality of farm animals. *Meat Science,* 77:36–45.

GenABEL project developers (2013). GenABEL: genome-wide SNP association analysis. R package version 1.8-0.https://cran.r-project.org/web/packages/GenABEL/index.html. Available from: https://cran.r-project.org/web/packages/RepeatABEL/citation.html

Gerardo, A., J. Fernandes, J.M. Guilherme, B.D. Valente, R. Carvalheiro, F. Baldi, D.A. Garcia, et al. 2016. Genomic prediction of breeding values for carcass traits in Nellore cattle. *Genetics Selection Evolution,* 48:7.

Gill, J.L., O. Matika, J.L. Williams, H. Worton, P. Wiener, and S.C. Bishop. 2010. Consistency statistics and genetic parameters for taste panel assessed meat quality traits and their relationship with carcass quality traits in a commercial population of Angus-sired beef cattle. *Animal,* 4:1–8.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. *Biometrics,* 27:857-874.

Granato, I. and R. Fristche-Neto. 2018. snpReady: Preparing Genomic Dataset in Order to Run Genomic Analysis. R package version 0.9.6 http://CRAN.R-project.org/packages=snpReady

Grobet, L., D. Poncelet, L.J. Royo, B. Brouwers, D. Pirottin, C. Michaux, F. Menissier, M. Zanotti, S. Dunner, and M. Georges. 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mammalian Genome,* 9:210-213.

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

Gronewald, K.W., S.C. Seideman, and W.D. Lamm. 1990. Composition and quality of beef from

steers sired by Piedmontese, Gelbvieh and Red Angus bulls. *Journal of Animal Science,* 68:1049-1060.

Grunert K.G., L. Bredahl, and K. Brunsø. 2004. Consumer perception of meat quality and implications for product development in the meat sector, a review. *Meat Science,* 66:259–272

Guerrero, A., C. Sañudo, P. Albertí, G. Ripoll, M.M. Campo, J.L. Olleta, B. Panea, S. Khliji, and P. Santolaria. 2013. Effect of production system before the finishing period on carcass, meat and fat qualities of beef. *Animal,* 7:2063-2072.

Hayes, B.J., P.J. Bowman, A.C. Chamberlain, and M.E. Goddard. 2009. Genomic selection in dairy cattle: Progress and challenges. *Journal of Dairy Science,* 92:433–443.

Hayes, B.J. and M.E. Goddard. 2014. Technical note: Prediction of breeding values using marker-derived relationship matrices. *Journal of Animal Science,* 86:2089–2091.

Habier, D., R.L. Fernando, K. Kizilkaya and D.J. Garrick. 2011. Extension of the bayesian alphabet for genomic selection. *BMC Bioinformatics,* 12:186.

Hocquette, J.F., D. Bauchart, D. Micol, R.Polkinghorne, and B. Picard. 2016 Beef quality. In Przybylski, W., and D. Hopkins (editors). Meat quality genetic and environmental factors, CRC Press, Taylor & Francis Group. Boca Raton, Florida, USA.

Honikel, K.O. 1998. Reference methods for assessment of physical characteristics of meat. *Meat Science,* 49:447-457.

Hornyak, Z., J.J. Frickh, and B. Furst-Waltl. 2008. Fleischqualitätsmerkmale beim Pinzgauer Rind. *Zuchtungskunde,* 80:175-185.

Hoshiba, H., K. Setoguchi, T. Watanabe, A. Kinoshita, K. Mizoshita, Y. Sugimoto, and A. Takasuga.

2013. Comparison of the effects explained by variations in the bovine *PLAG1* and *NCAPG* genes on daily body weight gain, linear skeletal measurements and carcass traits in Japanese Black steers from a progeny testing program. *Journal of Animal Science*, 84:529–534.

Jackman, P., W.W. Sun, and P. Allen. 2011. Recent advances in the use of computer vision technology in the quality assessment of fresh meats. *Trends in Food Science & Technology*, 22:185-197.

Jeong, J.Y., J.S. Kim, T.H. Nguyen, H.J. Lee, and M. Baik. 2013. Wnt/β-catenin signaling and adipogenic genes are associated with intramuscular fat content in the longissimus dorsi muscle of Korean cattle. *Animal Genetics*, 44:627–635. doi:10.1111/age.12061.

