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INNOVATION IN THE PIG BREEDING SECTOR: NEW TRAITS, NEW MODELS AND NEW METHODS IN BREEDING VALUE PREDICTION

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Non esiste il caso ne' la coincidenza,

noi camminiamo ogni giorno

verso luoghi e persone

che ci aspettavano da sempre.

G. Dembech

CONTENTS

SUMMARY

The most important income for the pig industry in Italy arises from the processing of meat in high-added-value typical products, mainly dry-cured hams having Protected Designation of Origin (**PDO**) label. Even through use of purebred pigs is not an exception, raw thighs are largely obtained from crossbred (**CB**) animals, which are slaughtered at heavy body weight (**BW**) (nearly 165 kg) and advanced age (270 d or more).

Breeding programs of pig lines linked to dry-cured ham production should focus on enhancement of growth performance, carcass traits and qualitative and technological properties of green thighs, in order to comply the rules dictated by the disciplinary of production. Moreover, the breeding goal (aggregate genotype) must be defined at the level of the "CB slaughter animal", so that the unfavourable effect on selection response due to genetic differences between purebreds (**PB**) and crossbreds, but also to environmental differences between nuclei and commercial farms, is alleviated. Given that breeding values (**EBV**) estimated using purebred phenotypes are often poorly related to the breeding goal defined at crossbred levels, the approach called "combined crossbred-purebred selection" is used in traditional genetic evaluation. It consists in estimating the EBV for the relevant traits using both PB and CB information. This approach is troublesome because it requires the availability of crossbred animals (half-sibs of the PB breeding candidates) which must be specifically reared as tested animals, and the phenotypic records of traits difficult and expensive to measured (as ham quality traits and curing losses). Moreover, EBV for members of a PB full-sibs family are identical when no PB individual information is available.

The main aim of this thesis was to provide new knowledge about innovative traits important in heavy pig industry and to propose new statistical models and genetic evaluation procedures to enhance the prediction of breeding values for carcass traits, and qualitative and technological properties of raw thighs.

The studies were carried out in collaboration with an Italian breeding company, Gorzagri s.s. (Fonzaso, Italy). It aims at producing boars (C21 line) and gilts (Goland line) used in commercial farms as sires and dams of CB pigs which are reared for production of PDO dry-cured hams. Genetic evaluation of C21 boar line is based on sib-testing program, where C21 nucleus boars are mating to a group of a CB Goland sows to produce CB paternal half-sib families (tested crossbred animals) that provide phenotypes on carcass and ham quality traits exploited in the genetic evaluation of C21 PB candidates.

The first part of this thesis aimed to investigate the prevalence and the genetic determinism of boar taint (BT), a new trait related to both pork quality and animal welfare which is becoming increasingly important for pig industries in EU countries after the decision to ceasing surgical castration of piglets from January,1 2018, and hence, the need to find alternative to reduce BT in entire male pigs.

In order to investigate the possibility to use genetic selection as tool to reduce BT in heavy pigs, the objectives of the study were: to evaluate the prevalence of BT in intact male pigs at 160 and 220 d of age, and to estimate the genetic parameters for BT compounds at the two different ages and the genetic relationships with growth traits.

Contents of androstenone (AND), skatole (SKA) and indole (IND) have been quantified by HPLC with fluorescence detection in biopsy samples of adipose tissues collected in-vivo from the neck area of 500 C21 entire male pigs at 160 and 220 d of age. In addition, for 100 of the investigated animals, BT compounds were quantified also in a sample of subcutaneous fat of raw thighs collected at slaughterhouse.

Contents of BT compounds measured in intact male pigs at 220 d were higher than those found at 160 d of age. In addition, in heavy animals, the percentage of samples that exceeded the sensory thresholds discriminating tainted from untainted carcasses was 2-fold that found for light pigs. Thus, the prevalence of BT is expected to be greater in mature and heavy pigs in respect to young and light pigs. The high phenotypic correlations between the contents of BT compounds measured in backfat and ham subcutaneous fat suggest that BT might be relevant also in PDO dry-cured hams. Medium-high heritabilities were found for contents of BT compounds at both ages, indicating that reduction of BT by means of genetic selection seems a valid alternative to surgical castration in pigs slaughtered at heavy BW. Genetic correlations between the contents of BT compounds measured at the two different ages were moderate to high. However, Spearman's rank correlations revealed that the breeding candidates ranked differently when measures of BT compounds taken at the different ages are used. Finally, weak genetic correlations were found between BT compounds and growth traits, indicating that selective breeding to decrease the contents of AND, SKA and IND is expected to have trivial effects on fat deposition and growth performance.

In the second section of this thesis the contribution of social genetic effects on variation of carcass and ham quality traits was investigated. In the current breeding program of C21 line the impact of the genetic effect of an individual on phenotypes of its pen mates (called social genetic effects or heritable social effects) is neglected. If present, social genetic effects are part of the total heritable variance and they affect the response to selection.

The aims of this study were to estimate (co)variance components for body weight adjusted at 270 d (**BW270**), carcass and ham quality traits using direct and competitive models, and to compare the ability of such models to fit the data.

The study was carried out on 9,871 CB finishing pigs raised in social groups containing from 4 to 7 individuals (6.1 pigs per group on average). Four sequential univariate animal models were compared through likelihood ratio test. REML estimates of covariance components were obtained for BW270, carcass backfat depth and lean meat (**CLM**), iodine number (**IOD**) and linoleic acid content of raw ham subcutaneous fat, subcutaneous fat depth in the proximity of semimembranosus and quadriceps femoris muscles, and linear scores for ham round shape (**RS**), subcutaneous fat (**SF**), and marbling.

Model comparison based on likelihood ratio test revealed that the model accounting for heritable social effects was significantly better than the best direct model for BW270, CLM, IOD, RS, and SF. The contribution of social genetic effects to the total heritable variance was large for CLM and BW270, whereas the one for ham quality traits was lower. The correlation between direct and social additive genetic effects was positive for BW270, but it was not significantly different from zero, suggesting independence between direct and social genetic effects for this trait. In contrast, a negative genetic covariance between direct and associative components was found for CLM, IOD, RS, and SF, which reduced the total heritable variance exploitable for genetic selection. The results obtained in this study suggest that social genetic effects affect variation in traits relevant for heavy pigs used in dry-cured hams manufacturing and, hence, the procedures for estimation of breeding values should take heritable social effects into account.

The third part of this thesis aimed to investigate new methods including genomic information in order to enhance the EBV prediction for traits related to carcass, ham quality and manufacturing of dry-cured hams. Genomic selection (**GS**) approach might overcome some of the drawbacks of current procedures for genetic evaluation of C21 line based on sib-testing.

The objective of the first study regarding GS was to develop and to investigate GS procedures based on single step BLUP (**SSBLUP**) methodology for genomic evaluation of PB breeding candidates of C21 boar line for traits relevant for dry-cured ham production.

Observations on BW270, carcass and ham quality traits were recorded from 11,488 CB finishing pigs. In addition, for 1,878 of the investigated animals, phenotypes for weight losses occurring during salting, resting and curing production stages were available. To constitute the reference population, 1,088 CB pigs, 136 nucleus boars (**C21_NB**: sires of CB tested pigs) and 500 C21 half-sibs of CB animals (**C21_HS**: half-sibs of CB tested animals) were genotyped for 8,826 single nucleotide polymorphisms using GGP Porcine LD Chip. Traditional evaluation was performed using BLUP methodology, whereas genomic evaluation was carried out using SSBLUP method which combine pedigree and genomic information in order to estimate genomic breeding values (**GEBV**) for both genotyped and ungenotyped animals. Pearson's correlation coefficients between EBV and GEBV ($r_{EBV,GEBV}$) were calculated in order to compare traditional and genomic evaluation. In addition, the 500 C21_HS with genotypic information were used to validate the prediction equation. Such animals mimic the condition of breeding candidates if genomic selection program

will be established for the C21 boar line, providing concrete information about the impact of genomic data in this boar line.

High $r_{EBV,GEBV}$ were observed for all the investigated traits for both CB animals, C21_NB, suggesting that genetic merit estimated using GS was consistent with breeding values prediction from traditional evaluation. Positive and moderate to high $r_{EBV,GEBV}$ were observed for C21_HS used in validation population. Differences in ranks of breeding candidates were found when they were based on traditional EBV or GEBV. In contrast to EBV, GEBV differed for members of a PB full-sibs family when they had genomic information. Thus, GS allows to choose the best animals based on "individual" genetic merit rather than "family" merit, increasing the accuracy of selection and reducing the rate of inbreeding. In addition, GS procedures might simplify genetic evaluation of PB breeding candidates of C21 boar line for traits difficult to measure, as ham weight losses during making process of dry-cured hams.

As found in the second part of this thesis, competitive models provided a better fit than classical direct model for BW270, CLM, IOD, RS and SF. Hence, for these traits, traditional genetic evaluation accounting for heritable social effects was compared to SSBLUP analysis performed using a competitive model. The dataset used in this study was the same described above. For animals in validation population, positive and moderate to high Spearman's correlation coefficients between direct EBV and direct GEBV were found for all the investigated traits, as well as between total breeding values and total genomic breeding values (**TGBV**). Hence, differences in ranks of breeding candidates were observed even when social genetic effects were included in the genomic evaluation. In fact, GS procedures accounting for heritable social effects allow to obtain individual TGBV rather than "family" TBV, increasing the benefit of GS for genetic evaluation of PB boar lines linked to dry-cured ham production.

Results of such studies are expected to favourably influence the competitiveness of the Italian pig breeding company involved in this research as well as the sustainability of the heavy pig industry.

RIASSUNTO

I profitti più rilevanti per l'industria suinicola italiana derivano dalla trasformazione della carne in prodotti tipici di alto valore economico, principalmente prosciutti crudi con Denominazione di Origine protetta (**DOP**). Le cosce fresche destinate alla produzione dei prodotti DOP derivano principalmente da soggetti ibridi che vengono macellati a pesi elevati (circa 165 kg) e ad età avanzate (almeno 270 giorni).
I programmi di selezione delle linee suine destinate alla produzione dei prosciutti crudi dovrebbero focalizzarsi sul miglioramento delle prestazioni di crescita, della qualità della carcassa e delle caratteristiche qualitative e tecnologiche delle cosce fresche, cosicché i requisiti dettati dai disciplinari di produzione siano soddisfatti. In questo scenario gli obiettivi di selezione devono essere definiti a livello di soggetto ibrido, piuttosto che a livello di linea pura. I valori genetici (breeding values, **EBV**) stimati utilizzando le informazioni fenotipiche dei soggetti di linea pura sono poco correlati con gli obiettivi di selezione definiti al livello dei soggetti ibridi. Per questo la valutazione genetica dei candidati riproduttori di linea pura attualmente avviene attraverso un approccio che combina informazioni derivanti sia da soggetti puri che da soggetti ibridi. Questo approccio presenta alcune criticità dovute alla necessità di allevare appositamente soggetti ibridi (mezzi fratelli dei candidati riproduttori di linea pura) per ottenere i rilievi fenotipici per caratteristiche che alcune volte sono difficili da misurare (come i caratteri di qualità della coscia o i cali di stagionatura). Inoltre, quando nella valutazione genetica non sono incluse informazioni fenotipiche rilevate sugli animali di linea pura, i EBV per i candidati riproduttori che appartengono alla stessa famiglia di fratelli pieni sono identici.

L'obiettivo principale della tesi è stato quello di fornire nuove conoscenze su caratteri innovativi per l'industria del suino pesante e di proporre nuovi modelli statistici e procedure di valutazione genetica per migliorare la predizione dei EBV per caratteri legati alla qualità della carcassa e delle cosce fresche destinate alla produzione dei prosciutti crudi DOP.
Gli studi qui presentati sono stati condotti in collaborazione con un'azienda suinicola italiana, Gorzagri s.s (Fonzaso, Italia). Essa ha come obiettivo la produzione di verri (linea C21) e scrofe (linea Goland) usati negli allevamenti commerciali come padri e madri di soggetti ibridi destinati alla produzione dei prodotti DOP. La valutazione genetica della linea parentale C21 si basa su un programma di sib-testing, dove i verri nucleo C21 sono accoppiati a un gruppo di scrofe ibride Goland per produrre gruppi di soggetti ibridi (mezzi fratelli dei candidati riproduttori) sfruttati per la misurazione dei fenotipi per i caratteri di qualità della carcassa e della coscia.

La prima parte della tesi si proponeva di studiare l'incidenza e il determinismo genetico dell'odore di verro, un nuovo carattere associato sia alla qualità della carne che al benessere animale, il quale sta diventando sempre più rilevante per l'industria suinicola italiana ed europea dopo la decisione di abbandonare la castrazione chirurgica dei suinetti maschi a partire dal 1 gennaio 2018. Questo impone la necessità di trovare valide alternative a questa pratica per ridurre l'odore di verro nei suini maschi interi.

Allo scopo di indagare la possibilità di sfruttare la selezione genetica per limitare l'odore di verro nel suino pesante, gli obiettivi dello studio sono stati: valutare la prevalenza dell'odore di verro in suini maschi interi di 160 e 220 giorni d'età e stimare i parametri genetici per i composti responsabili del problema alle due diverse età nonchè le relazioni genetiche che intercorrono tra questi e i caratteri legati all'accrescimento.

I contenuti di androstenone (**AND**), scatolo (**SCA**) e indolo (**IND**) sono stati quantificati tramite cromatografia liquida ad alta prestazione in microcampioni di tessuto adiposo prelevati in-vivo dalla regione del collo di 500 suini maschi interi della linea C21 al raggiungimento dell'età di 160 e 220 giorni. Inoltre, per 100 di essi, i composti sono stati quantificati anche in un campione di grasso di copertura della coscia prelevato in sede di macellazione.

Le concentrazioni dei tre composti misurati nel grasso di animali di 220 giorni sono risultati superiori a quelli riscontrati a 160 giorni di età. Inoltre, la percentuale di campioni che superano i limiti di accettabilità utilizzati per discriminare le carcasse affette o meno dall'odore di verro raddoppia rispetto a quella registrata negli animali di 160 giorni. Questo suggerisce che l'incidenza del problema dell'odore di verro è maggiore nei suini più pesanti e maturi da un punto di vista sessuale. Inoltre, un'elevata correlazione è stata riscontrata tra il contenuto di AND, SCA e IND nel grasso dorsale e nel grasso di copertura delle cosce, suggerendo che il problema può essere rilevante anche nei prosciutti crudi DOP. I composti responsabili dell'odore di verro hanno mostrato un'elevata ereditabilità ad entrambe le età, confermando che la selezione genetica potrebbe essere una valida soluzione per ridurre l'odore di verro anche in suini macellati a pesi elevati. Le correlazioni genetiche tra AND, SCA e IND misurati alle due diverse età sono risultate di grado moderato o elevato. Tuttavia, sono state riscontrate delle differenze nelle classifiche dei candidati riproduttori quando i fenotipi misurati alle due diverse età sono stati usati per la stima dei EBV. Infine, le correlazioni genetiche tra AND, SCA e IND e caratteri legati all'accrescimento sono risultate modeste, suggerendo che la riduzione della concentrazione di questi composti nel tessuto adiposo tramite selezione genetica non dovrebbe influenzare le prestazioni di crescita e la deposizione di grasso in suini non castrati.

Nella seconda parte di questa tesi è stato esaminato in contributo degli effetti sociali sulla variabilità di caratteri legati alla qualità della carcassa e della coscia in suini pesanti. Le attuali procedure di valutazione genetica della linea C21 non tengono in considerazione l'influenza che il genotipo di un animale

ha sul fenotipo dei suoi compagni di gruppo (effetto sociale). Se presenti, gli effetti sociali contribuiscono alla varianza ereditabile e quindi possono influenzare la risposta alla selezione. Lo studio si è quindi proposto di stimare le componenti di varianza per il peso a 270 giorni di età (**PESO270**), caratteri di qualità della carcassa e della coscia utilizzando modelli genetici "classici" e modelli competitive (che includono gli effetti sociali), nonché determinare la bontà dell'adattamento ai dati di questi modelli.

Lo studio è stato condotto su 9,871 suini ibridi Goland allevati in gruppi contenenti da 4 a 7 individui, con dimensione media del gruppo pari a 6.1 suini. Quindi, quattro modelli sequenziali sono stati utilizzati per la stima delle componenti di varianza.

Il confronto dei modelli ha rivelato che il modello competitive mostra un miglior adattamento ai dati rispetto ad un modello che considera solo gli effetti genetici additivi per il PESO270, la percentuale di carne magra in carcassa (**CM**), il numero di iodio, la globosità e lo spessore del grasso sottocutaneo misurati sulla coscia. Gli effetti sociali contribuiscono in modo considerevole alla varianza ereditabile per il PESO270 e CM, mentre hanno un effetto meno rilevante per gli altri caratteri esaminati. La correlazione tra la componente genetica additiva e quella sociale è risultata negativa per i caratteri legati alla qualità della carcassa e della coscia, causando una diminuzione della varianza ereditabile sfruttabile per la selezione di questi caratteri. In conclusione, lo studio ha dimostrato che gli effetti sociali contribuiscono alla variabilità di caratteri rilevanti nel suino pesante destinato alla produzione dei prosciutti crudi DOP e, quindi, le attuali procedure per la valutazione genetica della linea C21 dovrebbero essere modificate così da includere anche la componente sociale.

La terza parti della tesi ha riguardato lo studio di nuove procedure di valutazione genetica basate sulla selezione genomica (**SG**). L'obiettivo è quello di migliorare la predizione dei EBV per caratteri importanti nel suino pesante superando alcune delle criticità dell'attuale sistema di valutazione genetica della linea C21 basato su un programma di sib-testing.

In un primo lavoro, sono state studiate procedure di SG basate sulla metodologia single-step BLUP (**SSBLUP**). Le informazioni fenotipiche per caratteri riguardanti la qualità della carcassa e della coscia sono state registrate in 11,488 suini ibridi Goland. Per 1,878 animali sono stati inoltre rilevati i cali di peso della coscia durante le diverse fasi di trasformazione in prosciutto crudo. Allo scopo di costituire la popolazione di riferimento per lo sviluppo dell'equazione di predizione dei breeding values genomici (**GEBV**), 1,088 soggetti ibridi, 136 verri nucleo di linea C21 (**C21_VN**) e 500 soggetti C21 mezzi fratelli degli animali ibridi (**C21_MF**) sono stati genotipizzati per 8,826 polimorfismi a singolo nucleotide utilizzando il DNA chip GGP Porcine LD Chip. La valutazione genetica tradizionale è stata condotta sfruttando la metodologia BLUP, mentre la valutazione genomica si è basata su un approccio SSBLUP. L'equazione di predizione dei GEBV è stata validata utilizzando i 500 C21_MF con genotipo come popolazione di validazione. La struttura della popolazione di validazione qui investigata rispecchia un potenziale gruppo di candidati riproduttori della

linea C21 nel caso che procedure di SG siano realmente implementate nel programma di selezione di questa linea. Questo consente di avere delle informazioni concrete sull'impatto che l'inclusione delle informazioni genomiche ha sulla valutazione genetica della linea oggetto di studio. La valutazione genetica tradizionale e quella genomica sono state confrontate tra loro attraverso la correlazione tra EBV e GEBV ($r_{EBV,GEBV}$). Elevati coefficienti di correlazione tra EBV e GEBV sono stati osservati sia per i soggetti ibridi genotipizzati che per gli individui C21_VN, suggerendo che le stime dei GEBV sono simili a stime accurate dei EBV ottenute con metodi tradizionali. Per quanto concerne i soggetti inclusi nella popolazione di validazione, $r_{EBV,GEBV}$ sono risultate di entità minore rispetto ai due gruppi considerati in precedenza. Differenze nelle classifiche dei candidati riproduttori sono state osservate quando esse erano basate sui EBV piuttosto che sui GEBV. Tali differenze sono attribuibili all'abilità della metodologia SSBLUP di stimare GEBV individuali, anziché EBV identici per soggetti che appartengono alla stessa famiglia di fratelli pieni (quando essi hanno informazione genomica). Quindi l'utilizzo di un approccio di SG permette di scegliere i soggetti migliori in base a meriti genetici individuali piuttosto che "familiari", limitando il tasso di consanguineità e aumentando l'accuratezza della selezione. Inoltre, i risultati ottenuti rivelano che l'implementazione di procedure di SG nel programma di selezione della linea C21 consentirebbero di introdurre tra gli obiettivi di selezione anche caratteri di difficile misurazione come i cali di stagionatura.

Come descritto in precedenza, gli effetti sociali contribuiscono alla variabilità di caratteri rilevanti per il suino pesante e che modelli competitive forniscono un miglior adattamento ai dati per i caratteri PESO270, CM, il numero di iodio, la globosità e lo spessore del grasso sottocutaneo misurati sulla coscia. Quindi, per questi caratteri, sono state analizzate procedure di SG basate sulla metodologia SSBLUP che consideravano, oltre agli effetti genetici diretti, anche gli effetti sociali. Le informazioni fenotipiche e genomiche utilizzate sono le medesime descritte per il precedente studio. Differenze nelle classifiche degli animali inclusi nella popolazione di validazione sono state riscontrate sia quando esse erano basate su EBV e GEBV riferiti alla sola componente genetica diretta, che quando sono stati utilizzati i valori genetici e genomici totali. Quindi l'implementazione procedure di SG nel programma di selezione della linea C21 che utilizzano modelli competitive consentirebbero di sfruttare i vantaggi della SG già descritti in precedenza e contemporaneamente stimare più correttamente la varianza ereditabile totale considerando anche la componente associativa.

CHAPTER 1

General introduction

1.1 HEAVY PIGS FOR PRODUCTION OF DRY-CURED HAMS: THE PECULIARITY OF THE ITALIAN PIG INDUSTRY

In 2013 in Italy were reared about 8,561,000 pigs, making it the 7th country in Europe for pig production (IPQ-INEQ, 2014).

The most important income for the Italian pig industry arises from the processing of meat into high-added-value typical products, mainly dry-cured hams having Protected Designation of Origin (**PDO**) label. Nearly 70% of the Italian pig production consists in heavy pigs, slaughtered at heavy (nearly 165 kg) body weight (**BW**) and advanced age (270 d as a minimum) to obtain raw thighs to be processed into PDO dry-cured hams of high market value. The value of raw hams accounts for nearly 30% of total carcass value (CRPA, 2010) and end products generate sales for hundreds of millions Euro per year (CRPA, 2013). In order to safeguard the typicality and high quality of PDO dry-cured hams, specific rules about production stages and characteristics of raw thighs are dictated by disciplinary of production (e.g., Prosciutto di Parma PDO, 1992; Prosciutto di San Daniele, 1996).

Dry-cured hams are manufactured through a very long process that takes at least 10 months to complete. Important biochemical and enzymatic processes occur and determine the distinctive aroma and flavour of the end product. The three major production steps for the manufacture of Parma dry-cured ham (Prosciutto di Parma PDO, 1992) are:

1. SALTING. After removal of rind and excess subcutaneous fat, wet and dry salts are added to hams which are stored for 6-7 d in cold rooms at 1 to 4°C and 80% of humidity. Salt residues are removed and hams are sprinkled again with tiny amounts of salt. Hams are stored back in cold rooms where they remain for 15-18 d. During salting, hams lose about 3-5% of their initial weight.
2. RESTING. After removing salt residues, hams are stored in resting rooms for a period ranging from 60 to 90 d at about 1-5°C and 75% of humidity. During the resting phase, the weight loss is about 8-10% of the initial weight.
3. CURING. In the initial stage of curing, hams are hung on frames in properly ventilated rooms. Optimal ratios of internal to external humidity and internal humidity to product moisture ensure a gradual and continuing drying. During this period, the weight loss is about 8-10% of the initial

weight. The exposed surface of hams is softened with a mixture of minced lard and salt to prevent the over-dehydration of exposed muscular tissues. Then, hams are moved to the "cellars" which are cooler and less ventilated than pre-maturation rooms. During this stage, hams lose about 5% of their weight. Curing goes on for a minimum of 12 months since salting.

As salting and curing are not able to modify unfavourable characteristics or defects of raw thighs, qualitative and technological properties of green hams are key factors to obtain high-value end products. As a consequence, most rules, dictated by production disciplinary of PDO dry-cured ham, aim at optimizing the quality of raw thighs used in processing. The most important guidelines relate to weight and age at slaughter. Heavy slaughter weights (165 kg) stem, primarily, from the need to generate raw hams weighing at least 10 kg. Heavy weights at slaughter have been traditionally considered effective indicators of meat maturity as measured by carcass fat content. Improvements of weight gain potential due to selective breeding and enhanced feeding and management practices have progressively weakened this association and current guidelines for production of dry-cured hams restrict the age of slaughter pigs to a minimum of 9 months. Restrictions on weight and age at slaughter ensure optimal body tissue composition and exert favourable effects on restraint of weight loss during curing.

Raw thighs can be obtained from both purebreds and crossbreds. However, only individuals from Italian Large White and Italian Landrace breeds can be used as purebreds. Large White and Landrance purebreds from other countries or subjects of other breeds or synthetic lines can be used to generate crossbred slaughter pigs (Prosciutto di Parma PDO, 1992; Prosciutto di San Daniele, 1996). In 2013, nearly 90% of raw hams processed into PDO dry-cured hams have been obtained from crossbred animals (IPQ-INEQ, 2014).

Specific features are required for the amount and quality of subcutaneous fat of thighs. Depth of ham subcutaneous fat must be higher than 15 mm to prevent an excessive and rapid dehydration. In addition, linoleic acid content (expressed as a percentage of total fatty acids) and iodine number, assessed in subcutaneous fat samples including both the inner and the outer fat layers, must not exceed 15% and 70, respectively, in order to ensure an optimal quality of fat (white, firm and not oily). In 2013, 13,646,588 thighs have been certified for the production of PDO dry-cured hams (IPQ-INEQ, 2014).

1.2 BREEDING GOALS IN GENETIC IMPROVEMENT OF HEAVY PIGS

Breeding programs of pig lines linked to dry-cured ham production should focus on enhancement of growth performance, carcass traits, and qualitative and technological properties of raw thighs, in order to comply with the rules dictated by disciplinary of production. Moreover, the breeding goal (aggregate genotype) must be defined at the level of the "commercial" animal (slaughter pig).

Growth rate is an important trait to account for in breeding programs for heavy pigs. Indeed, very high weight gain potential is associated with very high carcass lean meat content and insufficient ham fat covering. As consequence, high curing losses and worsened sensorial characteristics of dry-cured ham are frequently observed in lines exhibiting very high growth rate potential (Bosi and Russo, 2004).

