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Effects of 17 performance, carcass and raw ham quality parameters on ham weight loss at first salting in heavy pigs, a meat quality indicator for the production of high quality dry-cured hams

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Running title: Ham weight loss at first salting and carcass and ham quality traits

Highlights

- Ham weight loss at first salting (HWLFS) was evaluated in Italian Large White pigs.
- HWLFS was affected by slaughter day and visible intermuscular fat of trimmed hams.
- Residual correlations between HWLFS and 17 traits were determined.
- HWLFS could be predicted by several parameters.
- Backfat thickness, lean cuts and pH_{24h} were the most important predictors.

Abstract

Ham weight loss at first salting (HWLFS) is a meat quality parameter used to assess the suitability of the hams for salting and seasoning. The relationships between HWLFS and 17 performance, carcass and raw ham quality parameters were investigated in 260 performance tested Italian Large White heavy pigs. HWLFS was affected by slaughter day and visible intermuscular fat of trimmed hams whereas sex did not affect its variability. Residual correlations of HWLFS with backfat thickness (BFT; r = -0.51) and lean cuts (LC; r = +0.51) were stronger than with ham weight at trimming (r = +0.40) and after first salting (r = +0.37). Significant correlations of HWLFS with fresh ham quality traits ranged from +0.16 to -0.25. BFT, LC and pH_{24h} were the main predictors of HWLFS in the regression model. Results from this study indicated that higher ham fat coverage and pH_{24h} and lower LC could reduce HWLFS of green hams for Protected Designation of Origin products.

Keyword: Heavy pig; Italian Large White; Ham quality; Ham weight loss at first salting; Phenotypic correlations.

1. Introduction

The Italian pig industry is mainly oriented towards the production of heavy carcasses to provide suitable raw meat material for typical processed products, including dry-cured hams of the Protected Designation of Origin (PDO) system. Italian heavy pigs are slaughtered at an average live weight of 160 kg (± 10%) and when they are at least nine months old (EEC, 1992). This long period is essential to obtain raw hams of the right weight, muscle maturity and subcutaneous fat (15-30 mm of thickness) that minimize seasoning losses of the hams over the processing period (Bosi & Russo, 2004). PDO dry-cured hams processing technology is standardized and involves only the addition of sea salt (which acts as a bacteriostatic agent, it reduces water activity and plays a role on

taste of the final product), the control of ambient conditions (i.e. temperature, relative humidity and air flow) and the duration of the ripening (Pagliarini et al., 2016; Parolari, 1996). Briefly, the processing of PDO dry-cured ham, such as Parma ham, includes the following steps: i) cooling, by keeping legs in cold rooms for 24 hours after slaughter to attain uniform temperature; ii) trimming, the procedure that removes external fat and rind needed to obtain the typical ham shape and to help the salting phase; iii) two salting steps (the first lasting seven days in a salting room; after which salt residuals are removed, legs are re-salted and stored for 15-18 days in another salting rooms); iv) resting for 60-90 days, to obtain a homogeneous salt distribution inside the leg muscles; v) washing from the excess of salt; and then drying; vi) pre-maturation period; vii) smearing or greasing; and then vii) final maturation (Pagliarini et al., 2016).

PDO dry cured ham production requires a processing time of at least 12 months (for PDO Parma ham) or 13 months (for PDO San Daniele ham). The key changes that occur during the processing period are related to water loss of the legs, salt intake, lipolysis and proteolysis (Čandek-Potokar & Škrlep, 2012). When the curing process is standardized, the final quality of dry cured hams (i.e. typical texture and flavour sensorial characteristics and processing yield) is primarily determined by the intrinsic characteristics of the fresh hams (Bosi & Russo, 2004). Sensory properties developed by the hams are mainly due to physical, and biochemical changes caused by endogenous proteolytic and lipolytic muscle enzymes that work during the drying and ripening/maturation phases(Toldrá, Aristoy, & Flores, 2000; Toldrá & Flores, 1998; Zhou, et al., 2019c,b).