Johnson, D.D., H.H. Van Horn, R.L. West, and B.J.R. Harris. 1992. Effect of calf management on carcass characteristics and palability traits of Veal Calves. *Journal of Dairy Science*, 75:2799-2804.

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.

Johnston, D.J., A. Reverter, D.L. Robinson, and D.M. Ferguson. 2001. Sources of variation in mechanical shear force measures of tenderness in beef from tropically adapted genotypes, effects of data editing and their implication for genetic parameter estimation. *Australian Journal of Experimental Agriculture*, 41:991-996.

Joo, S.T., G.D. Kim, Y.H. Hwang, and Y.C. Ryu. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science*, 95:828-836.

Juniper, D.T., E.M. Browne, A.V. Fisher, M.J. Bryant, G.R. Nute, and D.E. Beever. 2005. Intake, growth and meat quality of steers given diets based on varying proportions of maize silage and grass silage. *Animal Science*, 81:159-170.

Juriel, C., I. Ortigues-Marty, B. Picard, D. Micol, and J.F. Hocquette. 2006. The separate effects of the nature of diet and grazing mobility on metabolic potential of muscles from Charolais steers. *Livestock Science*, 104:182–192.

Karoui, R., G. Downey, and C. Blecker. 2010. Mid-infrared spectroscopy coupled with chemometrics: a tool for the analysis of intact food systems and the exploration of the molecular structure – quality relationships – a review. *Chemical Reviews*, 110:6144-6168.

Keller, M.C., P.M. Visscher, and M.E. Goddard. 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics*, 189:237-249.

King, J.W.B. and F. Menissier. 1982. Muscle hypertrophy of genetic origin and its use to improve beef production. Current Topics in Veterinary Medicine and Animal Science. Vol 16. Martinus Nijhoff Publishers.

King, D.A., S.D. Shackelford, N. Kalchayanand, and T.L. Wheeler. 2012. Sampling and aging effects on beef longissimus color stability measurements. *Journal of Animal Science*, 90:3596–3605. doi:10.2527/jas.2011-4871.

Kizilkaya, K., P. Carnier, A. Albera, G. Bittante, and R.J. Tempelman. 2003. Cumulative t-link threshold models for the genetic analysis of calving ease scores. *Genetics Selection Evolution*, 35:489-512.

Kluska, S., B. Ferreira Olivieri, M. Bonamy, H.L. Justino Chiaia, F.L. Braga Feitosa, M. Piatto

Berton, E. Peripolli, M.Vi. Antunes Lemos, R. Lara Tonussi, R. Barbosa Lôbo, C. de Ulhoa Magnabosco, F. Di Croce, J. Osterstock, A.S. Cravo Pereira, D. Prado Munari, L.A. Bezerra, F. Brito Lopes, and F. Baldi. 2018. Estimates of genetic parameters for growth, reproductive, and carcass traits in Nelore cattle using the single step genomic BLUP procedure. *Livestock Science*, 216:203-209.

Koohmaraie, M., J. Killefer, M.D. Bishop, S.D. Shackelford, T.L. Wheeler, and J.R. Arbona. 1995. Calpastatin-based method for predicting meat tenderness. In A. Ouali, D. Demeyer, & F. Smulders (Eds.), Expression of tissue proteinases and regulation of protein degradaion as related to meat quality (pp. 395–410). Utrecht, The Netherlands: ECCEAMST.

Küchenmeister, U., G. Kuhn, and K. Ender. 2000. Seasonal effects on Ca(2+) transport of sarcoplasmic reticulum and on meat quality of pigs with different malignant hyperthermia status. *Meat Science*, 55:239-45.

Lee, S.H., S. Clark, and J. Van Der Werf. 2017. Estimation Of Genomic Prediction Accuracy From Reference Populations With Varying Degrees Of Relationship. *Plos One*, 12:e0189775.

Lee, D.J., J.H. Hwang, J. Ha, G.E. Yu, and S. Kwon. 2018. Identification of a Bromodomain-containing Protein 2 (BRD2) Gene Polymorphic Variant and Its Effects on Pork Quality Traits in Berkshire Pigs. *Korean Journal for Food Science of Animal Resources*, 38:703–710.

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

Legarra A., C. Robert-Granié, P. Croiseau, F. Guillaume, and S. Fritz. 2011. Improved Lasso for genomic selection. *Genetics Research (Cambridge Core)*, 93:77–87.