With respect to traits related to ham quality, genetic evaluation should be focused on improvement of subcutaneous fat depth and fatty acid composition and round shape of thighs, so that excessive weight losses are avoided as well as rancidity processes.

Finally, the ham weight loss during making process should be another trait to consider in genetic improvement of heavy pigs. They represent the degree of dehydration occurring during all production stages and they are measured as difference between ham weight before salting and at the end of the process. For producer excessive weight losses represent an economical problem because they originate in lower weight of final product.

Carcass and ham quality can be measured only after slaughter, so they cannot be evaluated in breeding candidates. Moreover, determination of fatty acid content and iodine number requires expensive laboratory instruments and trained experts. In addition, assessment of weight losses is very time consuming (due the very long time needed for dry-cured ham production) making the evaluation of these traits very challenging.

1.1.2 Boar taint (BT): a new trait in genetic evaluation of heavy pigs

Boar taint is an unpleasant off-odour and off-flavour, released when heating or cooking meat obtained from some intact male pigs, which is considered undesirable by consumers (Bonneau, 1997). Boar taint is mainly due to accumulation in fat of three lipophilic compounds: androstenone (5-α-androst-16-en-3-one, **AND**), skatole (3-methylindole, **SKA**) and indole (**IND**) (Zamaratskaia and Squires, 2009). Androstenone is a sexual steroid hormone produced by the Leyding cells of the testis. Biosynthesis of AND is low in young pigs and progressively increases when approaching sexual maturity. Skatole and IND are produced by microbial degradation of the amino acid L-tryptophan in the large intestine of the pigs. The production of these indolic compounds is mainly regulated by the availability of tryptophan as resulting from the diet provision and turnover of the gut epithelium, by the protein to energy ratio of the diet and the activity of the intestine microbial population. Hepatic metabolism plays an important role in degradation of AND, SKA and IND and prevents the accumulation of these compounds in fat.

Mechanisms involved in perception of tainted meat and relationships between concentration of compounds responsible for BT, offensive odour and flavour perception and body fat levels are not straightforward. It seems clear that AND and indolic compounds are responsible of the urine/perspiration-like and faecal-like odours, respectively. However, sensory characterization of BT is very complex. Between-individuals variation in BT perception is large, depending on specific sensitivity to the

compounds. The threshold levels used for sensory perception of BT compounds are usually 0.5 - 1 μg/g for AND and 200 - 250 ng/g for SKA (Lundstrom et al., 2009). These threshold levels are used to discriminate carcasses affected by BT (which should be discarded) from not affected carcasses. Previous studies revealed that the proportion of carcasses from intact male pigs exceeding sensory thresholds levels for AND and SKA were about 30 and 11%, respectively, in light pigs used for pork production (Walstra et al., 1999; Strathe et al., 2013).

Several methods, based on the use of liquid or gas chromatography, mass spectrometry or immunological assays are currently available to quantify BT compounds in porcine fat or plasma. These methods are not applicable on the slaughter line because they require time-consuming sample preparation procedures and complex laboratory instruments and techniques (Aluwe et al., 2012). Boar taint in pork or pork-based products can be also evaluated by sensory analysis performed by trained panellists. However, large variation in perception of BT across individuals and the resulting low accuracy make application of this method troublesome (Haugen et al., 2012). Hence, collection of phenotypic information for BT in large samples of animals or at the population level is very difficult and expensive.

The most common practice to prevent BT is surgical castration of piglets performed few days after birth with no use of pain relievers. Such practice will be banned in the EU starting from January 1, 2018, as detailed in the European Declaration on Alternative to Surgical Castration signed by EU countries representatives in 2011. Hence, alternative strategies to reduce the risk of BT for pork from intact male pigs must be searched for.

At present, the only non-surgical method available to perform castration of male piglets is immunocastration. It relies on functional castration by means of a vaccine (Improvac^{TM}, Pfizer Ltd.) against the gonadotropin-releasing hormone which interrupts the hypothalamic-pituitary-gonadal axis and, as a consequence, the synthesis of testicular steroids. In light pigs, the vaccine is injected two times with the last injection occurring 4 to 6 weeks before slaughter. Although a number of studies (Zamaraskaia et al., 2008; Font i Furnols et al., 2009; Brunius et al., 2011) evidenced the effectiveness of this method in preventing BT, there are some concerns related to the proportion of pigs not responding to vaccination, the consumer's acceptability of pork obtained from treated animals and the safety risk for veterinarians, particularly when vaccination needs to be performed in pigs of heavy BW.

Genetic factors significantly affect the level of BT, so that selective breeding seems to be an excellent tool to reduce the content of AND, SKA and IND in adipose tissue of intact male pigs. Several studies estimated heritability of intermediate or high magnitude and positive genetic correlations for BT compounds (Robic et al., 2008). In light pigs, heritability estimates for AND ranged from 0.25 to 0.87 (Sellier, 1998; Tajet et al., 2006; Strathe et al., 2013), whereas estimates for indolic compounds were from 0.30 to 0.60 (Engelsma et al., 2007; Winding et al., 2012; Baes et al., 2013). In contrast to immunocastration, breeding is not a fast alternative to surgical castration. In addition, studies on the effects of selection to decrease BT compounds

concentration in fat tissues or plasma on carcass traits and reproductive performance are lacking. However, selective breeding seems a long-term solution to prevent BT suitable also for heavy pigs. Animals slaughtered at high BW would require an additional dose of vaccine resulting in increased costs and safety risks for veterinarians and decreased acceptability of the PDO dry-cured ham production system by consumers.

Previous studies regarding BT have been carried out on pigs slaughtered at 100-120 kg BW. To our knowledge, no study investigated variation in the content of BT compounds in heavy pigs. Due to increased fat deposition and degree of sexual maturity, concentrations of AND, SKA and IND in adipose tissue of heavy pigs are expected to be greater than those detected in light pigs and, as consequence, also the number of carcasses to be discarded for BT defect is expected to be greater. Several studies focused on the content of BT compounds in backfat. Raw hams are the most important meat cut for the Italian pig industry and, to date, no study assessed the content of AND, SKA and IND on covering fat of thighs.

1.3 TRADITIONAL GENETIC EVALUATION OF HEAVY PIGS

Traditional genetic evaluation is based on the use of phenotypic measures, collected from the breeding candidates and/or their relatives, and pedigree information to predict additive genetic values (estimate breeding value, **EBV**) using a linear mixed model method known as "Best Linear Unbiased Prediction" (**BLUP**, Henderson, 1984). The animals having the "best" EBV in relation to the breeding goal are selected and used as sires and dams of the next generation.

As previously discussed, raw hams for dry-cured ham production are prevalently obtained from crossbred animals. Hence, the breeding goal is defined at the level of the "crossbred slaughter animal", so that the unfavourable effect on selection response due to genetic differences between purebreds and crossbreds, but also to environmental differences between nuclei and commercial farms, is alleviated. Breeding values estimated using purebred phenotypes only are often poorly related to the breeding goal defined at the crossbred level (Cecchinato et al., 2010). To overcome such issues, the approach called "combined crossbred and purebred selection" (**CCPS**; Wei, 1992) has been proposed. It consists in estimating the breeding values for the relevant traits by making use of both purebred and crossbred phenotypic information. Combined crossbred-purebred selection increases the response to selection for crossbred performance in comparison with selection procedures focusing on purebred performance only. The following are possible drawbacks arising from CCPS:

- it requires the availability of crossbreds which are relatives (half-sibs) of the purebred breeding candidates. Commonly, such animals are not readily available and must be specifically generated to serve as tested animals (testing groups) providing phenotypic information used to estimate genetic merit of purebred breeding candidates;

- management of testing groups is very expensive as well as measurements of traits related to qualitative and technological properties of raw hams and BT;
- when no purebred individual information is available and all information is provided by testing groups of crossbred half-sibs (e.g., slaughter traits), EBVs for members of a purebred full-sibs family are identical. Hence, members of the same families are likely chosen as breeding animals and, as a consequence, the rate of inbreeding might increase (Bijma et al., 2001).

1.3.1 Competitive models: inclusion of social genetic effects in traditional genetic evaluation of heavy pigs

Interactions among individuals are common in both natural and domestic populations. Some livestock species, as poultry and swine, are commonly reared in groups so that interactions among group mates can affects phenotypes of the group members. The effects of an individual's genes on phenotypes of group mates are known as social genetic effects, indirect genetic effects or heritable social effects (Moore et al., 1997; Bijma et al., 2007a).

In classical quantitative genetics, the phenotype (P) of an individual is modelled as the sum of heritable (A) and non-heritable (E) components (Falconer and Mackay, 1996).

$$P_i = A_i + E_i$$

When social interactions occur, the model needs to be extended to incorporate social genetic effects. In such case, P_i is the sum of direct effects of the individual and social genetic effects exerted by each of the group mates of the individual (Griffing, 1967):

$$P_i = A_{D,i} + E_{D,i} + \sum_{j \neq i}^{n-1} (A_{S,j} + E_{S,j})$$

where $A_{D,i}$ is the direct breeding value (**DBV**) of individual *i,* $E_{D,i}$ is the non-heritable direct effect, $A_{S,j}$ is the social breeding values (**SBV**) of group member *j,* $E_{S,j}$ the non-heritable social effects, and *n* is the group size. The DBV refers to the heritable effect of an individual on itself (which is comparable to the "classical" breeding value), whereas the SBV accounts for the heritable effect of an individual on its group members.

Therefore, the total heritable impact of an individual on the mean trait value of the population (called total breeding value, **TBV**) is the sum of the individual's DBV and n-1 times its SBV (Bijma et al., 2007a).

$$TBV_i = DBV + (n-1)\, SBV$$

As in classical theory where the heritable variance exploitable in selective breeding is the variance of EBVs among individuals, the potential of a population to respond to selection for socially-affected traits is measured by the variance of TBVs among individuals (Bijma et al., 2007a):

$$\sigma^2_{TBV} = \sigma^2_{a_D} + 2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma^2_{a_S}$$

where $\sigma^2_{A,D}$ is the direct genetic variance, whereas $2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma^2_{a_S}$ quantifies the heritable variance due to social genetic effects. As a result, social genetic effects might contribute considerably to the

total heritable variance of a trait which is exploitable in selection, especially if animals are reared in large group. The sign of the additive genetic covariance between direct and social components ($\sigma_{a_D a_S}$) is a measure of competition vs. cooperation (Bijma et al., 2007b). Indeed, negative $\sigma_{a_D a_S}$ means that individuals with positive DBV have, on average, negative heritable effects on the phenotypes of their group mates. Conversely, positive $\sigma_{a_D a_S}$ may be interpreted as "heritable cooperation" where animals with positive DBV have, on average, positive heritable influences on the phenotypes of their group mates. When contributions of social genetic effects to trait variation are neglected, the heritable variation and the potential response to selection may be estimated incorrectly. Muir and Schinckel (2002) were the first ones to propose a specific mixed model, defined as competitive model, to account for both direct and social genetic effects when estimating variance components and breeding values.

Social genetic effects were studied in a wide range of animal species as pigs (Bergsma et al., 2008), poultry (Muir, 2005; Ellen et al., 2008), deer (Wilson et al., 2011) and mink (Alemu et al., 2014). With special focus on pigs, the contribution of social genetic effects was evaluated for growing-finishing traits as average daily gain (Arango et al., 2005; Chen et al., 2008; Bowman et al., 2010), feed intake, backfat thickness, muscle depth (Bergsma et al., 2008; Hsu et al., 2010), and androstenone content in fat (Duijvesteijn et al., 2012). Most of these investigations were on pigs of light BW (100-110 kg) fed ad libitum. No study investigated social genetic effects in heavy pigs used for dry-cured ham production. As previously detailed, the production of heavy pigs involves different conditions relative to light pigs, mainly related to a) the feeding strategy (restricted feeding from 75-80 kg BW onward), b) the achievement of a "heavy" slaughter weight (160 kg) and c) a constrained age at slaughter (not less than 270 d). Such differences might affect social interactions among pigs, the magnitude of social effects and, hence, the total heritable variation exploitable in selective breeding to enhance carcass and ham quality traits.

1.3.2 The breeding goal and the breeding program of the C21 boar line

Investigations presented in this thesis have been carried out in collaboration with an Italian breeding company, Gorzagri s.s. (Fonzaso, Italy). The company aims at producing boars (C21 line) and gilts (Goland line) used in commercial farms as sires and dams of crossbred pigs which are fattened and slaughtered at heavy BW for production of dry-cured hams. The C21 boar line was the line under study in this thesis.

The selection scheme of the C21 boar line consists in a nucleus farm (Riese Pio X, Italy) where pure C21 boars are produced and mated to pure C21 sows.

In addition to growth performance and residual feed efficiency, the current breeding goal of the C21 boar line is focused on enhancement of qualitative traits of raw hams and carcass. In particular, selective breeding is addressed to enhance the quality of ham fat covering, to an intermediate optimum for the

amount of subcutaneous fat and marbling in the raw thigh, to reduce excessive ham roundness, and to keep the average carcass lean meat content at the current value.

Genetic evaluation of C21 breeding candidates is based on a sib-testing program, where mating C21 nucleus boars to a group of a crossbred Goland sows produces crossbred paternal half-sib families providing phenotypic information on carcass and ham quality traits exploited in the genetic evaluation of C21 purebred candidates . Crossbred Goland sows originate from a cross involving boars of a synthetic line, derived from Large White and Pietrain breed, and sows of a Large White line selected for maternal ability and prolificacy. Hence, the "genetics" of crossbred animals involved in the sib-testing program for the C21 line is the same of fattening pigs sired by C21 boars in commercial farms. Tested crossbred animals are raised and fattened in a farm where feeding and management conditions mimic conditions of commercial farms, including rearing of pigs in groups. In the last fifteen years, the breeding program of the C21 line ensured favourable and satisfactory responses and a consistent enhancement of the breeding goal traits. However, current procedures for the estimation of breeding values in the C21 line do not take social genetic effects into account and the role of such effects for variation in traits under selection is unknown.

1.4 GENOMIC SELECTION AS INNOVATIVE METHOD FOR PREDICTION OF BREEDING VALUES

Over the last decade, traditional genetic evaluation procedures, based on the use of individual phenotypes and pedigree information, have been responsible for important improvements in several traits relevant in pig farming as growth rate, backfat thickness, feed efficiency and litter size (Eggen et al., 2012). Traditional breeding programs exhibit, however, some limitations and not only in pig lines originating fattening animals for PDO ham dry-curing. For example, in dairy cattle, evaluation of sex-related traits, like milk yield, are based on progeny tests which, on one hand, guarantee reliable predictions of genetic merit, but, on the other hand, require long time to provide EBVs of tested candidates, are responsible for long generation intervals and impair the efficiency of selection.

In order to overcome the drawbacks of traditional genetic evaluation methods, in the last 30 years genetic markers have been proposed to identify and map chromosomal regions affecting quantitative traits (**QTLs**), which could be exploited in selective breeding. Thank to the advance in molecular biology and technology, genome sequences are now available for several livestock species, including cattle and swine. Such advances allowed to discover hundreds of thousands genomic markers, mainly single nucleotide polymorphisms (**SNPs**), which led to a new technology: the DNA chips. At present, two DNA chips exhibiting different density of markers are commercially available for pigs: the Illumina Porcine60SNP beadchip (Ramos et al., 2009) and the GGP Porcine LD chip (Genomic Profiler for Porcine LD, GeneSeek Inc., a Neogen Co., Lincoln, NE).

In 2001, Meuwissen and colleagues proposed a new method for the prediction of breeding values, able to exploit the DNA chip technology and the availability of dense marker panels, called genomic

selection (**GS**). Hundreds or tens of thousands markers (SNPs), covering the whole genome, are evaluated simultaneously so that at least one marker is in linkage disequilibrium with the genes or QTLs affecting the trait and, potentially, all additive genetic variance is explained by the markers (Goddard and Hayes, 2007). The first step to implement GS in a breeding program is to collect individual phenotypes and DNA samples from a large group of animals (the reference population). Each animal of the reference population is then genotyped using a dense SNP panel. In the second step, such genotypic and phenotypic information are used to estimate the effects of each SNPs on the phenotypic value of the trait and to derive a prediction equation to estimate the genomic breeding values (**GEBVs**). After validation, the prediction equation can be applied to estimate GEBVs of breeding candidates (belonging to candidate population) that need to be genotyped, but not phenotype-recorded (Goddard and Hayes, 2009). In contrast to dairy cattle, breeding programs of purebred lines linked to dry-cured ham production define the breeding goal "at the crossbred level". In this case, animals of the reference population, which are genotyped and phenotype-recorded for traits of interest, are crossbreds animals generated by the lines for which genomic selection is planned. In particular, GS of purebred lines to enhance traits relevant at the crossbred level can be carried out through the following two steps (Dekkers, 2007): 1) estimation of SNPs effects on crossbred traits using phenotypes and genotypes assessed on crossbred animals belonging to a reference population; 2) estimation of GEBVs of purebred animals (only genotyping is needed) using SNPs effects previously estimated using data from the reference crossbred population.

Statistical methods in genomic prediction

The major challenge in genomic prediction is to choose the best statistical method to allow accurate predictions of GEBV for breeding candidates. The main difficulty, known also as "small n-large p" problem, is to estimate simultaneously a large number of marker effects in a single model when the number of phenotypic records is small (Calus et al., 2010). Genomic breeding values can be predicted using indirect or direct methods (Zhang et al., 2011). Indirect approaches estimate SNPs effects using data of the reference population and calculate the GEBVs of a genotyped breeding candidate (candidate population) by summing the effects at all marker genotypes. Marker effects can be estimated using different methods (e. g., BLUP, Bayes A, Bayes C and Bayes Lasso methods), which differ in the assumption about the probability distribution of marker effects (Hayes and Goddard, 2010). Direct approaches estimate GEBVs using mixed model equations (as the BLUP method used in classical genetic evaluation) and by replacing the relationship matrix based on the pedigree information with a relationship matrix constructed from genomic marker information. In particular, the single-step method, proposed by Legarra et al. (2009) and Christensen and Lund (2010), combines the genomic relationship matrix for genotyped animals with pedigree relationship matrix in a "extended relationship matrix" that allows to predict GEBVs for all animals in the pedigree (including also ungenotyped animals).

Advantages of GS

The main advantages of GS are:

- breeding candidates can be evaluated using the prediction equation, based only on genomic information. It makes easier the genetic evaluation for large numbers of candidates and traits for which individual phenotypes are difficult to measure;
- GEBVs can be estimate early in life because DNA sample can be collected even at birth. It reduces the generation intervals (this is the main benefit of GS in dairy cattle);
- increased accuracy of GEBVs, compared to traditional EBVs, especially for traits with low heritability.

Implementation of GS in breeding programs of pig lines linked to dry-cured ham production might overcome the drawbacks of CCPS approach described previously.

In particular:

- after SNPs genotype effects are estimated, prediction of purebred GEBVs can continue for several generations with no need of collection of phenotypic information. It might reduce the costs due to management of testing groups and collection of phenotypic information for traits expensive or difficult to measure for a large number of animals (like slaughter traits, ham quality and BT);
- no need of crossbred information (after SNPs effects have been estimated);
- prediction of EBVs can be made early in life, also for traits related to dry-cured ham manufacturing (like curing weight losses) whose measurement can be carried out at the end of curing only;
- GEBVs of purebred breeding candidates will be different for the members of full-sib families. Hence, selection of breeding candidates will be based on estimates of "individual" genetic merit within family rather than on values of "family" merit, increasing the accuracy of selection and reducing the rate of inbreeding.
- non-additive genetic effects (like dominance effects) can be explored and exploited.

Genomic selection has been implemented in dairy cattle since 2008 (Hayes et al., 2009; Van Raden et al., 2009). In pigs, GS has been recently applied to evaluation of growth performance (Christensen et al., 2012), carcass and ham quality traits (Wellman et al., 2013), reproductive traits (Cleveland et al., 2010; Forni et al., 2010) and BT (Azevedo et al., 2014) in pig lines linked to production of slaughtered pigs of light BW. Only one study, based on simulated data, is available in the literature about the implementation of GS in a selection scheme for heavy pigs based on sib testing of boars (Samorè et al., 2014). Studies making use of real data are currently lacking.

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CHAPTER 2

Aims

The general aim of this thesis was to provide increased knowledge about new traits relevant in heavy pig farming and to investigate innovative statistical models and genetic evaluation procedures to enhance the prediction of breeding values for traits related to the quality of the carcass and raw thighs used in production of PDO dry-cured hams.

The first part of this thesis provides original knowledge about prevalence and genetic aspects of boar taint compounds in heavy pigs. It aims at acquiring scientific knowledge to include this new trait, relevant for both meat and end product quality and animal welfare, in breeding programs of pig lines linked to production of dry-cured hams. In the second section, competitive models are compared to classical (direct) models to provide evidence about the contribution of heritable social effects on variation in carcass and ham quality traits and usefulness of models accounting for both direct and social genetic effects in genetic evaluation of heavy pigs boar lines. Finally, the third part of this thesis focuses on innovative selection procedures, incorporating genomic information in the estimation of breeding values for traits related to ham quality and manufacturing of dry-cured hams. It aims at acquiring scientific knowledge to implement genomic selection procedures into the breeding program of a boar line linked to dry-cured ham production, to overcome some critical issues of the current program.

Specific aims were:

1. to investigate the variation in the contents of compounds responsible for boar tainted pork, assessed in adipose tissue at two different ages and weights, and to estimate heritability and genetic correlations (Chapter 1);
2. to investigate the contribution of social genetic effects to variation in carcass traits and ham quality using competitive models, to estimate (co)variance components and genetic parameters and to compare rankings of breeding animals based on direct and competitive models (Chapter 2);
3. to acquire basic knowledge to develop genomic selection procedures for the evaluation and selection of C21 breeding candidates for carcass traits, ham quality and curing weight losses (Chapter 3);
4. to implement social genetic effects models in genomic selection procedures for carcass and ham quality traits (Chapter 4).

Results of such studies are expected to favourably influence the competitiveness of the Italian pig breeding company as well as the sustainability of the heavy pig industry.

CHAPTER 3
New traits relevant in heavy pig farming

Estimates of genetic parameters for content of boar taint compounds in adipose tissue of intact males at 160 and 220 days of age

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ABSTRACT

The objectives of this study were to investigate variation in content of BT compounds (androstenone (**AND**), skatole (**SKA**) and indole (**IND**)) quantified in fat from intact male pigs at 160 (110 kg) and 220 d (160 kg) of age, to estimate genetic parameters for AND, SKA and IND, and to investigate the genetic relationships between those compounds and grow traits.

A biopsy sample of backfat was collected in-vivo, using a biopsy device, from the neck area of 500 entire male pigs of C21 sire line (Gorzagri, Fonzaso), at the two different ages. In addition, a sample of raw ham subcutaneous fat was collected after slaughter in 100 of the investigated animals. Pig individual body weight (**BW**) were recorder at each sampling, whereas P2 backfat depth was measured only at 220 d. Quantification of AND, SKA and IND in fat was performed by HPLC with fluorescence detection. Estimates of (co)variance components and genetic parameters were performed through univariate and bivariate Bayesian analyses after logarithmic transformations of response variables.

Contents of BT compounds measured in intact male pigs at 220 d were higher than those found at 160 d of age. In addition, in heavy animals, the percentage of samples that exceeded the sensory thresholds discriminating tainted from untainted carcasses was 2-fold (67.3 %) that found for light pigs (32.3%). The phenotypic correlations between the contents of BT compounds measured in backfat and ham subcutaneous fat were 0.76, 0.88 and 0.70, for AND, SKA and IND, respectively. Medium-high heritabilities for BT compounds were observed for intact male pigs at both ages. In particular, in heavy pigs, heritabilities for AND, SKA and IND were 0.59, 0.60 and 0.69, respectively. Positive and moderate genetic correlations between the content of a BT compounds at 160 d and the one of the same compounds at 220 d of age were found. However, different rankings of animals were observed when breeding values for the content at 160 and 220 d were used. As consequence, performance testing should be based on phenotypes collected at advance age. Weak genetic correlations were observed between content of BT compounds and growth traits (BW at the two ages, BFT or average daily gain), indicating that selective breeding to reduce the risk of tainted pork is expected to exert trivial effects on growth performance and fat deposition.

Results suggest that the prevalence of BT is expected to be greater in mature and heavy pigs in respect to young and light pigs. In addition, BT seems to be a relevant problem also in PDO dry-cured hams, so that further investigation on consumer acceptability toward products obtained from intact male pigs are needed. The high heritability, the positive genetic correlations between AND, SKA and IND, and the trivial effects on growth traits provide the evidence that reduction of BT by means of genetic selection seems a valid alternative to surgical castration in pigs slaughtered at heavy BW.

Key words: boar taint, genetic parameters, heavy pigs, ham quality.

INTRODUCTION

Boar taint (**BT**) is an unpleasant flavour and odour released when meat or fat obtained from pubertal or sexually-mature intact male pigs (Bonneau, 1997) is heated making it undesirable for many consumers. Boar taint is mainly due to 3 lipophilic compounds: androstenone (5α-androst-16-en-3-one; **AND**), skatole (3-methylindole; **SKA**) and indole (**IND**) (Prelog and Ruzicka, 1944; Patterson, 1968; Vold, 1970).

Surgical castration of piglets with no use of pain relievers, the most common practice to prevent BT, will have to be banned on January 1, 2018 (European Declaration on Alternatives to Surgical Castration, 2011). Selective breeding is an option to reduce BT relying on moderate to high heritability of BT compounds and favourable genetic associations among AND, SKA and IND (Robic et al., 2008). Genetic parameters for BT compounds have been estimated in pigs of light BW (Grindfleck et al., 2011; Strathe et al., 2013). In Italy, pig farming focuses on heavy pigs used for production of Protected Designation of Origin (**PDO**) dry-cured hams. To comply with requirements dictated by guidelines for PDO dry-cured ham production, pigs must be slaughtered at 270 d of age and at an average BW of 160 kg (Bosi and Russo, 2004). Due to higher fat contents and sexual maturity compared to pigs of light BW, carcasses from heavy intact male pigs are expected to be more frequently tainted. Although several studies investigated the effect of BW on levels of BT compounds (Babol et al., 2004; Chen et al., 2007), no study investigated the genetic relationships between contents of BT compounds in fat measured at different ages and BW.

The aims of this study were to investigate variation in content of BT compounds measured in fat collected from intact male pigs at 160 (110 kg) and 220 d (160 kg) of age, to estimate genetic parameters for AND, SKA and IND at the two ages, and to investigate the genetic relationships between BT compounds and growth traits.