To produce suitable legs to maximize dry-cured ham processing yield, breeding programmes for Italian heavy pigs, beside performance traits (average daily gain and feed:gain ratio), carcass traits (backfat thickness, weight of neck and loin and weight of hams), and meat quality (visible intermuscular fat of trimmed hams), includes ham weight loss after the first salting step (HWLFS). This latter characteristic, that is determined just 8-9 days after slaughter, is a parameter used in the pig selection programmes with the aim to minimize the total weight loss of the hams up to the end

of the seasoning period. The weight losses at these two periods (at first salting and at the end of the processing period) have high phenotypic and genetic correlations (0.57-0.73 and 0.49-0.73, respectively (Bosi & Russo, 2004). Phenotypic correlations between HWLFS and traits related to performances, carcass and ham quality traits have been poorly investigated in heavy pig breeds (Bosi & Russo, 2004; Russo, Nanni Costa, Lo Fiego, & De Grossi, 1993).

The objectives of this study are 1) to investigate the phenotypic relationships between HWLFS and 17 parameters related to production performance, carcass and ham muscles in Italian Large White heavy pigs, 2) to determine the predictive parameters of HWLFS using a stepwise multiple regression analysis.

2. Material and methods

Animal care and slaughter of the animals used in this study were performed in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009] on the protection of animals during transport and related operations and at the time of the killing. All slaughter procedures were monitored by the veterinary team appointed by the Italian Ministry of Health and sampling occurred with the permission of the National Pig Breeder Association (ANAS; http://www.anas.it).

2.1. Animals

A total of 260 Italian Large White pigs (172 entire gilts and 88 castrated males obtained from 79 sires) were involved in this study. These pigs were from triplets of siblings of the same litter (two females and one castrated male) individually performance tested at the central station of ANAS for the genetic evaluation of a candidate boar from the same litter (sib-testing). The test period began when piglets were approximately 30 kg live weight and it ended when the pigs were slaughtered at about 8 months following the rules of the test station.

All pigs were genotyped for the ryanodine receptor 1 (*RYR1*) g.1843C>T mutation (Fujii et al., 1991), causing the Pale Soft and Exudative meat defect and for the p.R200Q mutation of the protein kinase AMP-activated non-catalytic subunit gamma 3 (*PRKAG3*) gene (Milan et al., 2000), causing the acid meat defect. Genotyping was carried out as previously described by Fontanesi et al. (2008).

2.2. *In vivo* performances of the animals

Nutritive level of the animals in the performance testing period till slaughter was *quasi ad libitum*, meaning that about 60% of the pigs were able to ingest the entire supplied ration. The feeding regime and the diets are already described by Fontanesi et al. (2010). Feed intake was individually recorded daily and live weight was measured every two weeks. The data obtained were then used to calculate average daily gain (ADG, kg/d) and feed/gain ratio (FGR) for each pig.

2.3. Slaughtering and carcass traits

The days before slaughter, the pigs were weighed in the performance station (thereafter referred as slaughter weight; SW, kg). Animals at the end of test were transported to a commercial slaughterhouse (six slaughtering days) located 24.5 km from the test station (the full sibs were slaughtered in two different slaughter days). After unloading, pigs were immediately stunned and bled in a lying position. Carcasses were then divided in commercial cuts. Within 3 h after slaughter, backfat thickness (BFT, mm) at the level of *Gluteus medius* muscle, weight of necks and loins (referred as lean cuts, LC; kg), and weight of raw hams (HW, kg) were measured in the commercial abattoir.

2.4. Ham technological traits

At a single processing plant, all raw hams were chilled for 24 h and then trimmed. The two hams of each pig were re-weighed at the end of the trimming line (mean trimmed ham weight;

158 HW_TR, kg), and after six days from the first salting step (HW_FS, kg). Mean ham weight loss
159 (HWL) after trimming [HWLTR = (HW - HW_TR), in kg] and after first salting [HWLFS =
160 (HW_TR - HW_FS), in kg] were then calculated.

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163 **2.5. Raw ham quality traits measurements**
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165 Visible intermuscular fat (VIF) is the level of fat deposition (infiltration) subjectively
166 evaluated on muscles of trimmed hams (which includes mainly *semitendinosus*, *semimembranosus*,
167 *biceps femoris*, *gastrocnemius* and *gracilis* muscles) by a trained personnel with the scoring system
168 described by Fontanesi et al. (2017). VIF in this trial was coded as follows: 0, when exposed muscle
169 area of both legs of the same pig did not show VIF; 1, when muscles from only one leg showed
170 VIF; 2, when muscles from both legs showed VIF.