Leiserson M.D.M., J.V. Eldridge, S. Ramachandran, and B.J. Raphael. 2013. Network analysis of GWAS data. *Current Opinion in Genetics & Development*, 23:6.

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.

Lin, C.Y., and C.S. Lin. 1994. Investigation of genotype-environment interaction by cluster analysis in animal experiments. *Canadian Journal of Animal Science*, 74:607-612.

Lisa, C., A. Albera, P. Carnier, and L. Di Stasio. 2013. Variability in Candidate Genes Revealed Associations with Meat Traits in the Piemontese Cattle Breed. *Italian Journal of Animal Science,* 12:46.

Liu, Z., M.E. Goddard, B. Hayes, F. Reinhardt, and R. Reents. 2016. Technical note: Equivalent genomic models with a residual polygenic effect. *Journal of Dairy Science*, 99:2016-2025.

Lo, A., H. Chernoff, T. Zheng, and S.H. Lo. 2015. Why significant variables aren't automatically good predictors. *Proceedings of the National Academy of Sciences (PNAS)*, 45:13892-13897

Luan, T., J.A. Wolliams, S. Lien, M. Kent, M. Svendsen, and T.H.E. Meuwissen. 2009. The Accuracy of Genomic Selection in Norwegian Red Cattle Assessed by Cross-Validation. *Genetics*, 183:1119–1126.

MacDougall, D.B. 1982. Changes in the colour and opacity of meat. *Food Chemistry*, 9:75–88.

Magalhaes, A.F.B., et al. 2018. Prediction of meat quality traits in Nelore cattle by near-infrared reflectance spectroscopy. *Journal of Animal Science,* 96:4229-37

Mantovani, R., M. Cassandro, B. Contiero, A. Albera, and G. Bittante. 2010. Genetic evaluation of type traits in hypertrophic Piemontese cows. *Journal of Animal Science,* 88:3504-3512.

Marshall, D.M. 1999. Genetics of meat quality. In Fries, R. and Ruvinsky, A. (eds.), The Genetics of Cattle. CAB International, Wallingford, Oxfordshire, UK, pp. 605–636.

McIlveen, H., and J. Buchanan, J. 2001. The impact of sensory factors on beef purchase and consumption. *Nutrition and Food Science,* 31:286–292.

Mehrban, H., D.H. Lee, M.H. Moradi, C.I. Cho, M. Naserkheil, and N. Ibáñez-Escriche. 2017. Predictive performance of genomic selection methods for carcass traits in Hanwoo beef cattle: impacts of the genetic architecture. *Genetics Selection Evolution*, 49:1.

Men, X., B. Deng, X. Tao, K. ke Qi, and Z. wei Xu. 2017. Wnt gene expression in adult porcine longissimus dorsi and its association with muscle fiber type, energy metabolism, and meat quality. *Journal of Integrative Agriculture,* 16:144–150.

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

Miller, S., D. Lu, G. Vander Voort, and I. Mandell. (2014). Genomic Prediction of Beef Tenderness in Canadian Beef Cattle. Proceedings, 10th World Congress of Genetics Applied to Livestock Production. Vancouver, British Colombia, Canada - 17 - 22 August 2014.

Minick, J.A., M.E. Dikeman, E.J. Pollak, and D.E. Wilson. 2004. Heritability and correlation estimates of Warner-Bratzler shear force and carcass traits from Angus-, Charolais-, Hereford-, and Simmental-sired cattle. *Canadian Journal of Animal Science*, 84:599–609.

Moon S.S., P.N. Seong, and J.Y. Jeong. 2015. Evaluation of Meat Color and Physiochemical Characteristics in Forequarter Muscles of Holstein Steers. *Korean Journal for Food Science of Animal Resources,* 35:646–652.

Moser, D.W., J.K. Bertrand, I. Misztal, L.A. Kriese, and L.L. Benyshek. 1998. Genetic parameter

estimates for carcass and yearling ultrasound measurements in Brangus cattle. *Journal of Animal Science*, 69:2542-2548.

Mudadu, M.A., et al. 2016. Genomic structure and marker-derived gene networks for growth and meat quality traits of Brazilian Nelore beef cattle. *BMC Genomics*, 17:1–16.