MATERIALS AND METHODS

Animal Care and Use Committee approval was obtained by the Local Health and Social Care Service n. 8 (Servizio Veterinario di Igiene degli Allevamenti e delle Produzioni Zootecniche, Montebelluna, Treviso, Italy) after transmission of procedures for in vivo collection of adipose tissue samples through biopsies ("Dichiarazione inizio progetto GENOTAINT", Protocollo 26B/2012). To guarantee animal welfare and health, all sampling procedures were carried out by a trained veterinarian using local anaesthesia.

Animals

The animals enrolled in this study were 500 purebred intact male pigs of the C21 Goland boar line (Gorzagri, Fonzaso, Italy). Besides growth and residual feed efficiency, the breeding goal for the C21 line

includes traits related to the quality of dry-cured hams as detailed by Cecchinato et al. (2008). Pigs were offspring of 27 boars and 226 sows, born between September 2012 and June 2013, reared in the same farm, and fed *ad libitum* conditions 14.41 % of CP and 3.07 kcal of ME/kg. Additive relationships were traced back for as many generations as possible, using the pedigree information available for the line and, for pigs with BT compounds phenotype, were computed on the basis of at least 8 generations of known ancestors.

Data records

A biopsy sample (0.5 g) of subcutaneous adipose tissue was collected in vivo from the neck area of each pig at the age of 160 and 220 d when average BW was 110 and 160 kg, respectively. For this purpose, the biopsy device developed by SUISAG (Sempach, Switzerland) and described in Baes et al. (2013) was used. Animals were subjected to local anaesthesia before performing the biopsy and the needle of the biopsy device and the skin were disinfected with 80% ethanol in distilled water. In addition, a sample of raw hams subcutaneous fat was collected after slaughter in 100 of the investigated animals. Adipose tissue samples were stored at -80 °C until chemical analysis. Pig individual BW was recorded at each sampling, whereas P2 backfat depth (**BFT**, mm) was measured, at 220 d of age only, above the last rib at approximately 6 cm from midline using an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco corporation, Minneapolis, USA). Average daily gain (**ADG**) from 160 to 220 d of age was calculated using BW records.

To extract BT compounds from fat, after removal of skin, hair and muscle tissue from each sample, the adipose tissue was melted in a microwave oven. Liquid fat (150 µl) was mixed with methanol (750 µl) and placed at -20°C for 20 min. Finally, centrifugation (5,000 rpm at 4°C for 5 min) and filtration of the liquid extract (0.45 µm RC-filter) were carried out before injection of samples into a HPLC instrument (SCL-10A VP, Shimadzu, with RF-10A XL fluorescence detector).

Two different chromatographic separations at 45°C column temperature were carried out for AND and indolic compounds using the same reversed-phase analytical column (Zorbax Eclipse Plus C18, 100 mm x 4.6 mm, 5 µm particle size, Agilent Technologies). For AND, a mixture of two solvents was used: solvent A consisted of 100% methanol and solvent B was 30% acetonitrile in water. The following gradient program with a flow rate of 1.5 ml/min has been used: starting condition of 60% solvent B kept for 2 min after injection, from 60 to 20% solvent B in 0.5 min, from 20 to 17.6% solvent B in 13 min, and linear return to the starting condition in 1 min. An excitation wavelength of 346 nm and an emission wavelength of 521 nm were used for fluorescence detection. Elution time for AND was 12 min. Before injection, an in situ AND derivatization was performed as described in Hansen-Møller (1994). Separations of SKA and IND were performed in isocratic condition using solvent B at a flow rate of 1.5 ml/min in 13 min. Fluorescence detection was performed at an excitation wavelength of 270 nm and emission wavelength of 350 nm.

Retention time for IND and SKA was 6.1 and 12 min, respectively. Repeatability, reproducibility and sensitivity of the analytical procedures were tested.

Statistical analysis

Concentrations of AND, SKA and IND were not normally distributed (Figure 1). Hence, a natural logarithmic transformation was applied to normalize the distribution of the data. The log-transformed data of AND (**LAND)**, SKA (**LSKA),** and IND **(LIND**) were then used in the statistical analysis.

Estimation of (co)variance components for the investigated traits was performed through univariate and bivariate Bayesian analyses. The univariate model was as follows:

$$\mathbf{y = Xb + Wa + e}$$

where **y** is a vector of observed phenotypes for a trait; **b** is an unknown vector of non-genetic effects which included the effects of the group in test (12 groups), the date of analysis (only for BT compounds; 57 dates of analysis for contents assessed at 160 d and 43 dates for contents assessed at 220 d), the linear effect of the age at weighing (covariate) for BW at 160 (**BW160**) and 220 d (**BW220**) only, and the linear effect of BW220 (covariate) for BFT only; **a** is an unknown vector of additive genetic effects of pigs (1,927 pigs); **X** and **W** are incidence matrices relating **b** and **a** to **y**, respectively; **e** is a vector of random residuals.

To investigate genetic relationships among traits, statistical inference was based on a set of bivariate analyses, which considered pairs of traits. The bivariate model was as follows:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \cdot \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \cdot \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where $\mathbf{y_1}$ and $\mathbf{y_2}$ are vectors of phenotypic records for trait 1 and 2, respectively; $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ are vectors of non-genetic effects as specified for the univariate model; $\mathbf{X_1}$ and $\mathbf{X_2}$ are known incidence matrices relating effects in $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ to $\mathbf{y_1}$ and $\mathbf{y_2}$, respectively; $\mathbf{a_1}$ and $\mathbf{a_2}$ are vectors of additive genetic effects of animals assumed to follow a multivariate normal distribution with $\begin{bmatrix} a_1 & a_2 \end{bmatrix}' \sim N(\mathbf{0}, \mathbf{G_o} \otimes \mathbf{A})$ where $\mathbf{G_0}$ is the (co)variance matrix for animal genetic effects, and **A** is the numerator of Wright's relationship matrix and N ( ) indicates a normal probability density; $\mathbf{W_1}$ and $\mathbf{W_2}$ are known incidence matrices relating additive genetic effects in $\mathbf{a_1}$ and $\mathbf{a_2}$ to $\mathbf{y_1}$ and $\mathbf{y_2}$, respectively; $\mathbf{e_1}$ and $\mathbf{e_2}$ are vectors of residual effects assumed to follow a multivariate normal distribution with $\begin{bmatrix} e_1 & e_2 \end{bmatrix}' \sim N(\mathbf{0}, \mathbf{R_o} \otimes \mathbf{I})$ where $\mathbf{R_0}$ is the (co)variance between residual effects for trait 1 and 2.

Marginal posterior distributions of (co)variance components and genetic parameters were estimated by performing numerical integration through the Gibbs sampler, as implemented in the program TM (Legarra et al., 2008). A unique Gibbs chain of 500,000 iterations was run for each analysis. One Gibbs sample was saved after 250 iterations and the length of burn-in was 50,000 iterations. Hence, the total number of

saved samples was 1,800. The posterior median was used as a point estimate of (co)variance components and genetic parameters. Lower and upper bounds of the highest 95% posterior probability density interval (HPD) for (co)variance components and genetic parameters were obtained from the estimated marginal densities.

The heritability (**h^2**) of a trait was calculated as $h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_e)$ where σ^2_a is the additive genetic variance and σ^2_e is the residual variance. Additive genetic and phenotypic correlations were estimated as $r_a = \sigma_{a1, a2} / (\sigma_{a1}\ \sigma_{a2})$ and $r_p = \sigma_{p1, p2} / (\sigma_{p1}\ \sigma_{p2})$, respectively, where $\sigma_{a1, a2}$ is the additive genetic covariance between trait 1 and trait 2, σ_{a1} and σ_{a2} are the additive genetic standard deviations of trait 1 and trait 2, respectively, $\sigma_{p1, p2}$ is the phenotypic covariance between trait 1 and 2, and σ_{p1} and σ_{p2} are the phenotypic standard deviations of trait 1 and 2, respectively.

In addition, for each compound responsible for BT, EBVs estimated at 160 d were correlated with EBVs at 220 d of age.

RESULTS AND DISCUSSION

Quantification of boar taint compounds

The sensitivity of the HPLC method used to separate and quantify BT compounds was 0.15 μg/g for AND and 3 ng/g for indolic compounds. These concentrations are lower than sensory thresholds proposed to distinguish tainted from untainted carcasses (Walstra et al., 1999; Lundstrom et al., 2009). In addition, the analytical technique showed high repeatability and reproducibility. Residual standard deviations (RSD) for repeatability were 0.03 μg/g, 2.05 ng/g and 2.10 ng/g for AND, SKA and IND, respectively; for reproducibility, RSD were 0.02 μg/g, 2.47 ng/g and 2.29 ng/g for AND, SKA and IND, respectively.

Descriptive statistics

Descriptive statistics for AND (**AND160**), SKA (**SKA160**) and IND (**IND160)** contents in adipose tissue at 160 and 220 d (**AND220**, **SKA220**, and **IND220**) of age, BW160, BW220, ADG and BFT are presented in Table 1.

Body weight at the first sampling was 106 ± 9.8 kg, which is consistent with average slaughter weight of pigs used for pork production in many European countries and, for this, with BW at which BT compounds were quantified in previous literature studies (e.g., Tajet et al., 2006). Body weight at 220 d was 154 ± 14.6 kg, in line with the slaughter weight of heavy pigs for the production of PDO dry-cured hams.

Contents of AND160 and SKA160 were comparable to those detected in studies performed on light pigs (Chen et al., 2007; Gregersen et al., 2012; Winding et al., 2012). In our study, average IND160 was greater than average SKA160 as well as than IND contents measured in previous studies in both backfat and ham subcutaneous fat samples. In literature, only Baes et al. (2013) assessed content of BT compounds in

live boars and reported that the difference between SKA and IND concentrations was lower than that detected in studies where compounds were quantified in samples collected at slaughter. Although pre-slaughter conditions might influence the metabolism of indolic compounds (Wesoly et al., 2014), this does not seems to be the reason of the higher amount of IND respect to SKA in our population. This is the first study that compare contents of the three BT compounds in adipose tissue sampled before and after slaughter, so that, further investigation are required.

Contents of AND, SKA and IND measured at 220 d were greater than those at 160 d, indicating an increased accumulation of BT compounds in adipose tissues of older and heavier pigs. As a consequence, in intact heavy pigs the prevalence of carcasses defective for BT is expected to be greater than that for intact male pigs slaughtered at 110 kg BW. Thresholds have been proposed to discriminate between tainted and untainted carcasses. Commonly used cut-off levels for AND and SKA are 1 μg/g and 250 ng/g, respectively (Walstra et al., 1999; Lundstrom et al., 2009). Given that both AND and SKA contribute to unpleasant off-flavour and off-odour, an exceeding content of just a single compound is responsible for a tainted carcass. In our study, 31.3 and 1.2% of samples collected at 160 d had contents of AND and SKA greater than the cut-off levels, and 32.3% of samples had a content of either AND or SKA exceeding the cut-off. The prevalence of tainted carcasses increased in older and heavier pigs. Indeed, 66.5 and 4.41% of samples collected at 154.6 ± 14.6 kg BW were above the thresholds for AND and SKA, respectively, whereas contents of either AND or SKA exceeding the cut-off were detected in 67.3% of boars. With respect to AND, quantification in intact males at 220 d of age guarantees that most animals are close to or at puberty and allows to discriminate boars with low risk to exhibit BT from animals with delayed sexual maturity.

Contents of AND, SKA and IND quantified in backfat samples were compared to the concentrations measured in raw ham subcutaneous fat samples. The phenotypic correlations between the contents of BT compounds measured in the two different anatomic regions were 0.76, 0.88 and 0.70, for AND, SKA and IND, respectively (data not presented in tables). This suggests that BT might be relevant also in PDO dry-cured hams. Given the notable economical value of these products for Italian pig industry, additional studies are needed to evaluate the possible effects of biochemical and enzymatic processes that occur during manufacturing of PDO dry-cured hams on contents of BT compounds and on perception of unpleasant flavours and odours.

Heritability of boar taint compounds and growth traits

Point estimates and 95% highest posterior density intervals of the marginal posterior density of (co)variance components and heritability for the logarithmic-transformed contents of BT compounds at 160 **(LAND160, LSKA160, LIND160)** and 220 d **(LAND220, LSKA220, LIND220)** of age obtained from univariate analysis are reported in Table 2.

Heritability of LAND160, LSKA160 and LIND160 was 0.39, 0.60 and 0.39, respectively. These values were similar to previous studies carried out on intact males of different breeds (Tajet, 2006; Grindfleck et al., 2011; Baes et al., 2013). Heritability of AND and IND increased in pigs at 220 d of age. It was 0.58 for LAND220 and 0.69 for LIND220 with a high probability (86 and 94%, respectively) to be greater than 0.40. For both traits, the additive genetic variance increased (+17 and +159% for for LAND220 and LIND220, respectively), whereas the residual variance decreased (-56 and -25% for for LAND220 and LIND220, respectively). In contrast, h^2 of LSKA220 was very similar to the estimate for LSKA160; in this case both additive genetic and residual variance increased. These results showed that BT compounds were highly heritable also in mature and heavy pigs.

Body weight at 160 d, BW220 and ADG exhibited a low or intermediate h^2. For BFT, h^2 was high, with 95% of probability to be greater than 0.40.

Estimates of (co)variance components and h^2 for all investigated traits obtained through bivariate analysis were consistent with parameters obtained from univariate models.

Genetic and phenotypic correlations for concentrations of boar taint compounds

Estimates of genetic and phenotypic correlations between concentrations of BT compounds are reported in Table 3. Genetic correlations between LAND160, LSKA160 and LIND160 were positive and consistent with estimates of previous studies (Engelsma et al., 2007; Grindflek et al., 2011; Strathe et al., 2013). Consistent with estimates reported in Baes et al. (2013), the genetic correlation between LAND160 and LIND160 was slightly higher than the one between LAND160 and LSKA160. The phenotypic correlation between LSKA160 and LIND160 was positive, of moderate magnitude (r = 0.5), and slightly lower than estimates reported in previous studies (Windig et al., 2012; Baes et al., 2013). Phenotypic correlations between LAND160 and log-transformed concentrations of indolic components at 160 d were small.

Magnitude of the genetic correlations between concentrations of BT compounds increased for measures assessed at 220 d of age. Point estimates for the genetic correlation between LAND220 and indolic compounds ranged from 0.56 (LSKA220) to 0.71 (LIND220). Such estimates had a 100% probability to be positive and a probability to be greater than 0.2 that was 85% or higher. The genetic correlation between LSKA220 and LIND220 was very similar to the correlation estimated using measures taken at 160 d of age and had a 100% probability to be greater than 0.2. These results and the estimated heritabilities indicate that selective breeding aimed at decreasing contents of AND and indolic compounds can be carried out more effectively in heavy than in light pigs.

The estimated phenotypic correlation between the content of a BT compound measured at 160 d and the one of the same compound at 220 d of age was 0.28, 0.26 and 0.34 for AND, SKA and IND, respectively. The probability that such correlations were greater than 0.20 was higher than 85%. The estimated genetic correlations between contents measured at 160 and 220 d had a probability of being

greater than 0.20 of 95, 88 and 99% for AND, SKA and IND, respectively. However, the probabilities that such correlations were greater than 0.9 was low suggesting that, albeit point estimates were of intermediate or high magnitude, concentrations of BT compounds in adipose tissue assessed at 160 and 220 d of age cannot be considered as repeated measures of the same trait. Spearman's correlations (data not presented in tables) between rankings based on estimated breeding values for the content at 160 and the one at 220 d were 0.69, 0.61 and 0.82 for AND, SKA and IND, respectively, providing evidence that breeding candidates ranked differently when using measures of BT compounds taken at different ages. As a consequence, performance testing programs based on phenotypes collected at young age would be less effective than programs where breeding candidates are tested at weight close to the target slaughter weight of their offspring. Early genetic evaluation of boars can decrease costs of testing and those arising from management of animals that will never be used for breeding. Due to decreased variation in the degree of sexual maturity, performance tests completing late in life would help to discriminate late-maturing boars from low-tainted animals and to increase accuracy of selection of breeding candidates. In addition, quantification of BT compounds at 220 d of age provides phenotypic information of breeding animals having BW similar to the slaughter weight of heavy pigs used for PDO dry-cured hams production.

Genetic and phenotypic correlations between growth traits and boar taint compounds

Estimated genetic correlations between BW160, BW220, BFT, or ADG and concentrations of BT compounds measured at 160 and at 220 d are presented in Table 4. These correlations were considered to be relevant when the probability of being greater than 0.1 (for positive estimates) or lower than -0.1 (for negative estimates) was 80% or greater. Phenotypic correlations were weak for all the investigated traits are not reported in tables.

For all additive genetic correlations, 95% highest posterior density intervals were wide , due to the limited number of records available, and the magnitude of the estimates was small, with the only exception of the correlation between SKA and BW. The genetic correlation between SKA and BW at 160 d was negative ($r_{=}$ -0.51) and had a probability of being lower than -0.1 of 99%. At 220 d, the correlation between the same traits was positive (with a 80% probability of being greater than 0.1).

Contents of BT compounds at both ages and BFT were genetically weakly correlated. Only the estimated correlation between BFT and SKA measured at 220 d (r = -0.24) was relevant. Point estimates for the genetic relationships between ADG and concentrations of BT compounds at 220 d were positive, but the probability that they were higher than 0.10 was lower than 80%. However, for indolic compounds, the probability was close to 0.8.

Weak genetic correlations between growth traits and BT compounds have been estimated in previous studies on intact males of light BW (Engelsma et al., 2007; Merks et al., 2010; Windig et al., 2012; Strathe et

al., 2013). Results of our study confirm that selective breeding to decrease the risk of tainted pork is expected to exert limited or trivial effects on growth performance and fat deposition.

In conclusion, compounds responsible for BT have been quantified in intact male pigs at 160 and 220 d of age. In particular, for the first time, AND, SKA and IND have been measured at BW comparable to the slaughter weight of heavy pigs used for production of PDO dry-cured hams. As expected, contents of BT compounds, as well as prevalence of samples exceeding quantitative thresholds used as a reference to identify tainted pork, increased at 220 d in comparison with values at 160 d. Medium-high h^2 estimates for BT compounds have been obtained for boars at both light and heavy BW. In addition, positive, albeit moderate, additive genetic correlations have been estimated between contents of BT compounds measured at the two ages. Based on the estimated genetic parameters, selective breeding of boars with low BT risk is an effective alternative to surgical castration also for the heavy pig industry. Due to rules imposed by the Disciplinary of Production of PDO products, others practices, expected to be effective in preventing risk of tainted pork, like the decrease of slaughter weight, cannot be applied in our scenario. Selective breeding, based on individual assessment of AND, SKA, and IND is a long-term solution, which might have undesirable side effects on reproductive performance of breeding animals due to the complex nature of traits underlying BT and their associations with fertility traits. For this purpose, estimates of genetic relationships between the concentration of BT compounds and reproductive traits, including libido, sexual behavior and maturity, should also be investigated. Conventional breeding programs might be supported by genomic information. Marker genotypes associated with low ability to accumulate AND, SKA and IND in adipose tissue could be exploited for development of genomic selection procedures to select individuals without routine recording of phenotypic information for contents of BT compounds. Raw thighs to produce PDO dry-cured hams are obtained to a large extent from crossbred pigs. Due to genetic differences between purebreds and crossbreds and environmental dissimilarities between nuclei and commercial farms (Cecchinato et al., 2010), possible differences in prevalence of BT and in genetic parameters might occur between the two pig populations. As reported in Lundström et al. (2009), the processing of raw meat can increase the acceptability of tainted pork. Thus, further evaluations of the content of BT compounds in fat of dry-cured hams and investigations on the consumer acceptability of hams from intact males would be useful to the heavy pig industry to better understand consequences of BT for the quality of processed products.

Table 1. Descriptive statistics for the investigated traits measured at 160 and 220 d of age (N = 500)[1]

Trait	Age at measurement									
	160 d					220 d				
	Median	Mean	SD	P1	P99	Median	Mean	SD	P1	P99
Boar taint compounds in backfat										
Androstenone, µg/g	0.71	0.98	0.85	0.15	4.51	1.47	1.73	1.21	0.35	5.91
Skatole, ng/g	35.43	51.56	49.93	6.09	295.95	48.56	75.89	98.53	8.75	605.23
Indole, ng/g	65.95	84.32	89.84	15.54	436.17	87.73	133.17	146.94	12.26	773.82
Boar taint compounds in raw ham subcutaneous fat										
Androstenone, µg/g						1.50	1.88	1.417	0.30	6.77
Skatole, ng/g						42.94	63.04	56.99	6.56	283.81
Indole, ng/g						82.95	139.50	173.00	6.13	950.53
Body weight, kg	106.1	106.4	9.8	84.0	132.0	153.9	154.6	14.6	122.0	196.0
Ultrasound backfat depth, mm						15.1	15.4	3.5	8.0	23.5
Average daily gain, g/d						864	868	205	321	1350

[1]P1: the 1st percentile, P99: the 99th percentile.

Table 2. Estimate and 95% highest posterior density interval (within parentheses) of variance components and heritability for the investigated traits[1,2]

Trait	Age											
	160 d						220 d					
	σ^2_a	σ^2_e	h^2	$P_{0.2}$	$P_{0.3}$	$P_{0.4}$	σ^2_a	σ^2_e	h^2	$P_{0.2}$	$P_{0.3}$	$P_{0.4}$
LAND	0.178	0.277	0.391	0.89	0.68	0.48	0.213	0.154	0.581	0.99	0.95	0.86
	(0.041, 0.382)	(0.133, 0.384)	(0.112, 0.722)				(0.072, 0.383)	(0.046, 0.255)	(0.265, 0.920)			
LSKA	0.396	0.259	0.604	1.00	0.99	0.91	0.445	0.297	0.601	1.00	0.97	0.89
	(0.172, 0.657)	(0.093, 0.410)	(0.316, 0.872)				(0.165, 0.786)	(0.087, 0.498)	(0.275, 0.908)			
LIND	0.154	0.239	0.391	0.94	0.74	0.48	0.400	0.180	0.691	1.00	0.98	0.94
	(0.052, 0.285)	(0.144, 0.316)	(0.150, 0.647)				(0.167, 0.670)	(0.007, 0.327)	(0.386, 0.994)			
BW	41.959	43.362	0.491	0.99	0.93	0.76	74.704	125.655	0.371	0.95	0.73	0.41
	(17.045,72.58)	(22.460,61.437)	(0.249, 0.772)				(29.955,141.920)	(81.543,162.724)	(0.153, 0.627)			
BFT							5.245	2.992	0.637	1.00	1.00	0.95
							(2.283, 8.679)	(0.686, 4.886)	(0.361, 0.935)			
ADG							7.255	25.801	0.220	0.57	0.27	0.12
							(1.658, 18.944)	(17.625, 31.813)	(0.058, 0.504)			

[1]LAND: natural logarithm of androstenone concentration (μg/g); LSKA: natural logarithm of skatole concentration (ng/g); LIND: natural logarithm of indole concentration (ng/g); BFT: P2 ultrasound backfat depth (mm) measured above the last rib at approximately 6 cm from midline using an A-mode ultrasonic device; ADG: average daily gain (g/d) from 160 to 220 d of age;

[2]σ^2_a: additive genetic variance; σ^2_e residual variance; $P_{0.2}$: posterior probability of the heritability being greater than 0.2; $P_{0.3}$: posterior probability of the heritability being greater than 0.3; $P_{0.4}$: posterior probability of heritability being greater than 0.4.