171 Subsequent measures on the hams were assessed on the *Semimembranosus* muscle (SM). On
172 the slaughter line, pH was measured at 2 h *post mortem* (pH_{2h}), and at 24 h *post mortem* (pH_{24h})
173 using a portable Crison pH-meter equipped with an Ingold Xerolite glass electrode (Model 5232,
174 Crison, Modena, Italy) and an automatic temperature compensation probe.

175 At 2 h *post mortem*, samples taken from SM were snap frozen in liquid nitrogen and freeze-
176 dried for the determination of muscle carbohydrates as described by Fontanesi et al. (2008). Briefly,
177 content of lactate was determined by UV method using the L-Lactic acid BioAnalysis kit
178 (Boehringer Mannheim/R-Biopharma, R-Biopharma GmbH, Darmstadt, Germany). Glycogen (first
179 degraded to glucose with amyloglucosidase from *Aspergillus niger)*, glucose and glucose-6-
180 phosphate were determined using the D-Glucose Enzymatic BioAnalysis kit (Boehringer Mannheim/R-
181 Biopharma, Milan, Italy) and expressed as µmol of lactic acid equivalent per gram of fresh muscle
182 (µmol/g). Glycolytic potential (GP), a measure of all components present in the muscle that can
183 potentially be converted into lactate during post-mortem glycolysis, was calculated as 2[glycogen +
184 glucose + glucose-6-phoshate] + [lactate] and expressed as µmol/g (Monin &
185 Sellier, 1985).

186 Activity of the endopeptidase cathepsin B activity (Catb activity) was analyzed on SM
187 samples collected at 24 h *post mortem*. Activity was measured using N-CBZ-L arginyl-L-arginine
188 as the fluorescent substrate (expressed as nmol of 7-amino-4-methylcoumarin released/min/g
189 muscle; nmol AMC/min/g. Parolari, Virgili, & Schivazappa, 1994; Russo et al., 2000; Schivazappa
190 et al., 2002).

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193 **2.6. Statistical analyses**
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195 Descriptive statistics (mean, standard deviation, minimum and maximum values, and
196 coefficient of variation) were calculated using the PROC MEANS procedure of Statistical Analysis
197 System (SAS Inst. INC., Cary, NC, USA, version 9.4.).

198 A multivariate analysis of variance was performed using the PROC GLM procedure of SAS.
199 The model included sex (castrated males and gilts) and VIF (three levels: 0, 1 and 2) as fixed effects
200 and slaughter day (six levels) as random effect. The least square means (LSM) and standard errors
201 (SE) were compared using the PDIFF option. Residual correlation coefficients were used to
202 determine the relationships between traits and were graphically displayed as heatmap using the
203 *gplots* package in R environment (R Core Team, 2018).

204 The observations of HWLFS were clustered using the FASTCLUS procedure in SAS. Two
205 classes of HWLFS referred as low (190 ± 21 g, range from 135 g to 221 g) and high (252 ± 24 g,
206 range from 224 g to 317 g) were defined. In this procedure, observations that were very close to
207 each other were assigned to the same cluster by an algorithm for minimizing the sum of squared
208 distances from the clustered means. The distribution of HWLFS clusters by VIF (absence and
209 presence of VIF, i.e. VIF = 0 and VIF > 0 including 1 and 2 classes of VIF) were analyzed using the
210 PROC FREQ procedure in SAS with the chi square test option. In this analysis two classes of VIF
211 were used (instead of three) since difference between LSM of HWLFS when VIF = 1 and VIF =2
212 was not significant.

213 Furthermore, the stepwise regression analysis of SAS was performed with the aim of selecting
214 input variables from a set of explanatory variables of performance, carcass, technological and meat
215 characteristics that most contributed to HWLFS. On the basis of the results of variance and cluster
216 analyses, the stepwise regression analysis was carried out for all pigs and separately for pigs
217 grouped based on visible intermuscular fat (VIF = 0 and VIF > 0). To assess multicollinearity, the
218 variance inflation factor option was used (values were all < 10). The *P* value, R^2 (partial and
219 cumulative) and root mean square error (RMSE) were used as parameters to determine the accuracy
220 of the prediction.