Mullen A.M., P.C. Stapleton, D. Corcoran, R.M. Hamill, and A. White. 2006. Understanding meat quality through the application of genomic and proteomic approaches. *Meat Science*, 74:3–16.

Murray, A.C. 1989. Factors affecting beef color at time of grading. *Canadian Journal of Animal Science*, 69:347-355.

Nair, M.N., S. Li, C.M. Beach, G. Rentfrow, and S.P. Suman. 2018. Changes in the Sarcoplasmic Proteome of Beef Muscles with Differential Color Stability during Postmortem Aging. *Meat and Muscle Biology*, 2:1.

Nishimura, S., T. Watanabe, K. Mizoshita, K. Tatsuda, T. Fujita, N. Watanabe, Y. Sugimoto, and A. Takasuga. 2012. Genome-wide association study identified three major QTL for carcass weight including the PLAG1-CHCHD7 QTN for stature in Japanese Black cattle. *BMC Genetics*, 13:40.

Ogawa, S., H. Matsuda, Y. Taniguchi, T. Watanabe, Y. Kitamura, I. Tabuchi, Y. Sugimoto and H. Iwaisaki. 2016. Genomic prediction for carcass traits in Japanese Black cattle using single nucleotide polymorphism markers of different densities. *Animal Production Science*, 57:1631–1636.

Ogawa, S., H. Matsuda, Y. Taniguchi, T. Watanabe, S. Nishimura, Y. Sugimoto, and H. Iwaisaki. 2014. Effects of single nucleotide polymorphism marker density on degree of genetic

variance explained and genomic evaluation for carcass traits in Japanese Black beef cattle. *BMC Genetics,* 15:15.

Organisation for Economic Co-Operation and Development 2017. Meat consumption (indicator).

Page, B.T., E. Casas, R.L. Quaas, R.M. Thallman, T.L. Wheeler, S.D. Shackelford, M. Koohmaraie, S.N. White, G.L. Bennett, J.W. Keele, M.E. Dikeman, and T.P.L. Smith. 2004. Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. *Journal of Animal Science,* 82:3474–3481.

Page, J.K., D.M. Wulf, and T.R. Schwotzer. 2001. A survey of beef muscle color and pH. J*ournal of Animal Science,* 79:678-687.

Pegolo, S., A. Cecchinato, S. Savoia, A. Albera, A. Brugiapaglia, L. Di Stasio, and G. Bittante. 2018. Genome-wide Association and Pathway Analysis of Carcass and Meat Quality Traits in Piemontese young bulls. *Manuscript in preparation*.

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

Pickrell, J.K., J.C. Marioni, A.A. Pai, J.F. Degner, B.E. Engelhardt, E. Nkadori, J.B. Veyrieras, M. Stephens, Y. Gilad, and J.K. Pritchard. 2010. Understanding mechanisms underlying human gene expression variation with RNA sequencing. *Nature*, 464:768–72.

Pimenetel E.C.G., and S. Konig. 2012. Genomic selection for the improvement of meat quality. *Journal of Animal Science,* 90:3418-3426.

Polkinghorne, R.J., and J.M. Thompson. 2010. Meat standards and grading A world view. *Meat Science,* 86:227–235.

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.

Prieto, N., D.W. Ross, E.A. Navajas, G.R. Nute, R.I. Richardson, J.J. Hyslop, G. Simm, and R. Roehe. 2009. On-line application of visible and near infrared reflectance spectroscopy to predict chemical–physical and sensory characteristics of beef quality. *Meat Science*, 83:96-103.

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.

Pryce, J.E., B.J. Hayes, S. Bolormaa, and M.E. Goddard. 2011. Polymorphic regions affecting human height also control stature in cattle. *Genetics*, 187:981–4.

Przybylski, W., and D. Hopkins. 2016. Meat quality genetic and environmental factors, CRC Press, Taylor & Francis Group. Boca Raton, Florida, USA.

Pszczola, M., T. Strabel, H.A. Mulder, and M.P. Calus. 2012. Reliability of direct genomic values for animals with different relationships within and to the reference population. *Journal of Dairy Science*, 95:389-400.

Qiao, T., J. Ren, C. Craie, J. Zabalza, C. Maltin, and S. Marshall. 2015. Quantitative prediction of beef quality using visible and NIR spectroscopy with large data samples. *Journal of Applied*

Spectroscopy, 82.