Table 3. Estimate, 95% highest posterior density interval (HPD95%) and posterior probability of the estimate being greater than 0.2 ($P_{0.2}$) for additive genetic (upper diagonal) and phenotypic (lower diagonal) correlations among concentrations of boar taint compounds quantified at 160 and 220 d of age in adipose tissue[1]

Age	**Trait**	**Parameter**	**Age**					
			160 d			**220 d**		
			LAND	LSKA	LIND	LAND	LSKA	LIND
160 d	LAND	Estimate	-	0.303	0.403	0.671	0.498	0.447
		HPD95%		-0.242, 0.844	-0.205, 0.902	0.211, 0.981	-0.135, 0.913	-0.209, 0.885
		$P_{0.2}$		0.64	0.73	0.95	0.80	0.79
	LSKA	Estimate	0.119		0.740	0.030	0.474	0.289
		HPD95%	-0.033, 0.257	-	0.340, 0.969	-0.509, 0.507	0.004, 0.877	-0.218, 0.733
		$P_{0.2}$	0.12		0.98	0.27	0.88	0.63
	LIND	Estimate	0.265	0.496		0.609	0.509	0.739
		HPD95%	0.147, 0.403	0.381, 0.600	-	0.174, 0.928	0.002, 0.895	0.360, 0.946
		$P_{0.2}$	0.86	1.00		0.95	0.88	0.99
220 d	LAND	Estimate	0.276	0.005	0.210		0.557	0.712
		HPD95%	0.146, 0.410	-0.144, 0.151	0.083, 0.342	-	0.100, 0.893	0.380, 0.929
		$P_{0.2}$	0.87	0.00	0.55		0.92	0.99
	LSKA	Estimate	0.102	0.257	0.200	0.233		0.773
		HPD95%	-0.034, 0.233	0.114, 0.392	0.063, 0.341	0.101, 0.367	-	0.494, 0.937
		$P_{0.2}$	0.06	0.80	0.50	0.69		1.00
	LIND	Estimate	0.072	0.131	0.336	0.435	0.430	
		HPD95%	-0.064, 0.208	-0.025, 0.271	0.216, 0.459	0.316, 0.536	0.325, 0.527	-
		$P_{0.2}$	0.02	0.18	0.97	1.00	1.00	

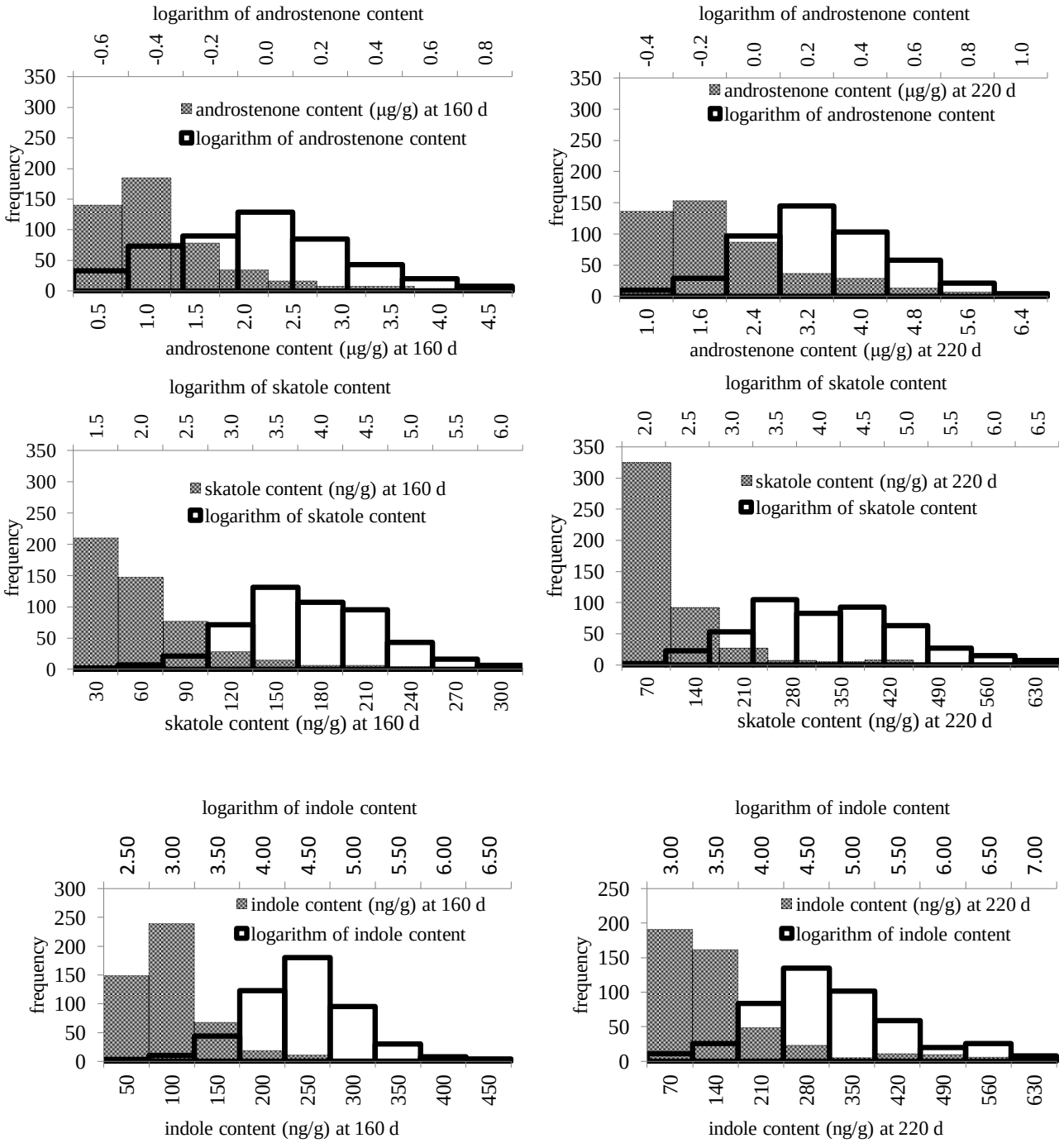
[1]LAND: natural logarithm of androstenone concentration (µg/g); LSKA: natural logarithm of skatole concentration (ng/g); LIND: natural logarithm of indole concentration (ng/g);

Table 4. Estimates, 95% highest posterior density interval (HPD95%) and posterior probability ($P_{|r|>0.1}$) of the estimate being greater than 0.1 (for positive estimates) or lower than -0.1 (for negative estimates) for additive genetic correlations between growth traits and concentrations of boar taint compounds quantified at 160 and 220 d of age in adipose tissue[1]

Growth trait	**Boar taint compound**	**Age at measurement of boar taint compounds**					
		160 d			**220 d**		
		Estimate	HPD95%	$P_{\|r\|>0.1}$	Estimate	HPD95%	$P_{\|r\|>0.1}$
BW160	LAND	0.162	-0.458, 0.664	0.58	-0.367	-0.746, 0.104	0.85
	LSKA	-0.510	-0.785, 0.058	0.95	0.241	-0.230, 0.694	0.71
	LIND	-0.142	-0.653, 0.515	0.55	-0.055	-0.489, 0.594	0.43
BW220	LAND	0.113	-0.535, 0.621	0.51	-0.113	-0.561, 0.435	0.52
	LSKA	-0.394	-0.787, 0.125	0.85	0.343	-0.167, 0.760	0.80
	LIND	0.057	-0.507, 0.700	0.46	0.281	-0.261, 0.895	0.71
BFT220	LAND	0.132	-0.470, 0.750	0.53	-0.052	-0.709, 0.540	0.45
	LSKA	0.136	-0.368, 0.611	0.55	-0.238	-0.801, 0.301	0.69
	LIND	-0.073	-0.603, 0.637	0.40	0.140	-0.390, 0.676	0.57
ADG160-220	LAND	-0.230	-0.820, 0.560	0.60	0.156	-0.564, 0.720	0.56
	LSKA	-0.040	-0.613, 0.665	0.42	0.354	-0.286, 0.889	0.76
	LIND	0.199	-0.432, 0.914	0.62	0.390	-0.310, 0.926	0.77

[1]LAND: natural logarithm of androstenone concentration (µg/g); LSKA: natural logarithm of skatole concentration (ng/g); LIND: natural logarithm of indole concentration (ng/g); BW160: BW (kg) at 160 d; BW220: BW (kg) at 220 d; BFT220: P2 ultrasound backfat depth (mm) measured at 220 d above the last rib at approximately 6 cm from midline using an A-mode ultrasonic device; ADG160-220: average daily gain (g/d) from 160 to 220 d of age.

Figure 1. Frequency distributions of boar taint compounds content assessed at 160 and 220 d and of their natural logarithmic transformation[1]



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CHAPTER 4
New models in genetic evaluation of heavy pigs

Direct and social genetic effects on body weight at 270 days, carcass and ham quality traits in heavy pigs

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ABSTRACT

The aims of this study were to estimate covariance components for BW at 270 d (**BW270**), carcass and ham quality traits in heavy pigs using models accounting for social effects and to compare the ability of such models to fit the data relative to models ignoring social interactions. Phenotypic records were from 9,871 pigs sired by 293 purebred boars mated to 456 crossbred sows. Piglets were born and reared at the same farm and randomly assigned at 60 d of age to groups (6.1 pigs per group on average) housed in finishing pens, each having an area of 6 m^2. The average additive genetic relationship among group mates was 0.11. Pigs were slaughtered at 277 ± 3 d of age and 169.7 ± 13.9 kg BW in groups of nearly 70 animals each. Four univariate animal models were compared: a basic model (**M1**) including direct additive genetic effects only, a model (**M2**) with non-heritable social group (pen) effects in addition to effects in M1, a model (**M3**) accounting for litter effects in addition to M2 and a model (**M4**) accounting for social genetic effects in addition to effects in M3. REML estimates of covariance components were obtained for BW270, carcass backfat depth (**BFT**) and lean meat (**CLM**), iodine number (**IOD**) and linoleic acid content (**LIA**) of raw ham subcutaneous fat, subcutaneous fat depth in the proximity of semimembranosus (**SFD1**) and quadriceps femoris (**SFD2**) muscles, and linear scores for ham round shape (**RS**), subcutaneous fat (**SF**), and marbling (**MB**). Likelihood ratio tests indicated that, for all traits, M2 fit the data better than M1 and that M3 was superior to M2 except for SFD1 and SFD2. Model M4 was significantly better than M3 for BW270 ($P < 0.001$) and CLM, IOD, RS, and SF ($P < 0.05$). The contribution of social genetic effects to the total heritable variance was large for CLM and BW270, ranging from 33.2 to 35%, whereas the one for ham quality traits ranged from 6.8 (RS) to 11.2% (SF). Direct and social genetic effects on BW270 were uncorrelated, whereas there was a negative genetic covariance between direct and social effects on CLM, IOD, RS, and SF, which reduced the total heritable variance. This variance, measured relative to phenotypic variance, ranged from 21 (CLM) to 54% (BW270). Results indicate that social genetic effects affect variation in traits relevant for heavy pigs used in dry-cured hams manufacturing. Such effects should be exploited and taken into account in design of breeding programs for heavy pigs.

Key words: carcass traits, genetic parameters, ham quality, heavy pigs, social genetic effects, variance components

INTRODUCTION

The potential of a population to respond to selection depends on heritable variation. In quantitative genetics theory, it is equal to the additive genetic variance of traits (Falconer and Mackay, 1996). In social species, variation in traits may also depend on social interactions between conspecifics (Moore et al., 1997). The effects of an individual's genes on phenotypes of group mates are known as social genetic effects, indirect genetic effects or heritable social effects (Moore et al., 1997; Bijma et al., 2007a). If

their contribution to the additive genetic variance is ignored, the heritable variation and the response to selection may be estimated incorrectly.

Effects of social genetic components were studied in a wide range of animal species as pigs (Bergsma et al., 2008), poultry (Muir, 2005; Ellen et al., 2008), deer (Wilson et al., 2011) and mink (Alemu et al. 2014). In pigs, the role of social genetic effects was evaluated focusing on variation in average daily gain (Arango et al., 2005; Chen et al., 2008; Bouwman et al., 2010), feed intake, backfat and muscle depth (Bergsma et al., 2008; Hsu et al., 2010), and androstenone content in fat (Duijvesteijn et al., 2012). Most of these investigations were on pigs of light BW and fed ad libitum. No study investigated social genetic effects in heavy pigs, which are fed in restricted conditions, slaughtered at 160 kg BW and used for production of Protected Designation of Origin (**PDO**) dry-cured hams (Bosi and Russo, 2004). The production of heavy pigs involves different conditions relative to light pigs. Such differences might affect social interactions among pigs raised in groups and the magnitude of social effects.

The aims of this study were to estimate covariance components for BW at 270 d, carcass and ham quality traits in heavy pigs using models accounting for social effects and to compare the ability of such models to fit the data relative to models ignoring social interactions.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not needed because animals providing data for the study were subjected to standard production conditions and no additional measurements were taken. Observations used in this study were from the sib-testing program of the C21 Goland sire line (Gorzagri, Fonzaso, Italy) and were registered at the farm where the program is carried out from 1998 to 2013. The farm operates in line with regulations of the Italian law on protection of animals.

Animals and data records

Observations on growth performance, carcass and ham quality traits used in this study were from 9,871 crossbred finishing pigs produced in the sib-testing program of the C21 Goland sire line (Gorzagri, Fonzaso, Italy). Pigs (4,759 gilts and 5,112 barrows) were offspring of 293 boars of the C21 line mated to 456 crossbred Goland sows. Crossbred sows originated from a cross involving boars of a synthetic line, derived from Large White and Pietrain breeds, and sows of a Large White line selected for maternal ability and prolificacy. Besides growth and residual feed efficiency, the breeding goal of the C21 line includes traits related to the quality of dry-cured ham as detailed by Cecchinato et al. (2008).

Crossbred piglets were born and reared at the same farm. Piglets were tail docked and male piglets were castrated within 5 d after birth. At 28 d of age, piglets were weaned and randomly assigned to groups of approximately 30 individuals. At 60 d of age, pigs were housed in finishing pens, each having an area of 6 m^2, and formed social groups containing from 4 to 7 individuals. Each pen was equipped with a nipple

drinker providing pigs with water continuously. Average size of social groups was 6.12 ± 0.82 and the average additive genetic relationship among social group members was 0.11 ± 0.02.

Finishing pigs were reared under consistent feeding conditions. Up to 75 kg BW, pigs were fed two diets *ad libitum*: diet A (17.6% CP and 13.2 MJ of ME/kg) was provided from 25 to 40 kg BW, whereas diet B (16.2% CP and 12.9 MJ of ME/kg) was fed up to 75 kg BW. From 75 kg BW onward, restricted feeding was used. From 75 to 110 kg BW, pigs were fed a diet containing 15.5% CP and 12.5 MJ of ME/kg, whereas CP content was reduced to 14% from 110 kg BW onward (Bonfatti et al., 2011). Major ingredients used to prepare the finishing diet were maize (450 g/kg ad fed), barley (312 g/kg as fed), wheat bran (98 g/kg as fed), sunflower meal (59 g/kg as fed), and durum wheat flour shorts (52 g/kg as fed). Lipid content was 33.9 g/kg as fed and linoleic acid content (**LIA**) was 14.7 g/kg as fed.

Pigs were slaughtered at 277 ± 3 d of age and 169.7 ± 13.9 kg BW in groups of about 70 animals each. All members of a social group were slaughtered in the same slaughter day. Final BW was adjusted to 270 d of age (**BW270**, kg) on the basis of individual linear regressions of BW on age estimated using 6 BW measures (at 60, 90, 135, 180, 245 d of age and the day before slaughter). The Fat-O-Meater optical probe was used to assess carcass backfat depth (**BFT**, mm) and loin depth. These measures were taken through a section of LM between the 10^{th} and 11^{th} rib, inlet 8 cm from the mid-line and exit 4 cm from the mid-line split. Carcass lean meat content (**CLM**, %) was estimated on the basis of the regression:

$$y = 45.371951 - 0.221432\,x_1 + 0.055939\,x_2 + 2.554674\,x_3$$

where x_1 is the Fat-O-Meater measure of backfat depth (including skin, mm), x_2 is the Fat-O-Meater measure of loin depth (mm), and $x_3 = x_2/x_1$. Subcutaneous fat linoleic acid content (**LIA,** %) and iodine number (**IOD**) of raw ham were estimated through calibration equations based on reflectance of trimmed fat measured with near-infrared spectroscopy in individual samples taken from the left thigh. Such calibration equations, developed through the years, provide very accurate estimates of LIA and IOD due to R^2 in cross-validation greater than 95%. Ham subcutaneous fat depth was measured in the proximity of semimembranosus (**SFD1**, mm) and quadriceps femoris (**SFD2**, mm) muscles using a gauge and a portable ultrasound system (Aloka SSD 500 equipped with the UST-5512 7.5 MHz linear transducer probe), respectively. One trained expert performed subjective evaluation of left thighs. All hams were scored, using a linear grading system, for round shape (**RS**; from 0 = low roundness to 4 = high roundness), subcutaneous fat (**SF**; from -4 = low depth to 4 = high depth), and marbling of the visible muscles of the thigh (**MB**; from 0 = low to 4 = high).

Pedigree information was available for all slaughtered pigs and for all C21 Goland boars, whereas only the sire, the maternal grandsire and granddam were known for the dams of the crossbred finishing pigs. Additive relationships between C21 Goland boars were traced back for as many generations as possible. For slaughter animals, additive relationships were computed on the basis of at least 6 generations of known ancestors. Sire and dam of each slaughter pig were unrelated.

Statistical analysis

Covariance components were estimated using average information REML procedures (Gilmour et al., 1995) based on the following univariate linear mixed models:

Model 1 (**M1**) : $\mathbf{y = Xb + W_D a_D + e}$,

Model 2 (**M2**) : $\mathbf{y = Xb + W_D a_D + Zg + e}$,

Model 3 (**M3**) : $\mathbf{y = Xb + W_D a_D + Zg + Vf + e}$,

Model 4 (**M4**) : $\mathbf{y = Xb + W_D a_D + W_S a_S + Zg + Vf + e}$,

where **y** is a vector of observed phenotypes for one trait; **b** is a vector of non-genetic fixed effects which included sex (female and castrated male) and slaughter group effects, **g** is a random vector of social group (animals grouped together in the same pen) effects, $\mathbf{a_D}$ is a random vector of direct additive genetic effects, **f** is a random vector of full-sibs family (i.e., individuals born in the same "biological" litter) effects, $\mathbf{a_S}$ is a random vector of heritable social effects, **e** is a vector of random residuals, and **X**, **Z**, $\mathbf{W_D}$, $\mathbf{W_S}$, and **V** are incidence matrices relating **b**, **g**, $\mathbf{a_D}$, $\mathbf{a_S}$, and **f** to **y**, respectively.

Assumptions on the probability distributions of social group effects, full-sibs family effects, and residuals were:

$$\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_g),\ \mathbf{f} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_f), \text{ and } \mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_e),$$

where $N(\ )$ indicates a normal distribution, **I** is an identity matrix of appropriate order, and σ^2_g, σ^2_f, and σ^2_e are variance components. In models M1, M2, and M3, direct additive genetic effects were assumed to be generated from the following probability distribution:

$$\mathbf{a_D} \sim N(\mathbf{0}, \mathbf{A}\sigma^2_{a_D})$$

where **A** is the numerator relationship matrix and $\sigma^2_{a_D}$ is the variance of direct additive genetic effects. In model M4, the assumption about the probability distribution of $\mathbf{a_D}$ was updated to take into account the covariance with $\mathbf{a_S}$. The vectors $\mathbf{a}_D$ and $\mathbf{a}_S$ were assumed to have a multivariate normal probability distribution:

$$\begin{bmatrix}\mathbf{a_D}\\ \mathbf{a_S}\end{bmatrix} \sim MVN(\mathbf{0}, \mathbf{A} \otimes \mathbf{C}),$$

where $\mathbf{C} = \begin{bmatrix}\sigma^2_{a_D} & \sigma_{a_D a_S}\\ \sigma_{a_D a_S} & \sigma^2_{a_S}\end{bmatrix}$, $\sigma^2_{a_S}$ is the variance of heritable social effects, $\sigma_{a_D a_S}$ is the covariance between direct and social genetic effects, and $\otimes$ denotes the Kronecker product.

Litter effects are routinely included in the genetic analyses of pig data, to account for non-genetic covariances between full sibs due to the shared maternal environment. In this study, due to the characteristics of the investigated traits, such covariances are expected to be trivial. The purpose of including full-sibs family (i.e., the "biological" litter) effects was of accounting for covariances between full sibs due to shared non-additive genetic effects. Estimates of covariance components were obtained

through the ASReml software (Gilmour et al., 2009). The ability of the investigated models to fit the data was compared through log-likelihood ratio tests (LRT), which used as test statistic:

$$LRT = -2(\text{LogL}_{\text{reduced model}} - \text{LogL}_{\text{full model}})$$

where LogL is the log-likelihood.

Based on Bijma et al. (2007b) and Bergsma et al. (2008), the phenotypic variance (σ^2_p) was computed as:

Model M1: $\sigma^2_p = \sigma^2_{a_D} + \sigma^2_e$,

Model M2: $\sigma^2_p = \sigma^2_{a_D} + \sigma^2_g + \sigma^2_e$,

Model M3: $\sigma^2_p = \sigma^2_{a_D} + \sigma^2_g + \sigma^2_f + \sigma^2_e$,

Model M4: $\sigma^2_p = \sigma^2_{a_D} + (n - 1)\, r\, [2\sigma_{a_D a_S} + (n - 2)\, \sigma^2_{a_S}] + (n - 1)\, \sigma^2_{a_S} + \sigma^2_g + \sigma^2_f + \sigma^2_e$,

where n is the average size of social groups and r is the average additive relationship among group mates.

For traits affected by heritable social effects, the total heritable variation exploitable in selection is the variance of total breeding values (**TBV**; Bijma et al., 2007a). The TBV is defined as:

$$\text{TBV}_i = a_{D_i} + (n - 1)a_{S_i}$$

and is the heritable effect of an individual on the phenotypic mean of a trait, which depends on the direct effect a_{D_i} of the individual on its phenotype and on the social effect a_{S_i} exerted on the phenotype of n-1 group mates. When a non-null covariance between direct and social genetic effects exists, the variance of TBV is:

$$\sigma^2_T = \sigma^2_{a_D} + 2\,(n - 1)\,\sigma_{a_D a_S} + (n - 1)^2\,\sigma^2_{a_S}$$

The ratio of σ^2_T to σ^2_p is called T^2 (Bergsma et al., 2008) and can be compared with the classical h^2 ($h^2 = \sigma^2_{a_D}/\sigma^2_p$) to evaluate the contribution of heritable social effects to the phenotypic variance. To evaluate the contribution of full-sibs family and social group effects (i.e., non-heritable social effects) to the phenotypic variance, we computed $f^2 = \sigma^2_f/\sigma^2_p$ and $g^2 = \sigma^2_g/\sigma^2_p$.

RESULTS AND DISCUSSION

Number of pigs, full-sibs families, social groups, slaughter groups and descriptive statistics for the investigated traits are presented in Table 1. Number of records, groups, and families was variable across traits because recording of individual phenotypes started at different times for different traits. The number of phenotypic records and social groups ranged from 4,191 to 9,871 and from 703 to 1,645, respectively. These numbers are much larger than minimum numbers suggested by Bijma (2010) to estimate heritable social effects when an optimum design is used.

Age and BW at slaughter were within common ranges for finishing pigs available in Italy for dry-cured ham production (Lo Fiego et al., 2000). Heavy BW at slaughter is primarily related to the need, for

raw hams weighing at least 10 kg, to comply with requirements dictated by guidelines for PDO dry-cured ham production. Final age of finishing pigs is constrained to a minimum of 9 mo to ensure optimal body tissue composition. Additional requirements (Bosi and Russo, 2004) relate to ham fat thickness (15 mm as minimum) and thigh subcutaneous fat quality assessed as iodine number (70 as maximum) and linoleic acid content on total fatty acids (15% as maximum).

Likelihood-ratio tests

The ability of the investigated models to fit the data was compared through likelihood-ratio tests using M1 (i.e., a model including fixed effects and the direct additive genetic effect of the animal) as the basic model. Results are presented in Table 2. Accounting for non-heritable social effects (social group effects) increased significantly (M2 vs. M1; $P < 0.018$ for SFD2 and $P < 0.001$ for the remaining traits) the ability of the statistical model to fit the data for all traits. In models investigating phenotypic variation among interacting individuals, social group effects are a computational-efficient alternative to fitting a residual covariance between group members (Bergsma et al., 2008; Chen et al., 2008), which is needed to avoid biased estimates of the genetic parameters (Bijma et al., 2007b; Chen et al., 2009).

In addition to social group and direct additive genetic effects, M3 accounted for the random effects of the full-sibs family (i.e., the "biological" litter). Litter effects are routinely included in the genetic analyses of pig data. The use of the "biological" or, alternatively, of the "nursed" litter in the model, to account for non-genetic covariances among full sibs, depends on the rate of cross-fostering and on the availability of detailed information on nursing. In this study, covariances among full sibs due to the shared maternal environment are expected to be trivial because trait values were measured very far from weaning. However, full sibs may share additional effects, other than those arising from the maternal environment or the additive gene action. Full sibs share not only alleles, but also genotypes and, as a consequence, non-additive genetic effects. These effects create a covariance among members of a full-sibs family and may lead to biased estimates of genetic variances and related parameters if not properly accounted for by statistical models. Hence, full-sibs family effects were included in M3 to account for such covariances. Model M3 exhibited enhanced performance, when compared with M2, in describing variation in phenotypes for most traits (M3 vs. M2; $P < 0.05$) with the only exceptions of SFD2 ($P = 0.136$) and SFD1 for which full-sibs family effects were borderline significant ($P = 0.067$).

Addition of social genetic effects to M3 (M4) enhanced model fitting for BW270 ($P < 0.01$) and CLM, IOD, RS, and SF ($P < 0.05$). Accounting for social genetic effects has been reported to enhance model performance in a number of recent studies on different species and traits (Bergsma et al., 2008; Chen et al., 2008; Ellen et al., 2008, Duijvesteijn et al., 2012; Alemu et al., 2014). In pigs, relevant social genetic effects have been detected for growth-related traits and feed consumption. The significant improvement of model

performance detected in this study for CLM, IOD, RS, and SF indicates that social interactions among heavy pigs affect also variation in carcass and ham quality traits.

Estimates of covariance components

Estimates of covariance components obtained with the 4 investigated models are presented in Table 3 for BW270 and carcass traits and in Table 4 for ham quality traits. Addition of social group (pen) effects to M1 decreased the estimated residual variance, but exerted minor effects on the estimate of the direct genetic variance, which, with M2, slightly decreased (BW270) or increased (BFT, CLM, and all ham quality traits). The decrease in the residual variance ranged from 2.8 (SFD2) to 8.6% (CLM). Hence, social group effects accounted, as expected, for non-heritable social variance and were essentially not confounded with direct additive genetic effects. Bergsma et al. (2008) detected a decrease in the estimated genetic, litter and residual variances when pen effects were included in models analyzing variation in growth rate and feed intake in pigs. They suggested that omitting pen effects from the models might inflate the estimated genetic variance due to the relatedness among pen mates. In our study, the average additive genetic relationship among group members was 40% lower than the average relatedness reported by Bergsma et al. (2008) and might explain differences between the 2 studies in the behavior of models including pen effects.

When full-sibs family (litter) effects were accounted for by the model (M3), no substantial change was observed, relative to estimates obtained with M2, in the non-heritable social and residual variance. However, direct additive genetic variances ($\sigma^2_{\mathbf{a_D}}$) decreased significantly. Such decrease was smaller, ranging from 4.5 to 9%, for ham quality traits than was for BW270 and carcass traits. Relative to M2, the decrease in $\sigma^2_{\mathbf{a_D}}$ estimated with M3 was of 15.2, 13.1, and 15.5% for BW270, BFT, and CLM, respectively. Because pigs were allocated to pens randomly, the probability of forming groups constituted by individuals from the same litter and the confounding of litter and social group effects were minimized. This enabled separation of non-heritable social and litter variances in the estimation process. Arango et al. (2005) reported that the direct additive genetic variance for daily gain in Large White gilts, when estimated accounting for non-heritable social effects in the model, but ignoring litter effects, was of magnitude similar to the sum of litter plus additive variance when both these sources of variation were taken into account in the analysis. As previously argued, ignoring contributions of full-sibs family effects to the overall variance might inflate the estimated direct genetic variances as a consequence of the covariance that non-additive genetic effects create among family members and might result in biased estimates of genetic parameters.

Model M4 included heritable social effects. Accounting for heritable social effects had trivial effects on the magnitude of the variances estimated for direct genetic, full-sibs family, and residual effects. However, in comparison with M3, the estimated variance for social group effects exhibited a large decrease

(-60.5%) for BW270 and a substantial increase for CLM (+35.9%), IOD (+35.6%), RS (+64.3%) and SF (+33.7%). To date, no study has reported an increase in the estimated social group variance when heritable social effects were included in the models. Conversely, a decrease in this variance, that might suggest a degree of confounding between social group effect and associative component (Cantet and Cappa, 2008), was observed with models used to analyze variation in growth rate and feed intake (Bergsma et al., 2008), daily gain in gilts (Arango et al., 2005), and concentration of androstenone in boars (Duijvesteijn et al., 2012).