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223 **3. Results and discussion**
224
225 **3.1. Description of the *in vivo* performance, carcass and technological traits**
226
227 All pigs were reared under the same environmental conditions and feeding regime and
228 subjected to the same pre- and post-slaughter handling, which are all important factors of variations
229 for the quality of hams (Čandek-Potokar & Škrlep, 2012). The pigs did not carry the g.1843T allele
230 of the *RYR1* gene (Fujii et al., 1991) and the p.200Q allele of the *PRKAG3* gene (Milan et al.,
231 2000), the two major negative mutations for meat quality traits in pigs (Čandek-Potokar & Škrlep,
232 2012; Cherel et al., 2010).

233 Descriptive statistics of the measured traits are reported in Table 1. The variability in HWLFS
234 as measured by the coefficient of variation (CV = 17.4%) was high. This ham quality parameter
235 was on average equal to 220 ± 38 g, corresponding to a loss percentage in this phase of 1.75 ±
236 0.28%. Similar mean values (1.93 ± 0.59%), but with higher variability (CV = 30.6%), were
237 previously reported by Schivazappa et al. (2002) in three Italian heavy pig breeds, Italian Duroc,
238 Italian Landrace and Italian Large White, with higher losses in this latter breed (2.16%).

239 Among the *in vivo* performance and carcass traits, BFT of *gluteus medius* showed the highest
240 variability (mean and SD = 26.5 ± 5.4 mm; CV = 20.5%), although the specific aim of selection of
241 heavy pigs is focused on maintaining a constant value of BFT because an insufficient fat covering

of the ham causes increased seasoning losses and negatively impacts organoleptic characteristics of dry cured hams (Bosi & Russo, 2004; Čandek-Potokar & Škrlep, 2012). Raw ham weight (HW) was on average 15.4 ± 1.3 kg (CV = 8.5%). After trimming the hams (with mean of 12.6 ± 1.1 kg) lost on average 2.8 ± 0.4 kg of their weight in fat and muscle, corresponding to a loss percentage at trimming phase of 18.3 ± 1.7%. Similar values of HW and HW_TR were previously reported in Italian Large White animals (Russo et al., 1993). In heavy pigs for Parma ham production, the trimmed legs should preferably weigh between 12 and 14 kg, but in no case be less than 10 kg (EEC, 1992).

3.2 Description of the ham quality traits

For visible intermuscular fat (VIF), defined as the subjective estimation of exposed fat depots between the ham muscle groups, 40.8% of the hams had absence of VIF in both legs while 25.0% showed the presence of VIF in one ham with the remaining 34.2% showing the presence of VIF in both hams.

The pH values were much less variable (pH_{2h}: CV = 4.1% and pH_{24h}: CV = 3.7%), in agreement with what was previously reported by other authors in both light and heavy pigs (Ramos, Serenius, Stalder, & Rothschild, 2007; Sturaro, Gallo, Noventa, & Carnier, 2008; Ventura et al., 2011). Several samples were not in the range of 5.6 - 6.2 recommended for pH_{24h} in fresh hams intended for curing (Arnau, 2004). Although the pigs did not carry the *PRKAG3* p.200Q allele, known to decrease the ultimate pH and pork quality characteristics (Cherel et al., 2010), eight samples exhibited low pH_{24h} values (<5.4) compatible with the acid meat defect. This indicated that low muscle pH_{24h} can occur in pigs that do not carry the negative allele of the *PRKAG3* gene. Conversely, nine samples had an insufficient decrease in the final pH (pH_{24h} above 6.2).