Ramayo-Caldas, Y., G. Renand, M. Ballester, R. Saintilan, and D. Rocha. 2016. Multi-breed and multi-trait co-association analysis of meat tenderness and other meat quality traits in three French beef cattle breeds. *Genetics Selection Evolution*, 48:37.

Renand, G. 1985. Genetic parameters of french beef breeds used in crossbreeding for young bull production. II – Slaughter performance. *Genetics Selection Evolution*, 17:265-282.

Ribeca, C., V. Bonfatti, A. Cecchinato, A. Albera, L. Gallo, P. and Carnier. 2014. Effect of polymorphisms in candidate genes on carcass and meat quality traits in double muscled Piemontese cattle. Meat Science, 96:1376-83.

Ribeca, C., V. Bonfatti, A. Cecchinato, A. Albera, F. Maretto, L. Gallo, and P. Carnier. 2013. Association of polymorphisms in calpain 1, (mu/I) large subunit, calpastatin, and cathepsin D genes with meat quality traits in double-muscled Piemontese cattle. *Animal Genetics*, 44:193-6.

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.

Rolf, M.M., D.J. Garrick, T. Fountain, H.R. Ramey, R.L. Weaber, J.E. Decker, E.J. Pollak, R.D. Schnabel, and J.F. Taylor. 2015. Comparison of Bayesian models to estimate direct genomic values in multi-breed commercial beef cattle. *Genetic Selection Evolution*, 47:23–30.

Rousseeuw, P.J. 1987. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics, 20:53–65.

Rutten, M.J.M., H. Bovenhuis, J.M.L. Heck, and J.A.M. van Arendonk. 2011. Predicting bovine

milk protein composition based on Fourier transform infrared spectra. *Journal of Dairy Science*, 94:5683–90.

Rutten, M.J.M., H. Bovenhuis, and J.A.M. Van Arendonk. 2010. The effect of the number of observations used for Fourier transform infrared model calibration for bovine milk fat composition on the estimated genetic parameters of the predicted data. *Journal of Dairy Science*, 93:4872–82.

Saatchi, M., M.C. McClure, S.D. McKay, M.M. Rolf, J. Kim, J.E. Decker, T.M. Taxis, R.H. Chapple, H.R. Ramey, S.L. Northcutt, S. Bauck, B. Woodward, J.C.M. Dekkers, R.L. Fernando, R.D. Schnabel, D.J. Garrick, and J.F. Taylor. 2011. Accuracies of genomic breeding values in American Angus beef cattle using K-means clustering for cross-validation. *Genetics Selection Evolution*, 43:40.

Saatchi, M., R.D. Schnabel, M.M. Rolf, J.F. Taylor, and D.J. Garrick. 2012. Accuracy of direct genomic breeding values for nationally evaluated traits in US Limousin and Simmental beef cattle. *Genetics Selection Evolution*, 44:38.

SAS. 2013. Statistical Analysis System Proprietary Software. Release 9.4. SAS Institute Inc., Cary, NC, USA.

Sañudo, C., E.S. Macie, J.L. Olleta, M. Villarroel, B. Panca, and P. Albertì. 2004. The effects of slaughter weight, breed type and ageing time on beef meat quality using two different texture devices. *Meat Science*, 66:925-932.

Sauvant, D., J.M. Perez, and G. Tran. 2004. Tables of Composition and Nutritional Value of Feed Materials: Pigs, Poultry, Cattle, Sheep, Goats, Rabbits, Horses and Fish. INRA Editions, Versailles, France.

Savoia, S., A. Albera, A. Brugiapaglia, L. Di Stasio, A. Cecchinato, and G. Bittante. 2018a. Heritability and genetic correlations of carcass and meat quality traits in Piemontese young bulls. *Meat Science,* submitted.

Savoia, S., A. Albera, A. Brugiapaglia, L. Di Stasio, A. Cecchinato, and G. Bittante. 2018b. Prediction of beef quality traits in the abattoir using portable and hand-held near infrared spectrometers: validation, repeatability and field testing. *Manuscript in preparation.*

Savoia, S., A. Brugiapaglia, A. Pauciullo, L. Di Stasio, S. Schiavon, G. Bittante, and A. Albera. 2018c. Characterization of beef production systems and their effect on carcass and meat quality traits. *Meat Science,* submitted.