Social genetic variances ($\sigma^2_{\mathbf{a_S}}$) were smaller than were estimates for $\sigma^2_{\mathbf{a_D}}$, but contributed markedly to the total heritable variance ($\sigma^2_{\mathbf{T}}$) in BW270 and CLM (Table 3). The contribution, as measured by the ratio $(\mathrm{n}-1)^2\sigma^2_{\mathbf{a_S}}/\sigma^2_{\mathbf{T}}$, where n is the average group size (6.1 in this study), ranged from 33.2 (CLM) to 35% (BW270). For ham quality traits (Table 4), contributions of $\sigma^2_{\mathbf{a_S}}$ to $\sigma^2_{\mathbf{T}}$ were smaller and ranged from 6.8 (RS) to 11.2% (SF). The correlation between direct and social additive genetic effects (data not reported in tables) was not significantly different from zero ($P > 0.05$) for BW270, suggesting independence between direct and social breeding values. The same correlation was negative and significantly different from zero ($P < 0.05$) for CLM (r = -0.72), IOD (r = -0.58), RS (r = -0.93), and SF (r = -0.64) indicating that, for these traits, pigs with positive direct breeding value affect negatively, through social genetic effects, the phenotype of their group mates. Moreover, the negative covariance between direct and social genetic effects reduces the total heritable variance exploitable in selective breeding. For CLM, IOD, RS, and SF, $\sigma^2_{\mathbf{T}}$ was 56.7, 75.5, 65.1, and 70.8 % of $\sigma^2_{\mathbf{a_D}}$, respectively. Ignoring heritable social effects acting on CLM and ham quality traits leads to an overestimation of the variance exploitable in selection and, as a consequence, of the response achievable in breeding programs. In Italy, CLM, SF, and IOD are important traits for heavy pigs (Bosi and Russo, 2004). Current guidelines for PDO dry-cured ham production dictate that thighs obtained from carcasses with CLM > 55%, exhibiting insufficient subcutaneous fat depth or IOD > 70 cannot be used in manufacture of PDO dry-cured hams. Currently, genetic evaluation of breeding candidates in sire lines used to produce heavy pigs is performed for all these traits with models neglecting heritable social effects and an overestimation of the actual response to selection might occur.

Estimates of genetic parameters

Estimates of genetic parameters are presented in Table 5 for BW270 and carcass traits and in Table 6 for ham quality traits. The proportion of phenotypic variation attributable to non-heritable social group (g^2) and full-sibs family (f^2) effects was small, irrespective of the model used, ranging from 2 to 6.7% and from 1.5 to 4.7%, respectively. The estimated direct heritabilities ($\mathrm{h}^2_{\mathrm{d}}$) were of intermediate magnitude for all models and traits, ranging from 0.231 ± 0.031 for BFT (M3) to 0.449 ± 0.038 for SFD2 (M1). When full-sibs family effects were neglected in the analysis (M1 and M2), $\mathrm{h}^2_{\mathrm{d}}$ were larger than those obtained with M3 and

M4, as a consequence of inflated estimates of variance due to direct genetic effects. Such difference in the estimated h_d^2 across models excluding or including full-sibs family effects was greater for BW270 than for the other traits. Accounting for heritable social effects (M4) had minor effects on the estimated h_d^2 which were similar to those obtained with M3. Social heritabilities (h_s^2) were very low for all traits. However, the contribution of the social genetic variance to the total phenotypic variance, as measured by the ratio $(n-1)^2\sigma_{as}^2/\sigma_p^2$, was small (ranging from 1.7 and 3.3%) for ham quality traits, but was 19 and 7% for BW270 and CLM, respectively (Table 5). In the literature, estimates of social genetic variance have been reported for different species and traits and for variable sizes of social groups and relatedness among group mates (Bijma, 2013). The proportion of total phenotypic variability attributable to social genetic effects has been described to be substantial for traits, like social dominance (Moore et al., 2002; Wilson et al., 2011; Sartori and Mantovani, 2013) or aggressiveness (Løvendahl et al., 2005; Wilson et al., 2009; Alemu et al., 2014), which require a social environment to be expressed. Heritable social effects have been reported to play a relevant role also in survival of laying hens (*Gallus domesticus*) due to group mates cannibalism and aggressions, whose expression is markedly affected by the social environment (Bijma et al., 2007b; Ellen et al., 2008). Body weight, carcass and ham quality traits are pig attributes not directly related to the social environment and a small estimated variance for social genetic effects on these traits, relative to characteristics tightly associated to the social environment, was partly expected (Moore et al., 1997). In this study, the model including heritable social effects (M4) performed better than M3 (Table 2) for BW270, CLM, IOD, RS and SF, but not for BFT, LIA, SFD1, SFD2, and MB. For BFT, M4 yielded a very small (1.7%) estimated contribution of the social genetic variance to the total phenotypic variance (Table 5). Consistent with findings of our study, Bergsma et al. (2008) and Hsu et al. (2010) did not observe any enhancement of model fitting when genetic effects attributable to social interactions were included in the model analyzing ultrasonic BFT in light pigs. The contribution of the total heritable variance to σ^2_p (**T^2**) estimated in this study for BW270 was 0.54 (Table 5), which is 1.57 times h_d^2 ($h_d^2 = 0.343$ with M4). This indicates that heritable social effects contribute considerably to the variation in BW270 exploitable in selection. An initial estimate of T^2 for daily BW gain in light pigs was reported by Bergsma et al. (2008) to be 0.71. In a subsequent study, however, Bergsma et al. (2013) estimated a substantially lower T^2, which is 37% lower (0.34 vs. 0.54) than that estimated in our study for BW270, suggesting a greater influence of heritable social effects on growth traits in heavy pigs when compared to light pigs.

To our knowledge, this is the first study investigating the role of social effects in heavy pigs traits variation. The production of heavy pigs involves a number of different conditions in comparison with light pigs. Such differences may lead to differences in interactions among group mates and, as suggested by Chen et al. (2008), in the magnitude of social effects. Feed restriction is imposed to heavy pigs in order to attain maximum protein growth conditional on optimal fat deposition in the carcass and fat covering in the

thighs (Bosi and Russo, 2004). For the animals involved in this study, feed restriction started when they reached 75 kg BW (Bonfatti et al., 2011). It can be argued that restricted feeding might emphasize, relative to ad libitum feeding, competition among pen mates even when optimal access to feed is guaranteed to all members of the group. Differences in age and weight at slaughter between light and heavy pigs imply differences in space allowance requirements. Thus, an increased level of competition, which may possibly lead to increased expression of aggressiveness, is expected when heavy pigs are housed under situations of high density (Martelli et al., 2003; Morrison et al., 2003). Alternatively, under fixed housing facilities, an optimum in space allowance may be pursued through a decrease in the average size of groups. Size of social group has been described as an important characteristic, able to affect the magnitude of associative effects (Bijma et al., 2007a). In our study, the average size of groups was small relative to that of other studies (Arango et al., 2005; Chen et al., 2008, Hsu et al. 2009), which implies that the time used to interact with each group mate was greater than that for pigs involved in other studies.

In contrast with BW270, the estimated T^2 for the other traits was lower than h_d^2, due to the negative covariance between direct and social genetic effects. Relative to the estimated h_d^2, T^2 exhibited a decrease ranging from 24.6 (IOD) to 43.3% (CLM). These results indicate competition between direct and social genetic effects. Because the response to selection is proportional to the square root of the genetic variance, here represented by $\sigma_{\mathbf{T}}^2$ (Falconer and Mackay, 1996), for the traits investigated in this study and with the only exception of BW270, the potential response is smaller than the expected on the basis of the estimated h_d^2.

In this study, estimation of variance components with a social model was feasible for the investigated traits. The results indicate that non-heritable and heritable social effects affect variation in a number of traits which are important in farming of heavy pigs. Such effects should not be neglected in the genetic evaluation program of breeding candidates of the investigated boar line even when social effects are not included in the breeding goal. Selective breeding focusing on both direct and social genetic effects, however, guarantees increased response from selection relative to breeding based on direct genetic effects only. Most studies on social genetic effects in pigs used phenotypic information collected in testing programs of breeding companies. Hsu et al. (2010) argued that conditions in testing programs of pig lines are not consistent with conditions of commercial farms. This raised the question of whether testing programs of purebred candidates will effectively improve social effects as expressed on commercial farms. In our study, social models were used to analyze records of pigs raised in a testing farm mimicking management and feeding conditions of a commercial farm. Phenotypes were not collected from the breeding candidates, but from their paternal crossbred half sibs. The genetic background of these animals is the same as the one of finishing pigs bred from boars of the C21 line in commercial farms. As a consequence, for our study, some conditions were comparable to those occurring in commercial farms. In

spite of such similarities, possible differences in the size of social groups and average relatedness among group mates cannot be easily overcome and make prompt extrapolation of results to commercial systems unfeasible.

Table 1. Number of pigs, full-sibs families, social and slaughter groups and descriptive statistics for the investigated traits[1]

Trait	Abbreviation	Pigs	Full sibs families	Social groups	Slaughter groups	Mean	SD	P1	P99
Body weight at 270 d, kg	BW270	9,871	1,839	1,645	146	164.2	13.3	132.9	196.3
Carcass traits									
Backfat depth, mm	BFT	8,343	1,557	1,389	124	27	5	17	41
Carcass lean meat, %	CLM	4,191	850	703	66	49.6	2.7	42.8	56.2
Ham quality traits									
Iodine number	IOD	8,055	1,733	1,352	141	66.1	2.6	57.9	74.1
Linoleic acid content, %	LIA	6,287	1,219	1,056	97	12.7	1.4	9.1	16.9
Subcutaneous fat depth, mm									
nearby semimembranosus muscle	SFD1	8,996	1,794	1,495	143	19	14	8	34
nearby quadriceps femoris muscle	SFD2	5,200	1,023	871	80	6	1	4	9
Round shape score	RS	9,362	1,787	1,556	142	1.78	0.85	0	4
Subcutaneous fat score	SF	9,362	1,787	1,556	142	-0.11	1.69	-4	4
Marbling score	MB	9,362	1,787	1,556	142	1.53	0.85	0	4

[1]P1: the 1st percentile; P99: the 99th percentile

Table 2. Likelihood-ratio tests for the investigated models[1,2]

Trait[2]	M2 vs. M1			M3 vs. M2			M4 vs. M3		
	-2 log likelihood	df	*P*	-2 log likelihood	df	*P*	-2 log likelihood	df	*P*
BW270	72.26	1	<0.001	38.58	1	<0.001	28.80	2	<0.001
Carcass traits									
BFT	44.92	1	<0.001	18.70	1	<0.001	1.12	2	0.571
CLM	27.08	1	<0.001	7.32	1	0.007	7.26	2	0.027
Ham quality traits									
IOD	21.82	1	<0.001	7.52	1	0.006	6.90	2	0.032
LIA	14.56	1	<0.001	4.42	1	0.036	3.80	2	0.150
SFD1	42.22	1	<0.001	3.36	1	0.067	2.98	2	0.225
SFD2	5.54	1	0.018	2.22	1	0.136	3.14	2	0.208
RS	10.88	1	<0.001	8.28	1	0.004	6.46	2	0.040
SF	29.42	1	<0.001	12.44	1	<0.001	6.46	2	0.040
MB	65.66	1	<0.001	12.18	1	<0.001	0.22	2	0.900

[1]All models included the fixed effect of sex and slaughter groups. Random effects were: the direct additive genetic effect of the pig for M1; the direct additive genetic effect of the pig and the social group effect for M2; the direct additive genetic effect of the pig, the social group and the full-sibs family effects for M3; the direct additive genetic effect of the pig, the social group and the full-sibs family effects, and the heritable social effects of the group mates for M4. In M4, direct and social genetic effects were assumed to be correlated;

[2]BW270: body weight at 270 d (kg); BFT: carcass backfat depth (mm); CLM: carcass lean meat content (%); IOD: iodine number; LIA: linoleic acid content (%); SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high);
[2]-2 log likelihood: χ^2 test statistic for the likelihood-ratio test = $-2(LogL_{reduced\ model} - LogL_{full\ model})$; df: degrees of freedom for the χ^2 test statistic.

Table 3. Estimates of variance or covariance components for BW at 270 d and carcass traits[1,2]

Parameter[3]	Model	BW270	BFT	CLM
σ_e^2	M1	104.371	13.554	3.477
	M2	96.532	12.667	3.178
	M3	97.107	12.744	3.268
	M4	96.631	12.490	3.102
σ_g^2	M2	9.042	0.922	0.276
	M3	8.930	0.932	0.273
	M4	3.527	1.022	0.371
$\sigma_{a_D}^2$	M1	72.435	7.806	2.367
	M2	71.686	7.869	2.442
	M3	60.792	6.835	2.063
	M4	60.205	7.015	2.074
σ_f^2	M3	8.281	0.762	0.216
	M4	8.292	0.746	0.204
$\sigma_{a_S}^2$	M4	1.280	0.014	0.015
$\sigma_{a_D a_S}$	M4	0.152	-0.119	-0.127
σ_T^2	M4	95.068	6.156	1.176

[1]BW270: body weight at 270 d (kg); BFT: carcass backfat depth (mm); CLM: carcass lean meat content (%);

[2]All models included the fixed effect of sex and slaughter groups. Random effects were: the direct additive genetic effect of the pig for M1; the direct additive genetic effect of the pig and the social group effect for M2; the direct additive genetic effect of the pig, the social group and the full-sibs family effects for M3; the direct additive genetic effect of the pig, the social group and the full-sibs family effects, and the heritable social effects of the group mates for M4. In M4, direct and social genetic effects were assumed to be correlated;

[3]σ_e^2: residual variance; σ_g^2: social group variance; $\sigma_{a_D}^2$: direct additive genetic variance; σ_f^2: full-sibs family variance; $\sigma_{a_S}^2$: social additive genetic variance; $\sigma_{a_D a_S}$: additive genetic covariance between direct and social effects;
σ_T^2: total heritable variance or variance of total breeding values $= \sigma_{a_D}^2 + 2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma_{a_S}^2$.

Table 4. Estimates of variance or covariance components for ham quality traits[1,2]

Parameter[3]	Model	IOD	LIA	SFD1	SFD2	RS	SF	MB
σ_e^2	M1	2.738	0.901	16.344	0.461	0460	1.698	0.396
	M2	2.601	0.861	15.389	0.448	0.445	1.614	0.364
	M3	2.613	0.864	15.396	0.449	0.446	1.615	0.366
	M4	2.523	0.850	15.275	0.446	0.438	1.582	0.368
σ_g^2	M2	0.145	0.042	1.032	0.015	0.014	0.090	0.033
	M3	0.143	0.041	1.031	0.015	0.014	0.089	0.033
	M4	0.194	0.049	1.357	0.006	0.023	0.119	0.035
$\sigma_{a_D}^2$	M1	2.157	0.606	9.020	0.377	0.258	1.086	0.276
	M2	2.166	0.609	9.037	0.377	0.261	1.089	0.278
	M3	2.014	0.570	8.583	0.360	0.243	1.001	0.253
	M4	2.032	0.567	8.106	0.359	0.235	0.982	0.245
σ_f^2	M3	0.112	0.030	0.370	0.012	0.016	0.072	0.018
	M4	0.111	0.030	0.403	0.012	0.016	0.073	0.018
$\sigma_{a_S}^2$	M4	0.006	0.002	0.014	0.003	0.0004	0.003	0.0002
$\sigma_{a_D a_S}$	M4	-0.064	-0.013	-0.318	-0.001	-0.009	-0.035	-0.002
σ_T^2	M4	1.532	0.479	5.238	0.421	0.153	0.695	0.225

[1]IOD: iodine number; LIA: linoleic acid content (%); SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high);

[2]All models included the fixed effect of sex and slaughter groups. Random effects were: the direct additive genetic effect of the pig for M1; the direct additive genetic effect of the pig and the social group effect for M2; the direct additive genetic effect of the pig, the social group and the full-sibs family effects for M3; the direct additive genetic effect of the pig, the social group and the full-sibs family effects, and the heritable social effects of the group mates for M4. In M4, direct and social genetic effects were assumed to be correlated;

[3]σ_e^2: residual variance; σ_g^2: social group variance; $\sigma_{a_D}^2$: direct additive genetic variance; σ_f^2: full-sibs family variance; $\sigma_{a_S}^2$: social additive genetic variance; $\sigma_{a_D a_S}$: additive genetic covariance between direct and social effects;
σ_T^2: total heritable variance or variance of total breeding values $= \sigma_{a_D}^2 + 2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma_{a_S}^2$.

Table 5. Estimates of contributions (SE within parentheses) of different effects in the models to the phenotypic variance of BW at 270 d and carcass traits[1,2]

Parameter[3]	Model	BW270	BFT	CLM
h_D^2	M1	0.410	0.365	0.405
		(0.028)	(0.029)	(0.040)
	M2	0.404	0.367	0.414
		(0.028)	(0.029)	(0.040)
	M3	0.347	0.321	0.354
		(0.029)	(0.031)	(0.045)
	M4	0.343	0.332	0.372
		(0.029)	(0.032)	(0.048)
g^2	M2	0.051	0.043	0.047
		(0.007)	(0.007)	(0.010)
	M3	0.050	0.044	0.047
		(0.007)	(0.007)	(0.010)
	M4	0.020	0.048	0.067
		(0.012)	(0.012)	(0.020)
f^2	M3	0.047	0.036	0.037
		(0.009)	(0.009)	(0.015)
	M4	0.047	0.035	0.037
		(0.009)	(0.009)	(0.015)
h_S^2	M4	0.007	0.001	0.003
		(0.002)	(0.002)	(0.003)
T^2	M4	0.542	0.292	0.211
		(0.081)	(0.078)	(0.121)

[1]BW270: body weight at 270 d (kg); BFT: carcass backfat depth (mm); CLM: carcass lean meat content (%);

[2]All models included the fixed effect of sex and slaughter groups. Random effects were: the direct additive genetic effect of the pig for M1; the direct additive genetic effect of the pig and the social group effect for M2; the direct additive genetic effect of the pig, the social group and the full-sibs family effects for M3; the direct additive genetic effect of the pig, the social group and the full-sibs family effects, and the heritable social effects of the group mates for M4. In M4, direct and social genetic effects were assumed to be correlated;

[3]$g^2 = \sigma_g^2/\sigma_p^2$ where σ_g^2 is the social group variance and σ_p^2 is the phenotypic variance; $h_D^2 = \sigma_{a_D}^2/\sigma_p^2$ where $\sigma_{a_D}^2$ is the direct additive genetic variance; $f^2 = \sigma_f^2/\sigma_p^2$ where σ_f^2 is the full-sibs family variance; $h_S^2 = \sigma_{a_S}^2/\sigma_p^2$ where $\sigma_{a_S}^2$ is the social additive genetic variance; $T^2 = \sigma_T^2/\sigma_p^2$ where $\sigma_T^2 = \sigma_{a_D}^2 + 2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma_{a_S}^2$ is the total heritable variance or variance of total breeding values and n is the average size of the social group.

Table 6. Estimates of contributions (SE within parentheses) of different effects in the models to the phenotypic variance of ham quality traits[1,2]

Parameter[3]	Model	IOD	LIA	SFD1	SFD2	RS	SF	MB
h_D^2	M1	0.441 (0.031)	0.402 (0.034)	0.356 (0.027)	0.449 (0.038)	0.359 (0.027)	0.390 (0.028)	0.411 (0.029)
	M2	0.441 (0.031)	0.403 (0.034)	0.355 (0.027)	0.445 (0.038)	0.362 (0.027)	0.390 (0.028)	0.412 (0.029)
	M3	0.413 (0.033)	0.379 (0.036)	0.338 (0.029)	0.430 (0.040)	0.339 (0.028)	0.361 (0.029)	0.378 (0.030)
	M4	0.427 (0.035)	0.383 (0.037)	0.330 (0.029)	0.430 (0.041)	0.338 (0.029)	0.364 (0.030)	0.370 (0.030)
g^2	M2	0.029 (0.007)	0.028 (0.008)	0.041 (0.007)	0.018 (0.008)	0.019 (0.006)	0.032 (0.007)	0.049 (0.007)
	M3	0.029 (0.007)	0.027 (0.008)	0.041 (0.007)	0.018 (0.008)	0.020 (0.006)	0.032 (0.007)	0.049 (0.007)
	M4	0.041 (0.012)	0.033 (0.013)	0.055 (0.012)	0.007 (0.014)	0.033 (0.010)	0.044 (0.011)	0.053 (0.011)
f^2	M3	0.023 (0.009)	0.020 (0.010)	0.015 (0.008)	0.015 (0.010)	0.022 (0.008)	0.026 (0.008)	0.026 (0.008)
	M4	0.023 (0.009)	0.020 (0.010)	0.016 (0.009)	0.015 (0.010)	0.023 (0.008)	0.027 (0.008)	0.027 (0.008)
h_S^2	M4	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)	0.003 (0.002)	0.001 (0.001)	0.001 (0.001)	0.0003 (0.001)
T^2	M4	0.322 (0.078)	0.324 (0.087)	0.213 (0.068)	0.404 (0.097)	0.220 (0.056)	0.257 (0.063)	0.340 (0.071)

[1]IOD: iodine number; LIA: linoleic acid content (%); SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high);

[2]All models included the fixed effect of sex and slaughter groups. Random effects were: the direct additive genetic effect of the pig for M1; the direct additive genetic effect of the pig and the social group effect for M2; the direct additive genetic effect of the pig, the social group and the full-sibs family effects for M3; the direct additive genetic effect of the pig, the social group and the full-sibs family effects, and the heritable social effects of the group mates for M4. In M4, direct and social genetic effects were assumed to be correlated;

[3]$g^2 = \sigma_g^2/\sigma_p^2$ where σ_g^2 is the social group variance and σ_p^2 is the phenotypic variance; $h_D^2 = \sigma_{a_D}^2/\sigma_p^2$ where $\sigma_{a_D}^2$ is the direct additive genetic variance; $f^2 = \sigma_f^2/\sigma_p^2$ where σ_f^2 is the full-sibs family variance; $h_S^2 = \sigma_{a_S}^2/\sigma_p^2$ where $\sigma_{a_S}^2$ is the social additive genetic variance; $T^2 = \sigma_T^2/\sigma_p^2$ where $\sigma_T^2 = \sigma_{a_D}^2 + 2(n-1)\sigma_{a_D a_S} + (n-1)^2\sigma_{a_S}^2$ is the total heritable variance or variance of total breeding values and n is the average size of the social group.

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Comparison of ranks of animals when direct or competitive models were used

In order to compare ranks of animals based on breeding values (**EBV**) estimated using a direct model (model M3) or, alternatively, a competitive model (model M4 providing EBV as $\mathrm{TBV_i} = \mathrm{a_{D_i}} + [\mathrm{n}-1]\mathrm{a_{S_i}}$ where $\mathrm{a_{D_i}}$ is the direct breeding value, $\mathrm{a_{S_i}}$ is the social breeding value and n is the group size), Spearman's correlation coefficients ($\boldsymbol{r_S}$) were computed for (1) animals with phenotypic record (crossbred pigs) and (2) for C21 nucleus boars (sires of crossbreds with high EBV accuracy) for all the investigated traits (Table 1A).

For BW270, r_S was 0.896 and 0.879 for crossbred pigs and C21 nucleus boars, respectively. The estimated r_S decreased to 0.569 for crossbred animals and to 0.652 for their sires when estimates were computed using data of the animals in the top (best) 10% of rank. These results indicate that the ranking of breeding candidates based on EBV provided by direct model differs largely from the ranking based on predictions from models accounting for both direct and social effects. They support the evidence of relevant contributions of heritable social effects on BW270 variation and highlight the importance of including social genetic effects in genetic evaluation programs of C21 breeding candidates.

Change in rankings for carcass and ham quality traits was limited and r_S were close to one (r_S > 0.97) for both crossbred pigs and nucleus boars. Similar correlations were found when only the top 10% animals with the best EBV were considered, suggesting no relevant difference in the ranking of breeding candidates when social genetic effects were neglected.

The different correlations obtained for BW270 compared to the other traits might be due to the diverse contribution of $\sigma^2_{\mathbf{as}}$ to $\sigma^2_{\mathbf{T}}$ for different traits.

Table 1A. Spearman's correlation coefficients ($\boldsymbol{r_S}$) between rankings based on EBVs from M3 and TBV from M4 for the investigated traits[1]

$\boldsymbol{r_S}$	Trait									
	BW270	BFT	CLM	IOD	LIA	SFD1	SFD2	RS	SF	MB
Full dataset										
crossbred	0.899	0.999	0.997	0.999	0.999	0.998	0.984	0.999	0.999	0.999
nucleus boars	0.879	0.999	0.997	0.998	0.998	0.999	0.970	0.999	0.999	0.999
10% best animals										
crossbred	0.569	0.994	0.978	0.970	0.986	0.987	0.985	0.982	0.988	0.998
nucleus boars	0.652	0.990	0.989	0.997	0.995	0.970	0.998	0.983	0.986	0.997

[1]BW270: body weight at 270 d (kg); BFT: carcass ultrasound backfat depth (mm); CLM: carcass lean meat content (%); IOD: iodine number; LIA: linoleic acid content (%); SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high).

CHAPTER 5
New methods in breeding values prediction

A single-step BLUP procedure for genomic evaluation of a boar line linked to dry-cured ham production

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ABSTRACT

The objective of this study was to develop and to investigate a genomic selection (GS) procedure based on single-step methodology (**SSBLUP**) for genetic evaluation of PB breeding candidates of a boar line for traits relevant for dry-cured ham production.

Individual phenotypes on growth performance, carcass and ham quality traits were recorded from 11,488 crossbred (**CB**) finishing pigs produced in the sib-testing program of the C21 Goland boar line (Gorzagri, Fonzaso, Italy). In addition, for 1,878 pigs enrolled in the study, observations on ham weight losses during the salting (**WLS**), resting, and curing (**WLC**) stages were available, as well as on total ham weight losses occuring during the complete manufacture process of dry-cured ham (**TWL**). A total of 1,088 CB pigs, 136 nucleus boars (**C21_NB**: sires of the CB genotyped animals) and 500 C21 half-sibs of CB animals (**C21_HS**) were genotypes for 8,826 single nucleotide polymorphisms (**SNP**) using the GGP Porcine LD Chip (Neogen Co., Lincoln, NE). After quality control, a total of 7,559 SNP and 1,687 pigs were available for genomic evaluation. Pedigree-based evaluation (**BLUP**) was performed using a model that included, in addition to slaughter group and sex as fixed, the random effect of social group and the additive genetic effects. Genomic evaluation was based on SSBLUP methodology and it was performed using the same model. In order to evaluate the consistence of genomic prediction, the 500 C21_HS (animals without own phenotypes but with genomic information, mimicking the real scenario if GS procedures will be implemented in selection scheme of C21 boar line) were used validation population. Pearson's (r) and Spearman's (r_S) correlation coefficients between traditional breeding values (**EBV**) and genomic breeding values (**GEBV**) were calculated for this group of animals.