Among the investigated traits, the highest variability of the fresh hams was observed for glycogen (CV = 47.7 %) lactate (CV = 28.2 %) and GP (CV = 22.1%). In pigs that do not carry the negative allele at the *RN* locus (i.e. animals with the recessive genotype at the *PRKAG3* p.R200Q

polymorphic site), the muscle glycogen level is mainly determined by husbandry and feeding practices of the animals, transport conditions, climatic and environmental factors, handling procedures at slaughter and muscle type (England et al., 2016; Larzul, Monin, Sellier & Le Roy, 1998; Scheffler & Gerrard, 2007). Lactate was the main component (56.4 ± 15.9 %) of GP, as already reported in other studies (Maribo, Støier, & Jørgensen, 1999; Przybylski, Sionek, Jaworska, & Santé-Lhoutellier, 2016). The contribution of lactate in GP reported in this study was lower than those in other investigations. This difference could probably be due to the difference in the sampling time (Maribo et al., 1999; Przybylski et al., 2016). However, according to Maribo et al. (1999) the determination of GP is not affected by the different *post mortem* sampling time.

In our samples, Catb activity (1.16 ± 0.23 nmol AMC min^{-1} g^{-1} meat; CV = 19.6%) was lower than what was on average obtained in other Italian heavy pigs (i.e. 1.36 nmol AMC min^{-1} g^{-1} and CV = 22.8%, Sturaro et al., 2008; 1.55 nmol AMC min^{-1} g^{-1} and CV = 18%, Virgili, Schivazappa, Parolari, Bordini, & Degni, 1998). Catb activity has been found to exhibit variability depending on genetic type, breeding, age at slaughter, type of muscle, season and, during the processing period of the legs, by several other factors such as salt, temperature, humidity and time of ripening (Arnau, Guerrero, & Sárraga, 1998; Mora, Escudero, & Toldrá, 2016; Sárraga, Gil, & García-Regueiro, 1993; Sturaro, Gallo, Noventa, & Carnier, 2008; Virgili, Parolari, Schivazappa, Bordini, & Borri, 1995; Zhou, et al., 2019a). Sturaro et al. (2008) reported a slight decrease in Catb activity when the carcass weight increased in crossbred of Large White boar line x crossbred sows (1.38 nmol AMC/min/g for lighter carcasses and 1.33 nmol AMC/min/g for heavier carcasses; $P < 0.05$). In addition, Sárraga et al. (1993) identified lower Catb activity in green hams from heavy pigs than light pigs, and they suggested that lower levels in heavy pigs could be attributed to their slower protein turnover.

3.2. Effects of sex, slaughter days and visible intermuscular fat

296 Investigated traits and parameters that were used in the correlation analyses were adjusted for
297 the effects of three different variation factors (Čandek-Potokar & Škrlep, 2011; Ramos et al., 2007)
298 such as sex, slaughter days and VIF. Probability values of factors included in the model and
299 coefficient of determination (R-square in percentage) are reported in Table 2. Coefficients of
300 determination was higher for HWLFS (32.0%).

301 Sex did not affect HWLFS as well as ADG, FGR and HW at different stages ($P > 0.05$;
302 Tables 2 and S1). Castrated males had thicker BFT and higher losses at trimming phase compared
303 to gilts. LC from gilts were heavier than males (Tab. S1). The thicker BFT of barrows and the
304 greater yields of lean cuts from gilts are in agreement with previous studies carried out on heavy
305 pigs (Latorre, García-Belenguer, & Ariño, 2008; Schiavon et al., 2015).

306 Sex did not show any effect on GP, its components, pH measurements and Catb activity of
307 fresh hams ($P > 0.05$), except for glycogen that was higher in castrated males. Other studies
308 reported no effect of sex on several meat quality traits, including some analyzed in this study such
309 as pH_{24h}, Catb activity and glycolytic components (Armero et al., 1999; Monin & Sellier, 1985;
310 Peloso, Lopes, Gomide, Guimarães, & Carneiro, 2010; Schivazappa, Virgili, & Parolari, 1992).
311 Nevertheless, Sturaro et al. (2008) identified slightly higher Catb activity in gilts than castrated
312 males.