Sbarra, F., R. Mantovani, A. Quaglia, and G. Bittante. 2013. Genetics of slaughter precocity, carcass weight, and carcass weight gain in Chianina, Marchigiana, and Romagnola young bulls under protected geographical indication. *Journal of Animal Science,* 91:2596-2604.

Schaeffer, L.R. 2006. Strategy for Applying Genome-Wide Selection in Dairy Cattle. *Journal of Animal Breeding and Genetics,* 123:218-23.

Schiavon, S., M. De Marchi, F. Tagliapietra, L. Bailoni, A. Cecchinato, and G. Bittante. 2011. Effect of high or low protein ration combined or not with rumen protected conjugated linoleic acid (CLA) on meat CLA content and quality traits of double-muscled Piemontese bulls. *Meat Science,* 89:133–142.

Schiavon, S., F. Tagliapietra, M. Dal Maso, L. Bailoni, and G. Bittante. 2010. Effects of low-protein diets and rumen-protected conjugated linoleic acid on production and carcass traits of growing double-muscled Piemontese bulls. *Journal of Animal Science,* 88:3372–3383.

Schiavon, S., F. Tagliapietra, G. Dalla Montà, A. Cecchinato, and G. Bittante. 2012. Low protein

diets and rumen-protected conjugated linoleic acid increase nitrogen efficiency and reduce the environmental impact of double-muscled young Piemontese bulls. *Animal Feed Science Technology,* 174:96–107.

Scollan, N.D., P.L. Greenwood, C.J. Newbold, D.R. Yáñez Ruiz, K.J. Shingfield, R.J. Wallace, and J.F. Hocquette. 2011. Future research priorities for animal production in a changing world. *Animal Production Science*, 51:1–5.

Seoane, J.R., F. Dumont, C.L. Girard, L. Bédard, and J.J. Matte. 1984. Effects of intraventricular injections of gamma-aminobutyric acid and related substances on feeding behavior in satiated sheep. *Canadian Journal of Physiology and Pharmacology*, 62:1296–1299.

Setoguchi, K., M. Furuta, T. Hirano, T. Nagao, T. Watanabe, Y. Sugimoto, and A. Takasuga. 2009. Cross-breed comparisons identified a critical 591-kb region for bovine carcass weight QTL (CW-2) on chromosome 6 and the Ile-442-Met substitution in NCAPG as a positional candidate. *BMC Genetics*, 10:43.

Sgoifo Rossi, C.A., G. Pastorello, L. Caprarotta, and R. Compiani. 2011. Piemontese, se c'è unifeed è più alto il peso carcassa [Piemontese, heavier carcass weight with total mixed ration]. *Informatore Zootecnico,* 1:22-29.

Shin, J.H., S. Blay, B. McNeney, J. Graham, J.H. Shin, S. Blay, B. McNeney, and J. Graham. 2006. LDheatmap: An R Function for Graphical Display of Pairwise Linkage Disequilibria Between Single Nucleotide Polymorphisms. *Journal of Statistical Software,* 16.

Sistema Informativo Veterinario [Veterinary Information System]. (2017). Teramo (IT): Ministero della Salute; [accessed 17 Jan 2018]. http://www.vetinfo.sanita.it

Snelling, W.M., M.F. Allan, J.W. Keele, L.A. Kuehn, R.M. Thallman, G.L. Bennett, C.L. Ferrell,

T.G. Jenkins, H.C. Freetly, M.K. Nielsen, and K.M. Rolfe. 2011. Partial-genome evaluation of postweaning feed intake and efficiency of crossbred beef cattle1,2. *Journal of Animal Science*, 89:1731–1741.

Soyeurt, H.F., F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. *Journal of Dairy Science*, 94:1657-67.

Sorbolini, S., G. Marras, G. Gaspa, C. Dimauro, M. Cellesi, A. Valentini, and N.P. Macciotta. 2015. Detection of selection signatures in Piemontese and Marchigiana cattle, two breeds with similar production aptitudes but different selection histories. *Genetics Selection Evolution*, 47:52.

Soulat, J., B. Picarda, S. Léger, and V. Monteils. 2016. Prediction of beef carcass and meat traits from rearing factors in young bulls and cull cows. *Journal of Animal Science*, 94:1712–1726.

Soulat, J., B. Picarda, S. Léger, and V. Monteils. 2018. Prediction of beef carcass and meat quality traits from factors characterising the rearing management system applied during the whole life of heifers. *Meat Science*, 140:88-100.