Estimates of heritability (h^2) were of moderate or intermediate magnitude, ranging from 0.226 for WLS to 0.603 for stearic acid content in ham subcutaneous fat. A decrease of h^2 was observed for all the investigated traits when estimates were provided by SSBLUP (from 1.5 for iodine number to 17.2% for WLC). High $r_{EBV,GEBV}$ were observed for the genotyped CB pigs and the genotyped C21_NB, suggesting that incorporating genomic information into breeding value prediction lead to estimate genetic merit that are consistent with accurate predictions from traditional methods. Positive $r_{EBV,GEBV}$ were found also for C21_HS in validation population, but the magnitude of the correlations was lower than CB and C21_NB previously evaluated (ranging from 0.361 for WLC to 0.778 for CW). Similar results were obtained when $r_{S_{EBV,GEBV}}$ were considered, suggesting differences in rankings of breeding candidates when genomic prediction are used in place of traditional BLUP. Such changes can be attributable to the ability of SSBLUP to estimate individual GEBV for members of full-sib families, allowing to select the best breeding candidates rather than the best families.

In conclusion, a GS approach based on SSBLUP methodology seems to be a suitable tool for genomic evaluation of C21 boar line. It might simplify the evaluation for traits difficult and expensive to measure, like TWL, to enhance the accuracy of selection and to reduce the rate of inbreeding.

Keywords: genomic breeding values, genomic selection, ham quality, heavy pigs, single-step BLUP

INTRODUCTION

The production of Italian heavy pigs aims at providing thighs for the production of Protected Designation of Origin (**PDO**) dry-cured hams (Bosi and Russo, 2004). Raw thighs are largely obtained from crossbred (**CB**) animals, slaughtered at heavy body weight (**BW**; approximately 160 kg) and advanced age (270 d or more). Breeding programs of boar lines linked to dry-cured ham production must focus also on the enhancement of quality and technological properties of raw thighs. If boars are used as sires of crossbred finishing pigs, the breeding goal of the line is defined at the crossbred level. Enhancement of traits relevant for dry-cured ham production through "traditional" selection procedures is difficult. Currently, evaluation of purebred (**PB**) boar lines linked to dry-cured ham production is based on sib-testing programs, where testing groups of CB half-sibs of PB breeding candidates, are organized, reared and slaughtered to collect phenotypic information used in a combined purebred-crossbred selection (**CCPS**; Wei, 1992). This approach accounts for genetic and environmental differences between PB and CB pigs, but it is troublesome due to costs associated to testing groups and collection of individual phenotypes. In addition, breeding values of breeding candidates belonging to a PB full-sib family are identical, leading to across-families selection and increased rates of inbreeding (Bijma et al., 2001). Implementation of breeding programs based on genomic selection (**GS**, Meuwissen et al., 2001) might facilitate the prediction of genetic merit and selection procedures in boar lines when traits measurable only after slaughter, as ham quality traits, and difficult to record, as traits related to the dry-cured ham making process, are in the breeding goal. Genomic breeding values (**GEBV**) of PB breeding candidates can be estimated with no use of CB phenotypes. Genomic selection has been recently applied in pigs slaughtered at light BW for genomic evaluation of growth performances (Christensen et al., 2012), carcass and meat quality traits (Wellman et al., 2013), reproductive traits (Cleveland et al., 2010; Forni et al., 2010) and boar taint (Azevedo et al., 2014).

The aim of this study was to develop and to investigate a GS procedure based on single-step methodology (**SSBLUP**) for genetic evaluation of PB breeding candidates of a boar line for traits relevant for dry-cured ham production.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not needed because animals providing data for the study were subjected to standard production conditions and no additional measurements were taken. Observations used in this study were from the sib-testing program of the C21 Goland boar line (Gorzagri, Fonzaso, Italy) and were registered at the farm where the program was carried out from 1998 to 2013. The farm operates in line with regulations of the Italian law on protection of animals.

Animals and data records

Individual phenotypes on growth performance, carcass and ham quality traits analyzed in this study were recorded for 11,488 CB finishing pigs produced in the sib-testing program of the C21 Goland boar line (Gorzagri, Fonzaso, Italy). Pigs (5,555 barrows and 5,933 gilts) were progeny of 343 C21 Goland boars mated to 524 Large White-derived CB sows. Crossbred sows originated from a cross involving boars of a synthetic line, derived from Large White and Pietrain breeds, and sows of a Large White line selected for maternal ability and prolificacy. Besides growth and residual feed efficiency, the breeding goal of the C21 line includes traits related to the quality of dry-cured ham as detailed by Cecchinato et al. (2008).

Crossbred piglets were born between February 2003 and January 2013 and reared at the testing farm. Piglets were tail-docked and male piglets were castrated within 5 d after birth. At 28 d of age, piglets were weaned and randomly assigned to groups of approximately 30 individuals. At 60 d of age, pigs were housed in finishing pens and formed social groups containing from 4 to 7 individuals. Each pen had an area of 6 m^2 and was equipped with a nipple drinker providing water continuously. Average social group size was 6.01 and within-pen average additive relationship was 0.110 ± 0.001.

Finishing pigs were reared under consistent feeding conditions, as detailed in Rostellato et al. (2015). Pigs were slaughtered at 279.1 ± 24.3 d of age and 167.9 ± 24.3 kg BW in groups of about 70 animals each. Final BW was adjusted at 270 d (**BW270**, kg) on the basis of individual linear regressions of BW on age estimated using 6 BW measures (at 60, 90, 135, 180, 245 d of age and the day before slaughter). After slaughter, carcasses were weighted (**CW**, kg) and the dressing percentage (**DP**, %) was computed as the ratio of CW to slaughter weight. The Fat-O-Meater optical probe was used to assess carcass backfat depth (**BFT**, mm) and loin depth. Carcass lean meat content (**CLM**, %) was estimated on the basis of the following regression:

$$y = 45.371951 - 0.221432\,x_1 + 0.055939\,x_2 + 2.554674\,x_3$$

where x_1 is the Fat-O-Meater measure of backfat depth (including skin, mm), x_2 is the Fat-O-Meater measure of loin depth (mm), and $x_3 = x_2/x_1$. The weight of right and left thighs of each animal was measured after trimming and averaged (**TTW**, kg). Iodine number (**IOD**), content of linoleic acid (**LIA**, %), of stearic acid (**SA**, %), of omega-6 fatty acids (**Ω6**, %), of polyunsaturated fatty acids (**PUFA**, %) and the ratio of monounsaturated fatty acids content to PUFA (**MO_PO**) were estimated through calibration equations based on near-infrared spectroscopy reflectance of trimmed fat measured in individual samples taken from the left thigh. Ham subcutaneous fat depth was measured in the proximity of semimembranosus (**SFD1**, mm) and quadriceps femoris (**SFD2**, mm) muscles using a portable ultrasound system (Aloka SSD 500 equipped with the UST-5512 7.5 MHz linear transducer probe) and a gauge, respectively. One trained panellist evaluated the quality of raw hams using a linear scoring system. All hams were scored, using a linear grid, for round shape (**RS;** from 0 = low roundness to 4 = high roundness), subcutaneous fat depth (**SF;** from -4 = low depth to 4 = high depth), marbling of the visible muscles of the thigh (**MB;** from 0 = low

marbling to 4 = high marbling), visible muscles colour (**FC**; from -4 = pale to 4 = dark) and amount of blood vessels (**BV**; from 0 = low to 4 = high).

For 1,878 pigs enrolled in the study, left thighs were processed to obtain dry-cured hams following the manufacturing process described in the disciplinary of production of Parma dry-cured ham (Prosciutto di Parma PDO, 1992). Raw thighs were weighted at the end of each production stage, so that ham weight losses during salting (**WLS, %**), resting (**WLR, %**) and curing (**WLC, %**) were calculated as difference between weight at the beginning and at the end of the stage and expressed as percentage of initial weight. In addition, the total weight loss at the end of curing (**TWL, %**) was computed.

Pedigree information was available for all slaughtered pigs and for all C21 Goland boars, whereas only the sire, the maternal grandsire and maternal granddam were known for the dams of CB finishing pigs. Additive relationships among CB finishing pigs, C21 Goland boars and CB dams were traced back for as many generations as possible. For slaughtered animals, additive relationships were computed on the basis of at least 6 generations of known ancestors. Sire and dam of each slaughter pig were unrelated, so that inbreeding at the CB level was 0.

Genotyping

To constitute the reference population to be genotyped, 1,088 CB pigs (314 barrows and 774 gilts) with phenotypic information for the investigated traits were selected. Selected animals were born from January 2010 and October 2012. They were offspring of 136 nucleus boars of the C21 line (about 12 CB pigs per boar) and 215 CB dams and were sampled from all the CB families reared and tested in that period so that each family had, on average, 2 members involved in the genotyping program. Such sampling strategy ensured that the reference population, used when developing GS procedures, was representative of the most recent “genetic features” of the C21 line and CB tested population. In addition, the 136 C21 nucleus boars (**C21_NB;** sires of CB tested pigs and C21 animals) and additional 500 C21 PB males (**C21_HS;** half sibs of CB tested animals) were genotyped. One ear tissue sample per pig was shipped to the GeneSeek Inc. (Lincoln, NE, USA) lab for DNA extraction and genotyping. Genotypes at 8,826 SNPs were assessed using the GGP Porcine LD chip (Genomic Profiler for Porcine LD, GeneSeek Inc., a Neogen Co., Lincoln, NE). SNPs exhibiting call rate < 0.9 or minor allele frequency < 5% and monomorphic SNPs were discarded. DNA samples with call rate < 0.9 and animals with parent-progeny genotype conflicts were excluded from the analysis.

Statistical analysis

Pedigree-based evaluation (**BLUP**) was performed for each trait using the following model:

$$\mathbf{y = Xb + Za + Wg + e}$$

where **y** is the vector of observed phenotypes for one trait; **b** is a vector of non-genetic fixed effects which included sex (male and female) and slaughter group effects, **g** is a random vector of social group (animals sharing the same pen) effects, **a** is a random vector of direct additive genetic effects, **e** is a vector of random residuals, and **X**, **W** and **Z** are incidence matrices relating **b**, **g** and **a** to **y**, respectively.

Assumptions on the probability distributions of social group effects and residuals were:

$$\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_g) \text{ and } \mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_e),$$

where $N(\ )$ indicates a normal distribution, **I** is an identity matrix of appropriate order, and σ^2_g and σ^2_e are variance components. Direct additive genetic effects were assumed to be generated from the following probability distribution:

$$\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma^2_a)$$

where **A** is the numerator relationship matrix and σ^2_a is the variance of direct additive genetic effects.

For each trait, heritability ($\mathbf{h^2}$) was computed as:

$$\mathbf{h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_g + \sigma^2_e)}$$

The contribution of social group effects to the phenotypic variance of a trait ($\mathbf{g^2}$) was calculated as:

$$\mathbf{g^2 = \sigma^2_g / (\sigma^2_a + \sigma^2_g + \sigma^2_e)}$$

Genomic evaluation was performed using SSBLUP method (Misztal et al., 2009; Legarra et al., 2009), where pedigree-based relationship matrix **A** was replaced by **H** matrix that combines pedigree and genomic information with the following inverse (Aguilar et al., 2010):

$$H^{-1} = A^{-1} \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

where $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix and $\mathbf{A_{22}^{-1}}$ is the inverse of the pedigree-based relationship matrix for the genotyped individuals. The G matrix was constructed as described in VanRaden et al. (2008) using allele frequencies calculated from genotyped animals and scaled, for compatibility with A, to have mean of diagonal and off-diagonals elements equal to A.

To compare "traditional" genetic evaluation and genomic predictions of breeding values, Pearson's correlations ($r_{EBV,GEBV}$) between EBV estimated from BLUP and GEBV from SSBLUP were computed for all genotyped CB pigs, all genotyped C21 nucleus and all C21 males and females.

In order to validate prediction equations providing GEBV, the 500 C21_HS with genotype information were used as validation population. Consistency of genomic predictions with "traditional" BLUP predictions was based on $r_{EBV,GEBV}$ computed for this validation set. In addition, Spearman's correlations (r_s) between rankings based on EBV and GEBV were calculated for this group of animals with the aim of comparing the rankings of breeding candidates based on traditional genetic evaluation and genomic predictions. Estimates of (co)variance components, EBV and GEBV were obtained by EM-REML methods using the BLUPF90 family programs (Misztal et al. 2008).

RESULTS AND DISCUSSION

Number of pigs, social and slaughter groups, and descriptive statistics for BW270, carcass and ham quality traits are reported in Table 1. The number of observations and groups was variable across traits because collection of individual phenotypes started at different times for different traits. The number of individual records ranged from 5,946 for CLM to 11,488 for BW270 and CW. The number of social groups varied from 1,010 for CLM to 1,922 for BW270 and CW. To guarantee high quality of dry-cured hams, raw thighs must comply with specific requirements dictated by guidelines issued by Consortia of PDO hams. Animals are slaughtered at 9 months of age or more and at heavy BW (160 kg or more). Averages for weight of trimmed thighs (> 10 kg), IOD (< 70), LIA (< 15%) and subcutaneous fat thickness (> 15 mm) for the sample investigated in this study were consistent with required characteristics of thighs for production of PDO dry-cured hams (Prosciutto di Parma PDO, 1992; Bosi and Russo, 2004). Descriptive statistics for ham weight losses occurring in the manufacturing process of dry-cured hams are presented in Table 2. During the making process, thighs loose nearly 28% of their initial weight (TWL). Specifically, hams loose 3.7% of their initial weight during salting (WLS), whereas during resting (WLR) and curing (WLC) the weight losses are 12.4 and 14.4%, respectively. Weight losses arise from dehydration occurring during the making process and are correlated to the degree thereof. Although dehydration is essential to prevent microbial proliferation and for the development of typical aroma and flavours, excessive weight losses result in reduced final weights and, as consequence, in reduced earnings for producers. Variation in ham weight losses observed in this study was consistent with ranges of values recommended by the disciplinary of production of PDO Parma dry-cured ham.

Single-step BLUP analysis

Genotype data were subjected to quality checks following criteria described in Materials and Methods section. Specifically, 162 SNPs were excluded for call rate < 0.9, 256 SNPs had MAF < 0.05 and 212 SNPs were monomorphic. In addition, 31 animals were excluded due to call rate < 0.9 and 6 animals were not further considered due to inconsistent parent-progeny relationships ascertained on the basis of genomic information. After editing, a total of 7,559 SNPs and 1,687 pigs were available for genomic evaluation.

The single-step method, proposed by Legarra et al. (2009) and Christensen and Lund (2010), extends the traditional pedigree-based BLUP method by incorporating marker information into the relationship matrix, in order to predict breeding values for both genotyped and non-genotyped animals in the pedigree. The construction of G matrix and combination of G and A matrices in H matrix are fundamental to obtain GEBV with high accuracy. Indeed, if G is inflated, deflated or incompatible with A, relative weighing of pedigree and genomic information will be incorrect and GEBV might be biased. In this study, the genomic relationship matrix was constructed using allele frequencies estimated from genotype data of reference animals to increase accuracy of genomic predictions, as suggested by Forni et al. 2011 and Chen et al.,

2011. Moreover, to increase the compatibility with A, the G matrix was scaled as described in Van Raden et al. (2008). The diagonal elements of G were centred to 1 (the average was 1.01, data not reported in tables), indicating lack of mistakes in genotypes and pedigree data or secondary population (Simeone et al. 2011). Such indication was confirmed by the high correlation between elements of G and A (r = 0.764, data not reported in tables).

Estimates of (co)variance components and genetic parameters

(Co)variance components and estimated parameters obtained from BLUP analysis are presented in Table 3. Both carcass and ham quality traits showed moderate or intermediate h^2, ranging from 0.267 for VESS to 0.603 for SA. The contribution of social group effects to the phenotypic variance was small, ranging from 2.2 for VESS to 6.8% for DP. The estimated h^2 for ham weight losses were of moderate magnitude, ranging from 0.226 for WLS to 0.310 for TWL, and were consistent with estimates reported in a previous study (Sturaro, 2004) for a different sample of CB Goland finishing pigs. Estimates of h^2 provided by SSBLUP (Table 4) exhibited limited variation compared to BLUP estimates. Estimates of h^2 consistently decreased (from 1.5 for IOD to 17.2% for WLC) when SSBLUP. For all traits, σ^2_a decreased (from 1.8 for IOD to 18.7% for WLC), whereas σ^2_e increased (from 0.4 for IOD to 4.8% for SFD2). For BW270, carcass and ham quality traits, g^2 slightly increased (from 2.2 for CW to 14.3% for SFD2) or did not change when marker information was included in genetic evaluation procedures. Conversely, for ham weight losses, g^2 decreased (from 4.3 for WLS to 9.0% for WLC) relative to BLUP estimates.

Comparison between BLUP and SSBLUP

Routine prediction of breeding values for the C21 boar line is based on a sib-testing program, where C21 nucleus boars are mated to a group of crossbred sows to sire CB animals providing phenotypic data used to predict genetic merit of C21 PB breeding candidates (half-sibs of CB tested animals). This ensures the availability of phenotypes that are i) specific of traits measurable only after slaughter, ii) measured on CB animals owning the same genetic background of pigs originated by C21 boars in farrow-to-feeder or farrow-to-finish commercial farms, and iii) are affected by non-genetic influences that are comparable to those arising in commercial farms. The accuracy of the estimated breeding values is not large (r = 0.4), but the length of the generation interval is short. Breeding values for TWL are an exception, because CB phenotypes can be measured only at the end of curing and such delayed availability of breeding values is responsible for prolonged generation intervals. This makes inclusion of TWL in the breeding goal of the line unfeasible. In the last 15 years, the sib-testing procedure and the organization of the current breeding program of the C21 line guaranteed consistent genetic trends for all breeding goal traits.

In order to compare genetic evaluation based on a traditional BLUP analysis and genomic predictions performed using SSBLUP, $r_{EBV,GEBV}$ were calculated as Pearson's correlations ($r_{EBV,GEBV}$: correlations between EBV and GEBV) or Spearman's correlations ($r_{s\ EBV,GEBV}$: correlations between rankings based on EBV and GEBV). High $r_{EBV,GEBV}$ were estimated for all traits (Table 5). For CB finishing pigs, owning both phenotypic and genomic information, $r_{EBV,GEBV}$ varied from 0.833 (WLR) to 0.933 (MO_PO). Large estimates for $r_{EBV,GEBV}$ were obtained also for the genotyped C21 nucleus boars. For genotyped C21 nucleus boars, $r_{EBV,GEBV}$ ranged from 0.779 (WLC) to 0.937 (Ω6). These results indicate that incorporating genomic information into breeding value prediction can lead to estimates of genetic merit that are consistent with accurate predictions from traditional methods. Indeed, BLUP breeding values for CB and C21 nucleus boars are predicted with high accuracy (approximately 0.7 for CB and 0.79 for C21 nucleus boars). Routine estimation of genetic merit for the C21 line is aimed specifically at predicting breeding values of PB breeding candidates and not those of CB or nucleus boars. Pearson's correlations estimated for the group of ungenotyped C21 animals (no nucleus boar in this group) were also large, ranging from 0.734 (WLR) to 0.972 (CW). Large estimates for $r_{EBV,GEBV}$ were expected for this group because missing genomic information makes GEBV similar to EBV. Hence, the estimated correlations for this group are not useful to assess the impact of genomic information on the evaluation of C21 breeding candidates in a GS scheme where genotypes are available for the breeding candidates.

The validation population used in this study (C21_HS) included 500 genotyped C21 PB intact males, which were phenotyped for concentrations of BT compounds and enrolled in the study presented in Chapter 3. Such animals do not own phenotypic records for the traits investigated in this study and mimic the condition of a C21 breeding candidate with availability of genomic information (i.e., the condition of a breeding candidate if a genomic selection program will be actually established for the line). Hence, the estimated $r_{EBV,GEBV}$ for this group provide information about the "actual" impact of genomic data on the evaluation of C21 breeding candidates in a GS scheme. The estimated $r_{EBV,GEBV}$ for the validation population are presented in Table 6. For all traits, such estimates were positive, of moderate to high magnitude, but smaller than estimates for CB and C21 nucleus boars . Body weight adjusted at 270 days, carcass and ham quality traits exhibited $r_{EBV,GEBV}$ that were greater than 0.6 or close to, with the exception of RS, MB, WLR, WLC and TWL. Spearman's correlation coefficients between rankings based on EBV and GEBV were very similar to $r_{EBV,GEBV}$ (varying from 0.361 for WLC to 0.778 for CW). These results indicate that differences in rankings of breeding candidates are expected when genomic predictions, based on SSBLUP for carcass traits and qualitative and technological properties of raw hams, are used in place of traditional BLUP predictions. Such changes can be attributed to the use of individual genomic information, in addition to phenotypes of the CB half-sib family, which leads to different predictions of genetic merit for the members of a full-sib PB family. In contrast to traditional pedigree-based predictions, GEBV allow to perform a within-family selection, whereas, traditional methods based on sib-testing lead to a between-

families selection. As depicted in Figure 1, GEBV for C21_HS (validation population) were different not only for different full-sib families, but also for members of the same family. Conversely, breeding values predictions obtained from traditional BLUP, exploiting phenotypes of CB half-sibs, are identical within the same family. The availability of individual GEBV leads to changes in the ranking of animals, so that the selected individuals are the best breeding candidates and not the best families. This is one of the main foreseeable advantages of GS when established for the breeding program of the C21 boar line, able to increase the accuracy of selection and control the rate of inbreeding.

Genomic selection has been recently applied in pig breeding focussing on growth performance (Christensen et al., 2012), meat quality (Wellman et al., 2013) and reproductive traits (Cleveland et al., 2010; Forni et al., 2010; Guo et al., 2014). With special focus on SSBLUP, Christensen et al. (2012) reported that SSBLUP provides more accurate predictions of breeding values compared to traditional genetic evaluation systems based only on phenotypes and pedigrees. Moreover, Guo et al. (2014) indicated that SSBLUP outperformed GBLUP, increasing the accuracy of genomic prediction for both genotyped and non-genotyped animals.

All previous studies investigating GS in swine have been performed on PB pig populations and, at our knowledge, this is the first study focusing on GS for genetic evaluation of PB breeding candidates when the breeding goal is set at the crossbred levels. Combined crossbred-purebred selection (CCPS) of PB breeding candidates for breeding goals defined at the crossbred levels is troublesome, not only for critical issues related to the rate of inbreeding. Breeding companies implementing CCPS must face additional costs arising from organization and management of testing groups and use of reserved logistics for tested animals. Moreover, collection of phenotypes for ham quality traits is very expensive and evaluation of traits related to the manufacture process of dry-cured ham is very challenging due to long time for curing to obtain final products. Although GS requires genotyping of breeding candidates, development of GS procedures might decrease costs associated to phenotyping and trait records and allow inclusion of traits, for which selective breeding is currently unfeasible (TWL), in the breeding goal.

The SSBLUP procedure implemented in our study assumed equal variance for SNPs effects. One question is whether the accuracy of genomic predictions would be greater with a methodology able to account for non-equal variances of markers effects. As a consequence, estimation of GEBV through Bayesian methods should be investigated. The model used for the single step approach investigated in this study didn't account for social genetic effects. Rostellato et al. (2015) reported that heritable social effects affect variation in traits relevant for heavy pig farming, suggesting the importance to include social genetic effects also in genomic evaluations.

In conclusion, a GS approach based on SSBLUP methodology seems to be a suitable tool for genetic evaluation and selection of the C21 boar line and, in general, for lines linked to PDO dry-cured ham production. Implementation of GS procedures in breeding programs of heavy pigs might overcome the limitations of current traditional genetic evaluation systems based on sib-testing programs. The availability

of individual GEBV for breeding candidates might reduce the rate of inbreeding, increase the accuracy of selection, simplify evaluation of traits difficult and expensive to measure, like ham quality and traits related to the manufacture process of dry-cured hams.

Table 1. Number of pigs, social and slaughter groups and descriptive statistics for the investigated traits[1]

Trait	Abbreviation	Pigs	Social groups	Slaughter groups	Mean	SD	P1	P99
Body weight adjusted at 270 d, kg	BW270	11,488	1,922	171	164.5	13.3	133.2	196.2
Carcass traits								
Carcass weight, kg	CW	11,488	1,922	171	140.5	11.8	112.2	169.0
Dressing percentage, %	DP	11,352	1,899	169	82.593	1.675	78.571	86.688
Carcass backfat depth, mm	BFT	10,041	1,688	150	26.6	5.1	17	40
Carcass lean meat, %	CLM	5,946	1,010	92	49.84	2.68	43.22	56.27
Ham quality traits								
Average trimmed thighs weight, kg	TTW	10,774	1,804	161	13.90	1.09	11.38	16.58
Iodine number	IOD	10,878	1,864	169	68.11	2.58	62.20	74.73
Linoleic acid content, %	LIA	8,614	1,463	126	13.810	1.392	10.918	17.511
Stearic acid content, %	SA	8,614	1,463	126	10.426	1.010	8.120	13.070
Omega-6 content, %	Ω6	8,614	1,463	126	14.184	1.412	11.213	17.889
Polyunsaturated fatty acids content, %	PUFA	8,614	1,463	126	15.921	1.573	12.656	20.045
Ratio between monounsaturated and polyunsaturated fatty acids contents	MO_PO	8,614	1,463	126	3.110	0.373	2.156	3.910
Subcutaneous fat depth, mm								
nearby semimembranosus muscle	SFD1	11,216	1,889	168	19	6	8	34
nearby quadriceps muscle	SFD2	7,412	1,259	109	6	1	4	9
Round shape score	RS	11,169	1,877	167	2	1	0	4
Subcutaneous fat score	SF	11,033	1,854	165	0	2	-4	4
Marbling score	MB	11,169	1,877	167	2	1	0	4
Fat colour score	FC	11,034	1,854	165	0	1	-4	3
Blood vessels score	VESS	11,169	1,877	167	1	1	0	3

[1]SD: standard deviation; P1: the 1st percentile; P99: the 99th percentile.