313 The visible intermuscular fat of trimmed hams was a significant source of variation for
314 HWLFS ($P < 0.001$; Tables 2 and S2). In particular, the pigs with absence of VIF in both hams
315 (VIF = 0) had significantly higher least square means of HWLFS compared to those of the pigs of
316 the other two classes of VIF (i.e. 1 = presence of VIF in one and 2 = presence of VIF in both hams;
317 $P < 0.001$). Furthermore, VIF affected the variability of other traits such as BFT, LC, HW_TR,
318 HW_FS, pH_{24h}, glycogen and GP (Table 2 and S2). The pigs with absence of VIF in both hams
319 (VIF = 0) had lower BFT and pH_{24h} and higher LC, HW_TR, HW_FS and glycogen than the pigs
320 with presence of VIF in at least one ham. The comparison between the least square means of the
321 extreme (0 and 2) classes of VIF resulted significant for GP ($P = 0.044$).

The day of slaughter (which may incorporate differences in pens, batch, and month of slaughtering) was a significant source of variation ($P < 0.05$) for all traits under study except for HW at trimming and at post first salting stages ($P = > 0.05$).

3.3 Correlations between HWLFS and *in vivo* performance, carcass and ham technological traits

HWLFS is a quality trait used to assess the aptitude of the meat for salting to obtain legs with optimal yield. Almost all correlations of HWLFS with *in vivo* performance, carcass and technological ham traits were significant (Figure 1. Table S3). As expected, HWLFS was negatively correlated with BFT ($r = -0.51$, $P < 0.0001$), confirming that an adequate level of BFT is beneficial to minimizing HW loss (Bosi & Russo, 2004). This is due to lower water content of adipose tissue than muscular tissue (5-15% vs 70-75%) and to the positive barrier effect of fat on water which drips out from the hams through diffusion and evaporation during the processing period (Bosi & Russo, 2004; Ramos et al., 2007). Conversely, HWLFS was positively correlated with LC ($r = +0.51$; $P < 0.0001$), indicating that the selection for carcass conformation (muscularity) tends to worsen this quality characteristic of the ham. The relationships between HWLFS and HW at different stages were positives and significant ($r = +0.34/+0.40$; $P < 0.0001$). Čandek-Potokar & Škrlep (2011) observed higher daily moisture losses in hams with heavier weight due to their larger contact area for exchange. Other studies showed no or very low correlations between HW and seasoning losses or yield (Čandek-Potokar & Škrlep, 2012; Ramos et al., 2007).

3.4 Correlations between HWLFS and ham quality traits

The correlations between HWLFS and fresh meat quality traits were generally low (Figure 1. Table S4). HWLFS was correlated negatively with pH_{24h} ($r= -0.25$. $P < 0.0001$). This result is in agreement with previous studies reporting higher weight losses during the initial phase of curing in hams exhibiting low pH_{24h} values (Čandek-Potokar & Škrlep, 2011; Schivazappa et al., 2002). The

correlations of HWLFS with glycogen (r = +0.22) and GP (r = +0.16) were significant (P < 0.05), indicating that higher glycogen and GP resulted in increased weight loss during the first salting process (Nanni Costa, Lo Fiego, Pantano, & Russo, 1998). The coefficient of correlation between HWLFS and GP observed in the current study was similar to those observed for commercial hybrid heavy pigs and Duroc x (Landrace x Large White) crossbreeds (Nanni Costa et al., 1998). These results confirmed the detrimental effect of high GP on ham production and pork quality attributes (Hamilton, Miller, Ellis, McKeith, & Wilson, 2003). HWLFS did not appear to be correlated with Catb activity.

3.5 Correlations between raw ham quality traits

Initial pH measured at 2 h was moderately correlated with pH_{24h} (r = +0.58. Table S4). The correlation between pH_{2h} and lactate was significant and negative (r = -0.48), no correlation was found between pH_{24h} and lactate. The correlation between glycogen and pH_{24h} (r = -0.50) suggested that glycogen, responsible for *post mortem* glycolysis, lead to hams with low pH_{24h}. The correlation between GP and pH_{24h} resulted negative (r = -0.54; P < 0.0001), confirming that an increased GP indicates a high level of final lactate that can be accumulated in the post mortem muscles (Hamilton et al., 2003; Maribo et al., 1999; Monin & Sellier, 1985; Przybylski et al., 2016). However, other authors found that GP was only weakly associated with pH_{24h} in a synthetic Duroc line (England et al., 2016).