Svishcheva, G.R., T.I. Axenovich, N.M. Belonogova, C.M. van Duijn, and Y.S. Aulchenko. 2012. Rapid variance components-based method for whole-genome association analysis. *Nature Genetics*, 44:1166–70.

Tatum, J.D., K.W. Gronewald, S.C. Seideman, and W.D. Lamm. 1990. Composition and quality of beef from steers sired by Piedmontese, Gelbvieh and Red Angus bulls. *Journal of Animal Science*, 68:1049-1060.

Tizioto, P.C., et al. 2013. Genome scan for meat quality traits in Nelore beef cattle. *Physiological Genomics*, 45:1012–1020.

Tizioto, P.C., C.F. Gromboni, A.R. de Araujo Nogueira, M.M. de Souza, M. de Alvarenga Mudadu, P. Tholon, A.Ô. do N. Rosa, R.R. Tullio, S.R. Medeiros, R.T. Nassu, and L. C. De Almeida Regitano. 2014. Calcium and potassium content in beef: Influences on tenderness and associations with molecular markers in Nellore cattle. *Meat Science*, 96:436–440.

Tøgersen G., J.F. Arnesen, B.N. Nilsen, and K.I. Hildrum. 2003: On-line prediction of chemical composition of semi-frozen ground beef by non-invasive NIR spectroscopy. *Meat Science*, 63: 515–523.

Turner, S.D. 2014. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv.

VanRaden, P.M. 2007. Genomic measures of relationship and inbreeding. *Interbull Annual Meeting Proceedings, Interbull Bullettin*, 37:33-36.

VanRaden, P.M. 2008. Efficient Methods to Compute Genomic Predictions. *Journal of Dairy Science*, 91:4414-4423.

VanRaden P.M., K.M. Olson, G.R. Wiggans, J.B. Cole, and M.E. Tooker. 2011. Genomic inbreeding and relationships among Holsteins, Jerseys, and Brown Swiss. *Journal of Dairy Science*, 94:5673-5682.

VanRaden P.M., C.P. Van Tassell, G.R. Wiggans, T.S. Sonstegard, R.D. Schnabel, J.F. Taylor, and F.S. Schenkel. 2009. Invited review: Reliability of genomic predictions for North American Holstein bulls. *Journal of Dairy Science,* 92:16–24.

Venter, J., et al. 2001. The Sequence of the Human Genome. *Science,* 291:1304–1351.

Verbeke, W., L. Van Wezemael, M.D. de Barcellos, J.O. Kugler, J.F. Hocquette, O. Ueland, and K.G. Grunert. 2010. European beef consumers' interest in a beef eating-quality guarantee insights from a qualitative study in four EU countries. *Appetite,* 54:289–296.

Vestergaard, M., N. Oksbjerg and P. Henckel. 2000. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. *Meat Science,* 54:177-185.

Veysset, P., M. Lherm, and D. Bébin. 2010. Energy consumption, greenhouse gas emissions and economic performance assessments in French Charolais suckler cattle farms: Model-based analysis and forecasts. *Agricultural Systems,* 103:41–50.

Veysset, P., M. Lherm, and D. Bébin. 2011. Productive, environmental and economic performances assessments of organic and conventional suckler cattle farming systems. *Organic Agriculture,* 1:1–16.

Visscher, P. M., S.E. Medland, M.A. Ferreira, K.I. Morley, G. Zhu, B.K. Cornes, G.W Montgomery, and N.G. Martin. 2006. Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. *PLoS Genet.* 2:e41.

Wang, W, Y. Peng, H. Sun, X. Zheng, and W. Wei. 2018. Spectral Detection Techniques for Non-Destructively Monitoring the Quality, Safety, and Classification of Fresh Red Meat. *Food Analytical Methods,* 11:2707-2730.

Ward, J.H. 1963. Hierarchical Grouping to Optimize an Objective Function. *Journal of the American Statistical Association,* 58:236-244.

Warner, R.D., P.L. Greenwood, D.W. Pethick, and D.M. Ferguson. 2010. Genetic and environmental effects on meat quality. *Meat Science,* 86:171–183.

Warris, P.D. 2000. Meat science: an introductory text. Wallingford (UK): CABI Publisher. Chapter 8, Post-mortem handling of carcasses and meat quality; p. 156-181.