Table 2. Number of pigs, social and slaughter groups and descriptive statistics for ham weight losses during the manufacture process of dry-cured ham[1]

Trait	Abbreviation	Pigs	Social groups	Slaughter groups	Mean	SD	P1	P99
Ham weight loss during salting, %	WLS	1,878	328	28	3.673	0.632	2.276	5.430
Ham weight loss during resting, %	WLR	1,878	328	28	12.401	1.074	9.976	15.019
Ham weight loss during curing, %	WLC	1,878	328	28	14.454	1.962	10.738	20.072
Total ham weight loss, %	TWL	1,878	328	28	27.802	2.359	23.172	34.251

[1]SD: standard deviation; P1: the 1st percentile; P99: the 99th percentile.

Table 3. Estimates of variance components and parameters for the investigated traits using BLUP[1,2]

Trait	σ_g^2	σ_a^2	σ_e^2	g^2	h^2
BW270	8.059	76.800	91.370	0.046	0.436
Carcass traits					
CW	6.006	57.070	73.770	0.044	0.417
DP	0.157	0.637	1.523	0.068	0.275
BFT	0.798	7.901	11.770	0.039	0.386
CLM	0.206	2.568	3.136	0.035	0.435
Ham quality traits					
TTW	0.053	0.562	0.540	0.045	0.487
IOD	0.189	2.174	2.899	0.036	0.413
LIA	0.053	0.671	0.789	0.035	0.443
SA	0.017	0.411	0.253	0.025	0.604
Ω6	0.056	0.700	0.839	0.035	0.439
PUFA	0.068	0.851	0.991	0.036	0.446
MO_PO	0.005	0.053	0.057	0.039	0.461
SFD1	0.963	9.742	16.260	0.036	0.361
SFD2	0.011	0.348	0.501	0.012	0.405
RS	0.013	0.259	0.432	0.018	0.368
SF	0.098	1.098	1.593	0.035	0.394
MB	0.033	0.253	0.371	0.050	0.385
FC	0.066	0.704	1.351	0.031	0.332
VESS	0.017	0.204	0.543	0.023	0.267
Ham weight losses					
WLS	0.008	0.074	0.246	0.024	0.226
WLR	0.019	0.226	0.733	0.020	0.231
WLC	0.066	0.680	2.070	0.024	0.241
TWL	0.136	1.458	3.102	0.029	0.310

[1]BW270: body weight adjusted at 270 d (kg); CW: carcass weight (kg); DP: dressing percentage (%); BFT: carcass backfat depth (mm); CLM: carcass lean meat content (%); TTW: average trimmed thighs weight (kg); IOD: iodine number; LIA: linoleic acid content (%); SA: stearic acid content (%); Ω6: omega-6 fatty acids content (%); PUFA: polyunsaturated fatty acid content (%); MO_PO: ratio between monounsaturated and polyunsaturated fatty acids contents; SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high); FC: subcutaneous fat colour linear score (from -4 = low to 4 = high); VESS: blood vessel linear score (from 0 = low to 4 = high); WLS: ham weight loss during salting; WLR: ham weight loss during resting; WLC: ham weight loss during curing; TWL: total ham weight loss;

[2] σ_g^2: social group variance; σ_a^2 : additive genetic variance; σ_e^2: residual variance; = $g^2 = \sigma_g^2 / \sigma_p^2$; $h^2 = \sigma_a^2 / \sigma_p^2$ where $\sigma_p^2 = \sigma_g^2 + \sigma_a^2 + \sigma_e^2$.

Table 4. Estimates of variance components and parameters for the investigated traits using single-step BLUP[1,2]

Trait	σ_g^2	σ_a^2	σ_e^2	g^2	h^2
BW270	8.348	70.010	95.450	0.048	0.403
Carcass traits					
CW	6.201	52.240	76.250	0.045	0.397
DP	0.156	0.620	1.531	0.068	0.269
BFT	0.822	7.610	11.950	0.040	0.373
CLM	0.206	2.203	3.384	0.035	0.380
Ham quality traits					
TTW	0.054	0.505	0.574	0.048	0.445
IOD	0.198	2.134	2.910	0.038	0.407
LIA	0.055	0.613	0.823	0.037	0.411
SA	0.017	0.395	0.261	0.026	0.587
Ω6	0.058	0.642	0.872	0.037	0.409
PUFA	0.071	0.773	1.037	0.038	0.411
MO_PO	0.005	0.050	0.059	0.040	0.438
SFD1	0.973	9.353	16.520	0.036	0.348
SFD2	0.012	0.312	0.526	0.014	0.368
RS	0.013	0.247	0.440	0.018	0.352
SF	0.101	1.047	1.619	0.037	0.378
MB	0.033	0.242	0.378	0.050	0.370
FC	0.067	0.655	1.382	0.032	0.312
VESS	0.017	0.194	0.549	0.023	0.255
Ham weight losses					
WLS	0.007	0.062	0.252	0.023	0.193
WLR	0.018	0.194	0.753	0.019	0.201
WLC	0.061	0.553	2.147	0.022	0.200
TWL	0.125	1.213	3.246	0.027	0.265

[1] BW270: body weight adjusted at 270 d (kg); CW: carcass weight (kg); DP: dressing percentage (%); BFT: carcass backfat depth (mm); CLM: carcass lean meat content (%); TTW: average trimmed thighs weight (kg); IOD: iodine number; LIA: linoleic acid content (%); SA: stearic acid content (%); Ω6: omega-6 fatty acids content (%); PUFA: polyunsaturated fatty acid content (%); MO_PO: ratio between monounsaturated and polyunsaturated fatty acids contents; SFD1: subcutaneous fat depth nearby semimembranosus (mm); SFD2: subcutaneous fat depth nearby quadriceps femoris (mm); RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high); MS: marbling linear score (from 0 = low to 4 = high); FC: subcutaneous fat colour linear score (from -4 = low to 4 = high); VESS: blood vessel linear score (from 0 = low to 4 = high); WLS: ham weight loss during salting; WLR: ham weight loss during resting; WLC: ham weight loss during curing; TWL: total ham weight loss;

[2] σ_g^2: social group variance; σ_a^2 : additive genetic variance; σ_e^2: residual variance; = $g^2 = \sigma_g^2 / \sigma_p^2$; $h^2 = \sigma_a^2 / \sigma_p^2$ where $\sigma_p^2 = \sigma_g^2 + \sigma_a^2 + \sigma_e^2$.

Table 5. Pearson's correlation coefficients[1] between BLUP estimated breeding value and genomic breeding values for genotyped crossbred pigs, genotyped C21 nucleus boars and ungenotyped C21 males and females

Trait	Genotyped crossbred	Genotyped C21 nucleus boars	Ungenotyped C21 males and females
Body weight adjusted at 270d, kg	0.923	0.932	0.946
Carcass traits			
Carcass weight, kg	0.920	0.934	0.972
Dressing percentage, %	0.898	0.921	0.920
Carcass ultrasound backfat depth, mm	0.909	0.919	0.878
Carcass lean meat, %	0.912	0.918	0.899
Ham quality traits			
Average trimmed thighs weight, kg	0.921	0.925	0.948
Iodine number	0.930	0.933	0.881
Linoleic acid content, %	0.929	0.935	0.893
Stearic acid content, %	0.957	0.932	0.938
Omega-6 content, %	0.928	0.937	0.895
Polyunsaturated fatty acids content, %	0.852	0.922	0.883
Ratio of monounsaturated to polyunsaturated fatty acids contents	0.933	0.935	0.899
Subcutaneous fat depth, mm			
nearby semimembranosus muscle	0.925	0.914	0.871
nearby quadriceps muscle	0.922	0.874	0.898
Round shape score	0.916	0.903	0.843
Subcutaneous fat score	0.922	0.923	0.903
Marbling score	0.921	0.889	0.827
Muscle colour score	0.915	0.893	0.889
Blood vessels score	0.892	0.907	0.865
Ham weight losses			
Ham weight loss during salting, %	0.869	0.874	0.847
Ham weight loss during resting, %	0.833	0.779	0.734
Ham weight loss during curing, %	0.847	0.781	0.764
Total ham weight loss, %	0.853	0.788	0.757

[1]All Pearson's correlation coefficients were positive and different from zero ($P < 0.05$);

[2]Round shape linear score: from 0 = low to 4 = high; subcutaneous fat linear score: from -4 = low to 4 = high; marbling linear score: from 0 = low to 4 = high; muscle colour linear score: from -4 = pale to 4 = dark; blood vessel linear score: from 0 = low to 4 = high.

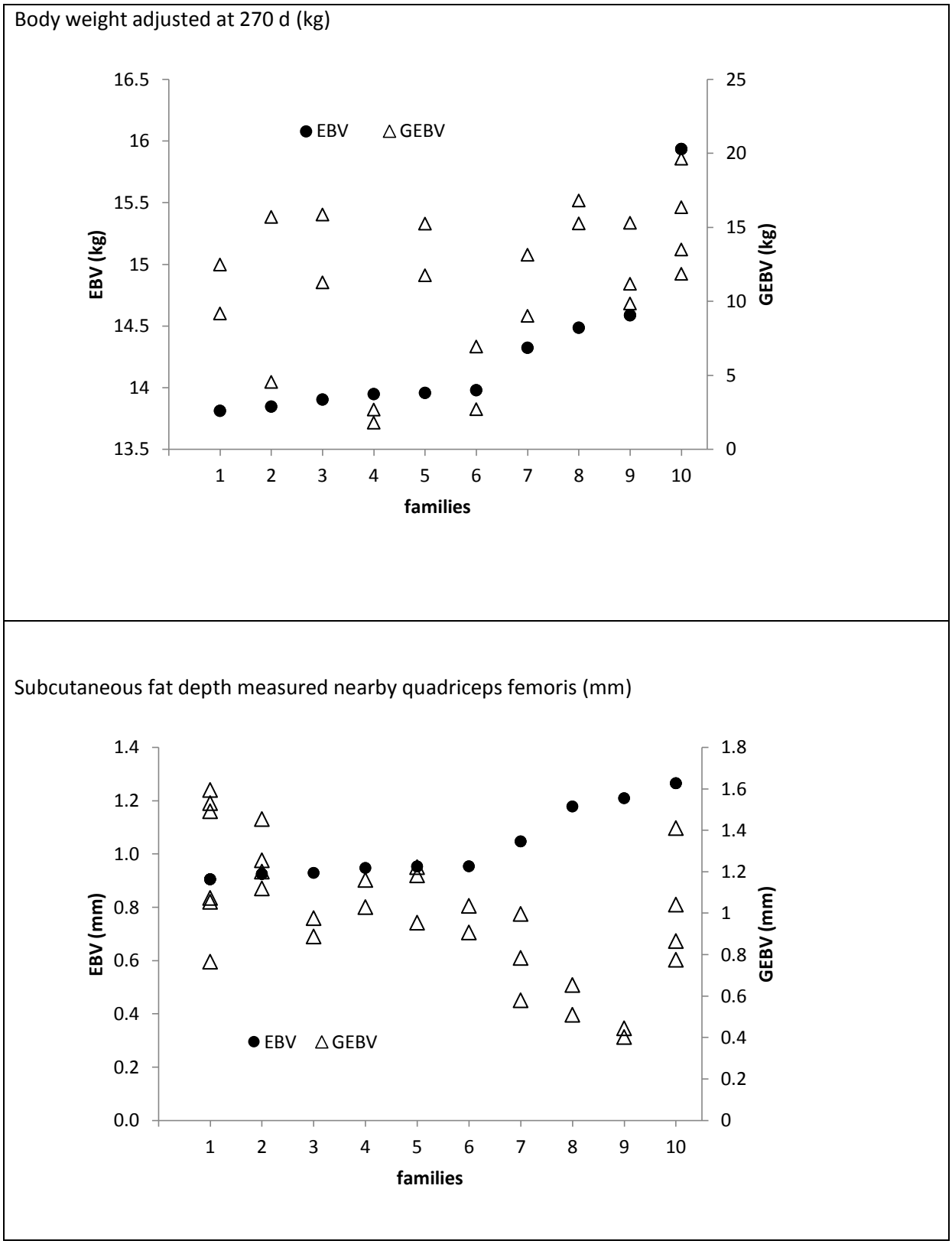
Table 6. Pearson's correlations between EBVs and GEBVs ($r_{EBV,GEBV}$) and Spearman's correlations between rankings based on EBV and GEBV ($r_{s\ EBV,GEBV}$) for animals in the validation population (N = 500) and for the top 10% animals with the best EBV for the trait

Trait	Full dataset		Top 10% animals
	$r_{EBV,GEBV}$	$r_{s\ EBV,GEBV}$	$r_{s\ EBV,GEBV}$
Body weight adjusted at 270d, kg	0.760	0.761	0.440
Carcass traits			
Carcass weight, kg	0.779	0.778	0.334
Dressing percentage, %	0.733	0.730	0.332
Carcass ultrasound backfat depth, mm	0.653	0.610	0.717
Carcass lean meat, %	0.716	0.702	0.494
Ham quality traits			
Average trimmed thighs weight, kg	0.758	0.757	0.478
Iodine number	0.622	0.566	0.500
Linoleic acid content, %	0.736	0.724	0.503
Stearic acid content, %	0.661	0.675	0.402
Omega-6 content, %	0.734	0.731	0.562
Polyunsaturated fatty acids content, %	0.727	0.722	0.334
Ratio of monounsaturated to polyunsaturated fatty acids contents	0.758	0.766	0.282
Subcutaneous fat depth, mm			
nearby semimembranosus muscle	0.635	0.602	0.367
nearby quadriceps muscle	0.694	0.719	0.344
Round shape score	0.377	0.322	0.133[†]
Subcutaneous fat score	0.601	0.576	0.329
Marbling score	0.443	0.397	0.180[†]
Muscle colour score	0.751	0.725	0.407
Blood vessels score	0.626	0.587	0.451
Ham weight losses			
Ham weight loss during salting, %	0.601	0.590	0.436
Ham weight loss during resting, %	0.530	0.478	0.406
Ham weight loss during curing, %	0.366	0.361	0.198[†]
Total ham weight loss, %	0.462	0.442	0.221

[1]All Pearson's and Spearman's correlations were positive and different from zero ($P < 0.05$);

[2]Round shape linear score: from 0 = low to 4 = high; subcutaneous fat linear score: from -4 = low to 4 = high; marbling linear score: from 0 = low to 4 = high; muscle colour linear score: from -4 = pale to 4 = dark; blood vessel linear score: from 0 = low to 4 = high.

Figure 1. Comparison between EBV and GEBV of members of the best 10 C21 families in the validation population (traits are BW270 and subcutaneous fat depth measured nearby quadriceps femoris)



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CHAPTER 6
New methods in breeding values prediction

Genomic evaluation of a boar line linked to dry-cured ham production using a single-step BLUP procedure including social genetic effects.

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ABSTRACT

The aim of this study was to implement social genetic effects in genomic selection (**GS**) procedure based on single-step BLUP (**SSBLUP**) methodology for genetic evaluation of PB breeding candidates of a boar line for body weight adjusted at 270 days (**BW270**), carcass and ham quality traits.

Individual phenotypes for BW270, carcass lean meat content, iodine number, ham round shape score (RS) and ham subcutaneous fat depth score were recorded from 11,488 crossbred (**CB**) finishing pigs. For these traits the model accounted for social genetic effects provided a better fit of the data respect to direct model. Animals were born and reared at the same farm, and randomly assigned at 60 d of age to groups from 4 to 7 individuals (average group size was 6.1 individuals, whereas the average additive genetic relationship among group mates was 0.11). A total of 1,088 CB pigs, 136 nucleus boars (**C21_NB** sires of the CB genotyped animals) and 500 C21 half-sibs of CB animals (**C21_HS**) were genotypes for 8,826 single nucleotide polymorphisms (**SNP**) using the GGP Porcine LD Chip (Neogen Co., Lincoln, NE). After quality control, 7,559 SNP and 1,687 pigs were available for genomic evaluation. Traditional genetic evaluation (**C_BLUP**) was performed using a competitive model that included in addition to sex and slaughter groups as fixed effects, the random effects of social group, full-sibs family, the additive and social genetic effects. Genomic evaluation was carried out using SSBLUP methodology accounting for social genetic effects (**C_SSBLUP**). In order to validate the prediction equation, the 500 C21_HS (animals without own phenotypes but with genomic information, mimicking the real scenario if GS procedures will be implemented in selection scheme of C21 boar line) were used as validation population. Pearson's (r) and Spearman's (r_s) correlation coefficients between direct breeding values (**dEBV**), direct genomic breeding values (**dGEBV**), total breeding values (**TBV**) and total genomic breeding values (**TGBV**) were computed in order to compare C_BLUP and C_SSBLUP. Estimation of (co)variance components, EBV and GEBV were obtained by EM-REML methods.

Modest differences in estimation of (co)variance components and parameters was found when a traditional competitive model was compare to a model that included both social genetic effects and genomic information, indicating that C_SSBLUP analysis is feasible for genomic evaluation accounting for heritable social effects. Large estimates for both $r_{dEBV,dGEBV}$ and $r_{TBV,TGBV}$ in both CB pigs and C21_NB suggested that estimates of genetic merit incorporating genomic information were consistent with accurate prediction from traditional methods. Positive $r_{dEBV,dGEBV}$ and $r_{TBV,TGBV}$ were found also for C21_HS in validation population, but the magnitude of the correlations was lower than CB and C21_NB previously evaluated. Spearman's rank correlation between dEBV and dGEBV in animals in validation set ranged from 0.313 for RS to 0.761 for BW270, whereas $r_{s_{TBV,TGBV}}$ varied from 0.380 for RS to 0.665 for BW270. It suggests differences in rankings of C21 breeding candidates when traditional evaluation and genomic prediction are considered. It can be attributable to the ability of C_SSBLUP to estimate individual dEBV, social genomic breeding values and, hence, TGBV.

In conclusion, the SSBLUP methodology seems to be a suitable tool to perform genomic evaluation accounting for both direct and social genetic effects. The opportunity to include heritable social effects might enhance the benefit of GS for evaluation of purebred pig lines linked to dry-cured ham production, thank the possibility to select the best individual based on their total genetic merit and to estimate more correctly the total heritable variation exploitable for selection.

Keywords: genomic breeding values, heavy pigs, ham quality, single-step BLUP, social genetic effects

INTRODUCTION

Performance of animals raised in groups may be affected by social interactions. The influence of the individual's genotype on phenotype of group mates is called social genetic effects (definite also heritable social effects or indirect genetic effects) (Moore et al., 1997; Bijma et al., 2007a). Social genetic effects are part of the total heritable variation, and if present, they could affect the response to selection. In 2002, Muir and Schinckel proposed the first mixed model equation, defined as competitive model, to account for both direct and social genetic effects to predict direct and social genetic variances and breeding values. Competitive models have been applied in pigs slaughtered at light body weight (**BW**) to estimate the influence of social genetic effects on average daily gain (Arango et al., 2005; Chen et al., 2008; Chen et al., 2009; Bowman et al., 2010) and meat quality traits (Bergsma et al., 2008; Hsu et al., 2010). With special focus on heavy pigs, which are slaughtered at 160 kg of BW and at least 270 d of age, Rostellato et al. (2015) revealed the influence of heritable social effects on variation of important carcass and ham quality traits, as well as, an increase in model fitting when competitive model was applied.

In Italy, heavy pigs are reared to obtain raw thighs to produce dry-cured hams having Protected Designation of Origin (**PDO**) label. Raw hams are largely obtained from crossbred (**CB**) pigs. When the breeding goal is definite at the level of the "crossbred slaughter animal", genetic evaluation of purebred (**PB**) breeding candidates of boar lines linked to dry-cured ham production is currently based on sib-testing. This approach allows to combine both crossbred and purebred information, but it shows some concerns related to the high costs for management of CB animals in testing groups, the difficulties in collection of phenotypes for traits difficult to record, and the increase of inbreeding due to identical breeding values for breeding candidates that are members of PB full-sibs families. To overcome these drawbacks, genomic selection (**GS**) proposed by Meuwissen et al. (2001) could be applied. Single-step BLUP (**SSBLUP**) methodology was investigate to predict the genomic breeding values (**GEBV**) for growth performance (Christensen et al., 2012) and reproductive traits (Guo et al., 2014) in light pigs. In addition, SSBLUP seems to be a suitable tool also for genomic evaluation of PB boar lines when traits related to ham quality and manufacture process of PDO dry-cured hams are considered (Chapter 5 of this thesis). However, at the best of our knowledge, no studies included social genetic effects on genomic evaluation.

The aim of this study was to implement social genetic effects in GS procedure based on SSBLUP methodology for genetic evaluation of PB breeding candidates of a boar line for body weight adjusted at 270 days (**BW270**), carcass and ham quality traits.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not needed because animals providing data for the study were subjected to standard production conditions and no additional measurements were taken. Observations used in this study were from sib-testing program of the C21 Goland sire line (Gorzagri, Fonzaso, Italy) and were registered at the farm where the program is carried out from 1998 to 2013. The farm operates in line with regulations of the Italian law on protection animals.

Animals and data records

Data records analyzed in this study were collected from 11,488 CB finishing pigs produced in the sib-testing program of the C21 Goland boar line (Gorzagri, Fonzaso, Italy). Pigs (5,555 barrows and 5,933 gilts) were progeny of 343 boars of the C21 line mated to 524 Large White-derived CB sows. Crossbred piglets were born between February 2003 and January 2013, and reared in the same farm. Piglets were tail docked and male piglets were castrated within 5 d after birth. At 28 d of age, piglets were weaned and randomly assigned to groups of approximately 30 individuals. At 60 d of age, pigs were housed in finishing pens and formed social groups containing from 4 to 7 individuals. Each pen had an area of 6 m^2 and it was equipped with a nipple drinker providing water continuously. Average social group size was 6.01 and within pen average additive relationship was 0.110 ± 0.001. Pigs were slaughter at 279.15 ± 24.26 d of age and 167.88 ± 24.26 kg of BW in groups of about 70 animals each.

The traits considered in this study were chosen on the basis of the model fitting assessed through likelihood ratio test. As found in Rostellato et al. (2015), the model accounted for social genetic effects provided a better fit of the data respect to direct model for traits described in the following. Final BW was adjusted at 270 d (**BW270**, kg) on the basis of individual linear regressions of BW on age estimated using 6 BW measures collected at 60, 90, 135, 180, 245 d of age and at the day before slaughter. Carcass lean meat content (**CLM**, %) was estimated on the basis of the regression:

$$y = 45.371951 - 0.221432\,x_1 + 0.055939\,x_2 + 2.554674\,x_3$$

where x_1 is the Fat-O-Meater measure of backfat depth (including skin, mm), x_2 is the Fat-O-Meater measure of loin depth (mm), and $x_3 = x_2/x_1$. On covering fat of hams, iodine number (**IOD**) was estimated through calibration equations based on reflectance of trimmed fat measured with near-infrared spectroscopy in individual samples taken from the left thigh. Moreover, round shape (**RS**, score from 0 = low roundness to 4 = high roundness) and subcutaneous fat depth (**SF**, score from -4 = low-depth to 4 = high depth) were assessed through a subjective evaluation of raw hams performed by trained experts.

Pedigree information was available for all slaughtered pigs and for all C21 Goland boars, whereas only the sire, the maternal grandsire and maternal granddam were known for the dams of CB finishing pigs. Additive relationships among CB finishing pigs, C21 Goland boars and CB dams were traced back for as many generations as possible. For slaughtered animals, additive relationships were computed on the basis of at least 6 generations of known ancestors. Sire and dam of each slaughter pig were unrelated, so that inbreeding at the CB level was 0.

More details regarding animals, data records and feeding conditions can be found in materials and methods of Chapter 5 of this thesis.

Genotyping

Genotypes for 8,826 SNPs were assessed using GGP Porcine LD chip (Genomic Profiler for Porcine LD, GeneSeek Inc., a Neogen Co., Lincoln, NE). To constitute the reference population to be genotyped, 1,088 CB pigs (314 barrows and 774 gilts born from January 2010 and October 2012) with phenotypic records for the investigated traits were selected. They were sampled from all the CB families reared and tested in that period so that each family had, on average, 2 members involved in genotyping program. In this way, the reference population was representative of the most recent "genetic feature" of the C21 line and CB tested population. In addition, 136 C21 nucleus boars (**C21_NB**: sires of CB tested pigs) and 500 C21 PB males (C21_HS: half-sibs of CB tested animals (**C21_HS**) were genotyped. One ear tissues sample for each animal was shipped to GeneSeek Inc. (Lincoln, NE, USA) for DNA extraction and genotyping. Samples and SNPs with call rate <0.90, SNPs with MAF <0.05 and monomorphic SNPs were discarded. After quality control, a total of 7,559 SNPs and 1,687 animals where qualified for genomic evaluation.

S*tatistical analysis*

Traditional genetic evaluation using competitive model (**C_BLUP**) was carried out for all investigate traits using the following model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{W_D a_D} + \mathbf{W_S a_S} + \mathbf{Zg} + \mathbf{Vf} + \mathbf{e}$$

where $\mathbf{y}$ is a vector of observed phenotypes for one trait; $\mathbf{b}$ is a vector of non-genetic fixed effects which included sex (female and castrated male) and slaughter group effects, $\mathbf{g}$ is a random vector of social group (animals grouped together in the same pen) effects, $\mathbf{a_D}$ is a random vector of direct additive genetic effects, $\mathbf{f}$ is a random vector of full-sibs family (i.e., individuals born in the same "biological" litter) effects, $\mathbf{a_S}$ is a random vector of heritable social effects, $\mathbf{e}$ is a vector of random residuals, and $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{W_D}$, $\mathbf{W_S}$, and $\mathbf{V}$ are incidence matrices relating $\mathbf{b}$, $\mathbf{g}$, $\mathbf{a_D}$, $\mathbf{a_S}$, and $\mathbf{f}$ to $\mathbf{y}$, respectively.

Assumptions on the probability distributions of social group effects, full-sibs family effects, and residuals were:

$$\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}\,\sigma_g^2),\ \mathbf{f} \sim N(\mathbf{0}, \mathbf{I}\,\sigma_f^2), \text{ and } \mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\,\sigma_e^2),$$

where *N*( ) indicates a normal distribution, ***I*** is an identity matrix of appropriate order, and σ_g^2, σ_f^2, and σ_e^2 are variance components. In competitive model, the assumption about the probability distribution of $\boldsymbol{a_D}$ was updated to take into account the covariance with $\boldsymbol{a_S}$. The vectors $\boldsymbol{a_D}$ and $\boldsymbol{a_S}$ were assumed to have a multivariate normal probability distribution:

$$\begin{bmatrix}\mathbf{a_D}\\ \mathbf{a_S}\end{bmatrix} \sim \text{MVN}\ (\mathbf{0}, \mathbf{A} \otimes \mathbf{C}),$$

where **A** is the pedigree-based matrix, $\mathbf{C} = \begin{bmatrix}\sigma^2_{a_D} & \sigma_{a_D a_S}\\ \sigma_{a_D a_S} & \sigma^2_{a_S}\end{bmatrix}$, $\sigma^2_{a_S}$ is the variance of heritable social effects, $\sigma_{a_D a_S}$ is the covariance between direct and social genetic effects, and $\otimes$ denotes the Kronecker product.