In agreement with the acidic properties of cathepsin B, pH_{24h} was negatively correlated with Catb activity. However, the magnitude of the correlation was limited (r = -0.19; P = 0.0024). Low pH_{24h} may favour the release of acid enzymes from the lysosomes, enhancing proteolytic activity in muscular tissue. Proteolysis and Catb activity is beneficial to the sensory quality of dry-cured hams, but high residual Catb at the end of processing period has been associated with textural (excessive softness, pastiness), flavor (i.e. bitter flavor), and appearance related (formation of white films on the ham surface) problems of dry-cured hams (Russo et al., 2000; Schivazappa et al., 2002; Virgili

et al., 1998). GP was more correlated with glycogen concentration (r= +0.75; P < 0.0001), than lactate accumulation (r = +0.40; P < 0.0001). This is not surprising given that GP was determined in the early *post mortem* period.

3.6 Cluster analysis and relationships between HWLFS and VIF

The distribution (count and frequency) of HWLFS clusters (low and high) by fatness classes of visible intermuscular fat (VIF = 0 and VIF > 0) was significant (P = 0.0012; Table S5). About 51% of the hams clustered in the group with high HWLFS were scored to have no VIF on both legs (Fig. 2). In the cluster based on low HWLFS, only 31.4% of samples were scored to have no VIF.

These results confirmed that, beside subcutaneous level (BFT), HWLFS is partially influenced by intermuscular fat depot in the hams. However, an excess in VIF is a defect that depreciates the final product, as sliced hams with high intermuscular fat content are not accepted by consumers (Kouba & Sellier, 2011). Thus, HWLFS and VIF appear to be antagonistic traits in ham quality.

3.7 Stepwise multiple regression analyses predicting HWLFS

Stepwise multiple regression analyses were performed to identify explanatory variables of performance, carcass, technological and raw ham quality traits that were most effective in predicting HWLFS (Table 3). For the entire sample, the predictors that satisfied the P < 0.05 to enter into the regression model were BFT, LC, pH_{24h}, SW and HW, which explained 55.9% of the variation in HWLFS. BFT and LC accounted for 33.0% and 13.8% of the variation, respectively, confirming the importance of these variables in the prediction of HWLFS. As expected, BFT, pH_{24h} and SW had negative regression coefficients, confirming that higher values of these traits are beneficial for lower losses during the curing process.

In the sample with absence of visible intermuscular fat (VIF = 0), 50.5% of the variation was explained by BFT, HW_TR and pH_{24h}. BFT (27.4%), and HW_TR (20.2%) explained a higher proportion of the variation. The predictors when VIF was present in one or both hams (VIF > 0) were BFT, LC and pH_{24h} explaining 50.6% of the variation in HWLFS. BFT and LC explained 34.2 and 11.8% of the variation, respectively.

According to our data the aptitude of the ham for seasoning is mainly affected by an increase in BFT and pH_{24h} and a decrease in LC supporting the notion that the right proportion of BFT, LC, VIF, and SW is required to prevent inappropriate losses during ripening.

The phenotypic correlations and stepwise regression analyses reported in this study provided information that can be used to minimize the inappropriate loss of weight during the curing of raw hams.

4. Conclusions

This study identified significant phenotypic residual correlations between HWLFS and 17 parameters, including performance, carcass and ham quality traits.

From the results a higher SM muscle pH_{24h} is desirable and associated with improved quality of meat as well as ham yield in the early stage of salting. Furthermore pH_{24h} was among the main explanatory variables influencing HWLFS, aside BTF and LC.

BFT alone explained a higher proportion of the variability in HWLFS, which confirms the importance of this variable in predicting HWLFS. In addition to BFT, VIF of trimmed hams appears to influence HWLFS and could be used as quality criteria in the evaluation of weight loss during the curing process. These results further pointed out the importance to use mature animals and carcasses of the right weight to obtain raw hams that could be suitable for the salting and seasoning processes.

Conflict of interest

436 The authors declare that they do not have competing interests.
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Table 1

Descriptive statistics of traits measured in 260 Italian Large White pigs.