Wheeler, T.L., L.V. Cundiff., R.M. Koch, and J.D. Crouse. 1996. Characterization of Biological Types of Cattle (Cycle IV): Carcass Traits and Longissimus Palatability. *Journal of Animal Science,* 74:1023-1035.

Wiedemair, V., M. De Biasio, R. Leitner, D. Balthasar, and C.W Huck. 2018. Application of design of experiment for detection of meat fraud with a portable near-infrared spectrometer. *Current Analytical Chemistry,* 14:58-67.

Wolcott, M.L, D.J. Johnston, S.A. Barwick, C.L. Iker, J.M. Thompson, and H.M. Burrow. 2009. Genetics of meat quality and carcass traits and the impact of tender stretching in two tropical beef genotypes. *Animal Production Science,* 49:383-398.

Xia, J., H. Fan, T. Chang, L. Xu, W. Zhang, Y. Song, B. Zhu, L. Zhang, X. Gao, Y. Chen, J. Li, and H. Gao. 2017. Searching for new loci and candidate genes for economically important traits through gene-based association analysis of Simmental cattle. *Scientific Reports,* 7:42048.

Xia, J., X. Qi, Y. Wu, B. Zhu, L. Xu, L. Zhang, X. Gao, Y. Chen, J. Li, and H. Gao. 2016. Genome-wide association study identifies loci and candidate genes for meat quality traits in Simmental beef cattle. *Mammalian Genome,* 27:246–255.

Xiong, Z., D.W. Sun, X.A. Zeng, and A. Xie. 2014. Recent developments of hyperspectral imaging systems and their applications in detecting quality attributes of red meats: A review. *Journal of Food Engineering,* 132:1.13.

Yang, J., B. Benyamin, N.P. McEvoy, S. Gordon, A.K. Henders, D.R. Nyholt, P.A. Madden, A.C. Heath, N.G. Martin, G.W. Montgomery, M.E. Goddard, and P M. Visscher. 2010. Common

SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, 42:565–569.

Zamora-Rojas, E., A. Garrido-Varo, E. De Pedro-Sanz, J.E. Guerrero-Ginel, and D. Pérez-Marín. 2013. Prediction of fatty acids content in pig adipose tissue by near infrared spectroscopy: At-line versus in-situ analysis. *Meat Science*, 95:503-511.

Zamora-Rojas, E., D. Pérez-Marín, E. De Pedro-Sanz, J.E. Guerrero-Ginel, A. Garrido-Varo. 2012. In-situ Iberian pig carcass classification using a micro-electro-mechanical system (MEMS)-based near infrared (NIR) spectrometer. *Meat Science*, 90:636-642.

Zheng, M., Y. Huang, J. Ji, S. Xiao, J. Ma, and L. Huang. 2018. Effects of breeds, tissues and genders on purine contents in pork and the relationships between purine content and other meat quality traits. *Meat Science*, 143:81–86.

Zheng, X., Z. Ju, J. Wang, Q. Li, J. Huang, A. Zhang, J. Zhong, and C. Wang. 2011. Single nucleotide polymorphisms, haplotypes and combined genotypes of LAP3 gene in bovine and their association with milk production traits. *Molecular Biology Reports*, 38:4053–4061. doi:10.1007/s11033-010-0524-1.

Zwambag, A., M. Kelly, F. Schenkel, I. Mandell, J. Wilton, and S. Miller. 2013. Heritability of beef tenderness at different aging times and across breed comparisons. *Canadian Journal of Animal Science*, 93: 307-312.

CURRICULUM VITAE

Simone Savoia was born on the 5h of January 1985 in Turin (Italy). After graduation of his Bachelor in "Livestock Science" at the University of Torino (Italy) in 2009, he was employed by the Italian Piemontese cattle Breeders Association (ANABORAPI) to work as geneticist at the "Research and Development" Office. While working, he continued his education enrolling in the Master in "Livestock Science and Technologies" at the University of Torino (Italy) obtaining the degree of MSc with honors. He then attended several national and international post-graduate courses in the context of "Animal Breeding and Genetics". In 2015 he started his PhD project in "Animal Science" at the University of Padova (Italy), which he combined with the activities at ANABORAPI. The research underlying the PhD was conducted within the project "QualiPiem" - Innovative tools for selection of meat quality in Piemontese breed.