Genomic evaluation including social genetic effects were performed using SSBLUP methodology (**C_SSBLUP**) where the A was replaced by H matrix that combine pedigree and genomic information with the following inverse (Aguilar et al., 2010):

$$H^{-1} = A^{-1}\begin{bmatrix}0 & 0\\ 0 & G^{-1} - A_{22}^{-1}\end{bmatrix}$$

where G^{-1} is the inverse of genomic relationship matrix and A_{22}^{-1} is the inverse of the pedigree-based relationship matrix for genotyped individuals. G matrix was constructed as described in VanRaden et al. (2008) using allele frequencies calculated from genotyped animals and scaled for compatibility with A to have mean of diagonal and off-diagonals elements equal to A matrix.

Phenotypic variance (σ_p^2) was calculated as suggested by Bijma et al. (2007b) and Bergsma et al. (2008):

$$\sigma_p^2 = \sigma^2_{a_D} + (n-1)\, r\left[2\sigma_{a_D a_S} + (n-2)\sigma^2_{a_S}\right] + (n-1)\,\sigma^2_{a_S} + \sigma^2_g + \sigma^2_f + \sigma^2_e$$

where n is the average size of social groups and r is the average additive relationship among group mates.

Direct heritability ($\mathbf{h_D^2}$) was computed as:

$$h^2_D = \frac{\sigma^2_{a_D}}{\sigma^2_p},$$

whereas social heritability ($\mathbf{h_S^2}$) was calculated as:

$$h^2_S = \frac{\sigma^2_{a_S}}{\sigma^2_p}$$

For traits affected by heritable social effects, the total heritable variation exploitable in selection is the variance of total breeding values (**TBV**; Bijma et al., 2007a). The TBV is defined as:

$$TBV_i = a_{D_i} + (n-1)a_{S_i}$$

and is the heritable effect of an individual on the phenotypic mean of a trait, which depends on the direct effect a_{D_i} of the individual on its phenotype (a_{D_i} is also called direct breeding value; **dEBV**) and on the social effect a_{S_i} exerted on the phenotype of n-1 group mates (a_{S_i} is also defined social breeding value; **sEBV**).

When a non-null covariance between direct and social genetic effects exists, the variance of TBV ($\boldsymbol{\sigma_T^2}$) is:

$$\sigma^2_T = \sigma^2_{a_D} + 2\,(n-1)\,\sigma_{a_D a_S} + (n-1)^2\,\sigma^2_{a_S}$$

In order to evaluate the contribution of social genetic effects to the σ_p^2, the ratio between σ_T^2 and σ_p^2 (called $\mathbf{T^2}$; Bergsma et al., 2008) was computed. To evaluate the contribution of full-sibs family and social group effects (i.e., non-heritable social effects) to the phenotypic variance, $f^2 = \sigma_f^2/\sigma_p^2$ and $g^2 = \sigma_g^2/\sigma_p^2$ were computed.

To compare C_BLUP and C_SSBLUP evaluations, Pearson's correlation coefficients between dEBV and direct genomic breeding values (**dGEBV**) ($r_{dEBV,dGEBV}$), and between TBV and total genomic breeding values (**TGBV**) ($r_{TBV,TGBV}$) were calculated for CB animals and C21_NB.

In order to validate the prediction equations for estimation of GEBV, the 500 C21_HS with genotypic information were enrolled to constitute the validation population. In order to assess the consistency of genomic prediction, $r_{dEBV,dGEBV}$ and $r_{TBV,TGBV}$ were computed for this set of animals. In addition, Spearman's rank correlations between dEBV and dGEBV ($r_{s_{dEBV,dGEBV}}$), and between TBV and TGBV ($r_{s_{TBV,TGBV}}$) were computed in order to investigate possible variations in the rankings of animals when traditional evaluation or genomic prediction are considered.

Estimation of (co)variance components, EBV and GEBV were obtained by EM-REML methods using BLUPF90 family programs (Misztal et al., 2008).

RESULTS AND DISCUSSION

Number of pigs, slaughter and social groups, full-sib families and descriptive statistics for the investigate traits were presented in Table 1. The number of phenotypic records varied from 5,946 for CLM to 11,488 for BW270, because recording of individual phenotypes started at different times for different traits. The number of social groups ranged from 1,010 for CLM to 1,922 for BW270 and the number of full-sibs families varied from 1,182 for CLM to 2,154 for BW270. Bijma et al. (2010) proposed a minimum number of individuals (1000-2000 animals) and social groups (250-500) needed to estimated correctly social genetic effects when an optimum design is used. The dataset investigated comprised an amount of pigs and social groups much higher than the suggested numbers, making it suitable for estimation of social genetic effects.

Estimates of (co)variance components and parameters

(Co)variance components and parameters obtained through C_BLUP and C_SSBLUP analysis were presented in Table 2. Results from C_BLUP were consistent with a previous study about the contribution of social genetic effects on carcass and ham quality traits in heavy pigs (Rostellato et al., 2015) using a subgroup of the investigated animals.

When heritable social effects were investigated using SSBLUP approach, social group variances decreased for BW270 (-63.1%), IOD (-17.9%) and RS (-8.3%), whilst they slightly increased for CLM (+2%) and SF (+1.3%). Hence, the contribution of social group effect on σ_p^2 varied from 0.012 for BW270 to 0.041 for CLM. Full-sibs families variances increased for all traits (from 3.1% for BW270 to 18.8% for CLM) when

C_SSBLUP was performed. The contribution of full-sibs family effect on σ^2_p augmented respect to C_BLUP, ranging from 0.023 for IOD and RS to 0.051 for BW270 and CLM.

When genomic evaluation was carried out, $\sigma^2_{a_D}$decreased for all investigated traits. Direct heritabilities calculated from C_SSBLUP ranged from 0.313 for BW270 to 0.382 for IOD, decreasing from 0.5% for IOD to 15.2% for CLM respect to C_BLUP. A comparable reduction of heritability was also found when SSBLUP approach was applied using traditional direct model (Chapter 5 of this thesis). As expected, $\sigma^2_{a_S}$ were smaller than $\sigma^2_{a_D}$. When genomic information were used, $\sigma^2_{a_S}$ increased for all investigated traits, except for SF. Social heritabilities ranged from 0.002 for IOD to 0.006 for BW270. In comparison to C_BLUP, a large increase of h^2_S was found for CLM, due to great variation in $\sigma^2_{a_S}$ (+250%). The covariance between direct and associative components was positive for BW270, suggesting that individuals with positive effects for this trait exerted, on average, positive social effects on BW of their group mates. In contrast, negative $\sigma_{a_D a_S}$ were found for the other traits, indicating that pigs with positive direct breeding values affect negatively the phenotype of their group mates for CLM, IOD, RS and SF (Bijma et al., 2007b). When C_SSBLUP were performed, $\sigma_{a_D a_S}$ decreased for BW270 (-0.65%) and IOD (-53%), while it largely increased for RS (+25%) and CLM (+41%). No variation in $\sigma_{a_D a_S}$ was found for SF. The total heritable variance exploitable for selection estimated using C_SSBLUP decreased for all investigated traits (from 4.4% for RS to 12.1% for CLM), excepted for IOD (+23.9%). The contribution of σ^2_T to σ^2_p ranged from 0.253 for RS to 0.515 for BW270. In comparison to C_BLUP, T^2 decreased for all investigated traits (from -2.3% for BW270 to -9.1% for CLM), saved for IOD (+27.1%). Due to negative $\sigma_{a_D a_S}$, T^2 were lower than h^2_D for CLM (-2.7%), IOD (-4.7%), RS (-26.8%) and SF (-18.9%). In contrast, T^2 exceed h^2_D for BW270 (it was 1.64 times h^2_D) confirming the relevant contribution of social genetic effects on total heritable variation available for selection for BW270 (Rostellato et al., 2015).

Modest differences in estimation of (co)variance components and parameters was found when a traditional competitive model was compare to a model that included both social genetic effects and genomic information. It suggests that C_SSBLUP analysis is feasible for genomic evaluation accounting for heritable social effects for the investigated traits.

Comparison of traditional and genomic evaluation using competitive model

In order to compare C_BLUP and C_SSBLUP analyses, $r_{dEBV,dGEBV}$ and $r_{TBV,TGBV}$, were computed for CB animals and C21_NB (Table 3). Both of these groups of animals presented in the pedigree showed high accuracy in EBV (that is 0.7 for CB and 0.8 for C21_NB). High $r_{dEBV,dGEBV}$ were found for all investigate traits. For CB pigs, having both phenotypic and genotypic information, $r_{dEBV,dGEBV}$ ranged from 0.897 for BW270 to 0.916 for SF. For genotyped C21_NB (owing only genomic information) it varied from 0.891 for RS to 0.932 for IOD. These correlations were consistent with the Pearson's correlation coefficients between

EBV and GEBV estimated using a direct model (Chapter 5 of this thesis). Pearson's correlation coefficients between TBV and TGBV for CB animals ranged from 0.820 for CLM to 0.915 for IOD, whereas for genotyped nucleus boars it varied from 0.824 for CLM to 0.909 for IOD. Large estimates for both $r_{dEBV,dGEBV}$ and $r_{TBV,TGBV}$ suggested that estimates of genetic merit incorporating genomic information were consistent with accurate prediction from traditional methods. In fact, both of the two groups of animals showed high accuracy in EBV (that is 0.7 for CB and 0.8 for C21_NB). With special focus on $r_{TBV,TGBV}$, high values of these correlation indicates that both dGEBV and sGEBV were similar to estimates from traditional competitive models.

In order to validate the prediction equation, 500 C21_HS with genomic information were used as validation population. In fact in breeding programs of sire lines linked to dry-cured ham production, the breeding candidates are represented by the half-sibs of CB animals reared in testing groups. As consequence, this validation set mimicked the real scenario when PB breeding candidates of C21 boar line will be evaluated using GS procedures and, hence, provide concrete information about the impact of genomic data in genetic evaluation of the investigated boar line. Pearson's and Spearman's correlation coefficients between dEBV and dGEBV, and between TBV and TGBV were calculated for animals in validation population. High $r_{dEBV,dGEBV}$ were found for all traits, excepted for RS ($r_{dEBV,dGEBV}$ = 0.359) (Table 3). Comparable correlations were found for the investigated traits when classical direct model was used (Chapter 5 of this thesis). Pearson's correlations between TBV and TGBV were similar to $r_{dEBV,dGEBV}$, varying from 0.428 for RS to 0.682 for CLM. Spearman's correlation coefficients between dEBV and dGEBV varied from 0.313 for RS to 0.761 for BW270, indicating differences in ranks of animals when the breeding values were estimated using traditional competitive model and SSBLUP accounted for heritable social effects. Similar Spearman's correlations were found between TBV and TGBV, ranging from 0.380 for RS to 0.667 for BW270. Hence, rankings of breeding candidates changed even when they were based on TBV rather than on TGBV. The differences in ranks of animals can be due to the ability of genomic evaluation to estimate different GEBV and TBV for breeding candidates having genotypic information, though they are members of a PB full-sibs family. In fact, in traditional evaluation based on sib-testing, half-sibs of CB animals with same sire and dam, have identical breeding values. This is one of the major disadvantages of the traditional approach because the rate of inbreeding could increase (Bijma et al., 2001). When C_BLUP evaluation was performed, both dEBV and sEBV were identical for C21_HS that were members of a PB full-sib family. As consequence, also the TBV (calculated as $TBV_i = a_{D_i} + (n-1)a_{S_i}$) differed only between families. In contrast, when social genetic effects were included in SSBLUP, different dGEBV, sGEBV, and hence TGBV, were estimated for each breeding candidate with genomic information. Therefore, the implementation of C_SSBLUP procedures allows to select the best individuals rather than the best families even when social genetic effects are considered.

As reported in Ellen et al. (2014), at present, the optimum design of reference population (e. g. the number of social groups, the group structure and the relatedness within groups) for genomic evaluation including heritable social effects is unclear. In this study the amount of pigs having both phenotypic and genotypic information was just borderline to the minimum number indicated by Bijma et al. (2010). In addition, genomic evaluation has been performed using SSBLUP methodology which combines information from both genotyped and non-genotyped animals, making feasible the estimation of social genetic effects even when few animals are genotyped. However, future investigation about the design of reference population are needed, especially when different statistical methods to estimates genomic breeding values are used.

In conclusion, the SSBLUP methodology seems to be a suitable tool to perform genomic evaluation accounting for both direct and social genetic effects. The opportunity to include heritable social effects in genomic evaluation might allow to enhance the benefits of GS in breeding programs of boar lines liked to dry-cured ham production. As reported in Chapter 5 of this thesis, the implementation of GS procedures in breeding programs of heavy pigs might allow to include important traits overly difficult or expensive to measured in the breeding goal and to control the rate of inbreeding. In addition, the inclusion of heritable social effects in GS procedures, might increase the accuracy of selection thank the availability of individual TGBV and the possibility to estimate more correctly the total heritable variation exploitable for selection.

Table 1. Number of pigs, full-sibs families, social and slaughter groups, and descriptive statistics [1] for the investigate traits [2].

	Trait				
	BW270	CLM	IOD	RS	SF
Pigs	11,488	5,946	10,878	11,169	11,033
Full-sibs families	2,154	1,182	2,125	2,100	2,072
Social groups	1,922	1,010	1,864	1,877	1,854
Slaughter groups	171	92	169	167	165
Mean	164.5	49.84	68.11	2	0
SD	13.3	2.68	2.58	1	2
P1	133.2	43.22	62.20	0	-4
P99	196.2	56.27	74.73	4	4

[1] SD: standard deviation; P1: the 1st percentile; P99: the 99th percentile;

[2] BW270: body weight adjusted at 270 d (kg); CLM: carcass lean meat content (%); IOD: iodine number; RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high).

Table 2. Estimates of variance or covariance components and parameters for the investigated traits using competitive BLUP (C_BLUP) and competitive single-step BLUP (C_SSBLUP) analysis [1,2]

Parameter	C_BLUP					C_SSBLUP				
	BW270	CLM	IOD	RS	SF	BW270	CLM	IOD	RS	SF
σ^2_g	6.073	0.197	0.251	0.012	0.088	2.239	0.201	0.206	0.011	0.089
σ^2_f	9.044	0.240	0.104	0.015	0.071	9.320	0.285	0.114	0.016	0.080
$\sigma^2_{a_D}$	63.54	2.230	1.955	0.241	0.996	56.880	1.829	1.932	0.228	0.940
$\sigma_{a_{Da_S}}$	0.926	-0.048	-0.067	-0.008	-0.039	0.920	-0.068	-0.031	-0.010	-0.039
$\sigma^2_{a_S}$	1.104	0.006	0.007	0.001	0.010	1.128	0.021	0.009	0.002	0.010
σ^2_e	93.940	3.101	2.778	0.429	1.569	95.850	3.266	2.811	0.433	1.591
σ^2_T	99.758	1.899	1.465	0.182	0.857	93.630	1.670	1.852	0.174	0.802
g^2	0.032	0.034	0.049	0.017	0.032	0.012	0.036	0.041	0.016	0.033
f^2	0.048	0.042	0.020	0.021	0.026	0.051	0.051	0.023	0.023	0.030
h^2_D	0.335	0.387	0.384	0.346	0.366	0.313	0.328	0.382	0.331	0.348
h^2_S	0.006	0.001	0.001	0.001	0.004	0.006	0.004	0.002	0.003	0.004
T^2	0.527	0.329	0.288	0.261	0.315	0.515	0.299	0.366	0.253	0.297

[1] σ^2_g: social group variance; σ^2_f: full-sibs family variance; $\sigma^2_{a_D}$: additive genetic variance; $\sigma_{a_{Da_S}}$: additive genetic covariance between direct and social effect; $\sigma^2_{a_S}$: social additive genetic variance; σ^2_e: residual variance; σ^2_T: variance of total breeding values = $\sigma^2_{a_D}$ + 2(n-1) $\sigma_{a_{Da_S}}$ + (n-1)^2 $\sigma^2_{a_S}$ where n is the average size of the social group; $g^2 = \sigma^2_g/\sigma^2_p$; $f^2 = \sigma^2_f/\sigma^2_p$; $h^2_D = \sigma^2_{a_D}/\sigma^2_p$; $h^2_S = \sigma^2_{a_S}/\sigma^2_p$; $T^2 = \sigma^2_T/\sigma^2_p$,

σ^2_p: phenotypic variance = $\sigma^2_p = \sigma^2_{a_D} + (n-1)\, r\left[2\sigma_{a_{Da_S}} + (n-2)\sigma^2_{a_S}\right] + (n-1)\,\sigma^2_{a_S} + \sigma^2_g + \sigma^2_f + \sigma^2_e$ where r the average additive genetic relationship among group mates.

[2] BW270: body weight adjusted at 270 d (kg); CLM: carcass lean meat content (%); IOD: iodine number; RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high).

Table 3. Pearson's (r) and Spearman's correlation coefficients (r_S)[1] between direct breeding values (dEBV), direct genomic breeding values (dGEBV), total breeding values (TBV) and total genomic breeding values (TGBV) for the investigated traits[2].

	Trait				
	BW270	CLM	IOD	RS	SF
Genotyped crossbreds					
$r_{dEBV,dGEBV}$	0.910	0.897	0.927	0.911	0.916
$r_{TBV,TGBV}$	0.856	0.820	0.915	0.895	0.855
Genotyped C21 nucleus boars					
$r_{dEBV,dGEBV}$	0.922	0.908	0.932	0.891	0.917
$r_{TBV,TGBV}$	0.858	0.824	0.909	0.874	0.835
C21 half-sibs in validation population					
$r_{dEBV,dGEBV}$	0.760	0.724	0.618	0.359	0.605
$r_{TBV,TGBV}$	0.657	0.682	0.580	0.428	0.580
$r_{s_{dEBV,dGEBV}}$	0.761	0.712	0.565	0.313	0.584
$r_{s_{TBV,TGBV}}$	0.665	0.667	0.515	0.380	0.586

[1] all correlation coefficients were positive and different from zero ($p > 0.05$);

[2] BW270: body weight adjusted at 270 d (kg); CLM: carcass lean meat content (%); IOD: iodine number; RS: round shape linear score (from 0 = low to 4 = high); SF: subcutaneous fat linear score (from -4 = low to 4 = high).

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CHAPTER 7
General conclusions and future perspectives

Main objectives of this thesis were to provide increased knowledge about new traits, relevant for heavy pig farming, and to investigate new statistical models and genetic evaluation procedures to enhance the efficiency in the prediction of breeding values for traits related to the quality of the carcass and of raw thighs used in production of PDO dry-cured hams.

The first part of this thesis focused on boar taint (**BT**), a new trait related to both pork quality and animal welfare which is becoming increasingly important for pig farming in EU countries after the voluntary declaration of ceasing surgical castration of male piglets by January, 1 2018 and the resulting need to find alternatives to prevent BT. In Chapter 3, results of a study where contents of androstenone (**AND**), skatole (**SKA**) and indole (**IND**) have been repeatedly quantified in adipose tissue of intact male pigs at 160 and 220 d of age have been discussed. At the second sampling, animals reached a body weight (**BW**) comparable to the slaughter weight (roughly 165 kg BW) of finishing pigs used for production of Protected Designation of Origin (**PDO**) dry-cured hams. Contents of compounds responsible for BT and the percentage of samples that exceeded the sensory thresholds discriminating tainted from untainted carcasses increased in mature and heavy pigs in comparison with young and light pigs. Hence, prevalence of BT in heavy pigs is expected to be greater as well as is greater the relevance of the problem for the Italian pig industry in comparison with other EU countries where pigs are slaughtered at about 110 kg BW.

In addition, the large correlation between contents of BT compounds in backfat and those in ham subcutaneous fat (this thesis) implies that BT is a critical issue for the quality of raw hams also. This is of concern for the Italian pig industry, which mainly focuses on manufacturing raw thighs into PDO dry-cured hams of high market value. Processing of tainted raw hams might result in huge economical losses due to low consumer's acceptability of end-products of impaired quality. Thus, additional studies are required to evaluate effects of biochemical and enzymatic processes, occurring during curing, on perception of flavours and odours associated to BT.

At present, pork products having PDO, "Traditional Specialities Guaranteed" or "Protected Geographic Indication" labels benefit of derogations on rules dictated by the European declaration on alternatives to surgical castration of pigs (2011). As a consequence, surgical castration is still allowed for animals reared for this purpose, but application of anaesthesia is mandatory. Surgical castration with pain relief is not, however, considered economically sustainable and cost-effective in the Italian pig production system. In the last years, the Italian pig industry is focusing also on production of pigs slaughtered at

intermediate BW (140 kg) providing thighs manufactured into cooked hams or non-PDO dry-cured products. No derogation on rules dictated by the European declaration will cover this type of production system. In view of a total relinquishment of surgical castration of male piglets and the lack of dispensations for typical products, it is fundamental for the Italian pig industry to identify valid alternatives to surgical castration before 2018. Estimation of (co)variance components and genetic parameters, presented in Chapter 3, revealed that selective breeding seems to be a promising method to reduce contents of BT compounds. In contrast to other practices to prevent BT, like immunocastration and slaughter at young age, breeding for boars with low risk to develop BT seems to be a suitable long-term alternative to surgical castration also for pigs slaughtered at heavy BW.

Additional investigations are required in order to evaluate possible undesirable side effects on reproductive performance of boars and sows. Traditional selective breeding might be supported by genomic information to identify genes and polymorphisms associated with low contents of AND, SKA and IND. In this way, the potential undesirable effects, linked to AND reduction, on reproductive performance of boars and sows might be mitigated and the influence of selection against BT on carcass and ham quality traits in CB intact male pigs might be investigated. Finally, if raw thighs will be processed to obtain dry-cured hams, a sensory analysis might be performed in order to evaluate the consumer's perception and acceptability of dry-cured hams obtained from intact males with reduced levels of BT compounds.

In the second part of this thesis, competitive models were used to investigate the contribution of social genetic effects on variation of BW adjusted at 270 d (**BW270**), carcass and ham quality traits in heavy pigs (Chapter 4). This was the first study that explored the influence of heritable social effects in pigs fed in restricted condition (from 75-80 kg BW onward), slaughtered at heavy BW and advanced age. Differently from light pigs investigated in all studies available in the literature, such different conditions might increase the magnitude of social genetic effects. Competitive models provided better fit to the data in comparison with models ignoring heritable social effects for BW270, lean meat content (**CLM**), iodine number, ham round shape and ham subcutaneous fat thickness scores, indicating that social interactions among heavy pigs affect variation in growth traits, carcass and ham quality traits.

Estimation of (co)variance components and parameters revealed a large contribution of heritable social effects to variation in BW270 and CLM. With special focus on BW270, the contribution of total heritable variance to phenotypic variance ($\mathbf{T^2}$) was one and half time the magnitude of direct heritability ($\mathbf{h_D^2}$) of the trait, suggesting an increase of the heritable variation exploitable in selection due to social genetic effects. In addition, for BW270, differences in rankings of animals were detected when total breeding values from competitive models were used in place of breeding values estimated with direct models, confirming relevant contributions of social genetic effects on variation in this trait.

Conversely, heritable social effects had smaller influence on variability in backfat thickness and ham quality traits. However, due to negative covariances between direct and social components, T^2 were lower than h_D^2 for carcass and ham quality traits, reducing the overall potential to respond to selection for these traits.

In conclusion, the current procedures for estimation of breeding values in C21 boar line should be revised to take social genetic effects into account and to exploit more efficiently the potential to respond to selection.

The last part of the thesis aimed at investigating new genetic evaluation procedures based on genomic selection (**GS**) to improve the efficiency in prediction of breeding values for candidates of the C21 boar line. Currently, genetic evaluation of purebred (**PB**) boar lines linked to dry-cured ham production is based on sib-testing programs, where testing groups of CB half-sibs of PB candidates, are reared and slaughtered to collect phenotypic information used in combined crossbred-purebred selection programs (**CCPS**; Wei, 1992). Although this approach increases the response to selection in crossbred performance in comparison with procedures focusing on purebred performance only, it is troublesome due to high cost of management of testing groups and collection of phenotypic information.

Genomic selection procedures based on single-step BLUP (**SSBLUP**) seems to be a suitable tool for genetic evaluation and selection of the C21 boar line and the implementation in the breeding program of the line might convey several advantages. One major benefit of GS is that phenotypic information and sib-testing programs are no more needed after estimation of SNPs effects and development of the prediction equation (Goddard and Hayes, 2009). With special focus on the C21 line, GS might decrease costs for organization and management of testing groups and make the evaluation for traits difficult to measure easier. As a consequence, traits relevant in the manufacture process of PDO dry-cured hams can be included in the breeding goal of the line, given that breeding candidates can be evaluated exclusively using genomic information available early in life. In this way, GS might also simplify genetic evaluations for content of BT compounds, favouring selective breeding as an alternative to surgical castration of male piglets. Indeed, quantification of AND, SKA and IND requires expensive laboratory instruments (Aluwe et al., 2012) and collection of fat samples from live breeding candidates of high BW might increase safety risks for veterinarians. Implementation of GS procedures might increase the accuracy of selection and reduce the rate of inbreeding, thank to the ability of SSBLUP to provide predictions of individual genetic merit (**GEBV**) of PB candidates in place of "family" breeding values estimated by pedigree-based methods. Different rankings of breeding candidates where observed when traditional EBV or genomic GEBV were used. The availability of individual GEBV rather than "family" EBV allows the selection of the best individuals within each family rather than the best families. Given that SSBLUP seems appropriate even when social genetic effects are included in the model (Chapter 6), rankings based on "individual" genomic total breeding values might enhance the accuracy of selection also for traits affected by heritable social effects.

The SSBLUP approach used in our studies to estimate GEBV assumed equal variance of SNPs effects. Further investigations are needed in order to evaluate whether different assumptions about the probability distribution of markers effects (like in Bayesian methods) enhance the consistency of genomic predictions. Finally, genomic information might be exploitable in order to investigate non-additive genetic effects, as dominance effects. The opportunity to account for and to exploit non-additive genetic effects might improve the accuracy of genomic evaluation, especially when non-additive effects exert important contribution to the total genetic variation in the trait. To date, studies on inclusion of dominance effects in genomic evaluation are lacking in the scientific literature.

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