Traits	Mean	Standard deviation (SD)	Min	Max	CV (%)
Production (in vivo performances) and carcass traits					
Average daily gain, ADG; kg/d	0.777	0.083	0.494	0.960	10.7
Feed:gain ratio, FGR	3.95	0.38	3.25	5.77	9.6
Slaughter weight, kg; SW	148.8	11.5	108.3	173.6	7.7
Slaughter age, d; SA	239.7	8.8	220	305	3.7
Backfat thickness of *gluteus medius* m., BFT; mm	26.51	5.43	13.00	42.00	20.5
Necks and loins weight (referred as lean cuts**)**, LC; kg	28.59	2.78	19.56	36.80	9.7
Raw ham weight, HW; kg	15.41	1.31	11.03	19.24	8.5
Ham technological traits[1]					
Ham weight after trimming, HW_TR; kg	12.59	1.10	9.00	16.05	8.8
Ham weight post first salting, HW_FS; kg	12.37	1.09	8.84	15.74	8.8
Ham weight loss, HWL					
Trimming, HWLTR; kg	2.82	0.36	1.94	3.99	12.8
First salting, HWLFS; g	220	38	135	317	17.4
Raw ham quality traits[2]					
pH at 2 h *post mortem*, pH_{2h}	5.94	0.24	5.41	6.80	4.1
pH at 24 h *post mortem*, pH_{24h}	5.67	0.21	5.30	6.54	3.7
Glycogen[3]	46.92	22.40	7.33	135.74	47.7
Lactate[3]	56.40	15.92	23.28	116.93	28.2
Glycolytic potential, GP[3]	103.32	22.85	49.23	182.73	22.1
Cathepsin B activity, Catb[4]	1.16	0.23	0.6	2.0	19.6

[1] Averaged from left and right hams.

[2] Measured on the *Semimembranosus* muscle.

[3] Expressed as µmol of lactic acid equivalent per g of fresh muscle (µmol/g).

[4] Expressed as nmol of 7-amino-4-methylcoumarin release/min/g muscle (nmol AMC/min/g).

Table 2

Effects of sex, slaughter day and visible intermuscular fat of trimmed hams on traits measured in Italian Large White pigs. Probability of the effects and coefficient of determination (R^2) of the model.

Traits[1]	Sex (df = 1)	Slaughter day (df = 5	VIF (df = 2)[2]	R^2, %
	P values[3]			
Average daily gain, ADG; kg/d	0.393	**0.002**	0.700	8.2
Feed:gain ratio, FGR	0.262	**<0.001**	0.466	17.8
Slaughter weight, kg; SW	0.335	**0.024**	0.312	5.8
Slaughter age, d; SA	0.191	0.400	0.571	3.6
Backfat thickness of *Gluteus medius* m., BFT; mm	**0.010**	**<0.001**	**0.001**	16.9
Lean cuts, LC; kg	**0.041**	**0.035**	**0.049**	8.3
Ham weight,				
Raw, HW; kg	0.724	**0.031**	0.054	5.6
Trimming, HW_TR; kg	0.358	0.122	**0.017**	5.4
Post first salting, HW_FS; kg	0.363	0.162	**0.026**	4.9
Ham weight loss				
Trimming, HWLTR; kg	**<0.001**	**<0.001**	0.553	18.2
First salting, HWLFS; g	0.472	**<0.001**	**<0.001**	32.0
pH_{2h}	0.768	**0.015**	0.446	6.2
pH_{24h}	0.726	**<0.001**	**0.031**	12.0
Glycogen; µmol/g	**0.049**	**0.002**	**0.001**	11.5
Lactate; µmol/g	0.518	**0.001**	0.238	8.9
Glycolytic potential, GP; µmol/g	0.147	**0.018**	**0.044**	7.7
Cathepsin B activity, Catb; nmol AMC/min/g	0.569	**0.031**	0.803	5.0

[1] Traits are defined in Table 1.

[2] VIF= subjective assessment of visual intermuscular fat of trimmed hams (0= absence in both hams, 1= presence in one ham, 2= presence in both hams).

[3] Significant effects ($P < 0.05$) are in bold.

Figure 1

Heatmap showing the residual correlations of ham weight loss at first salting (HWLFS) with production, carcass and ham technological traits of Italian Large white pigs. Traits are defined in Table 1. Data of residual correlations are shown in Tables S3-S5.

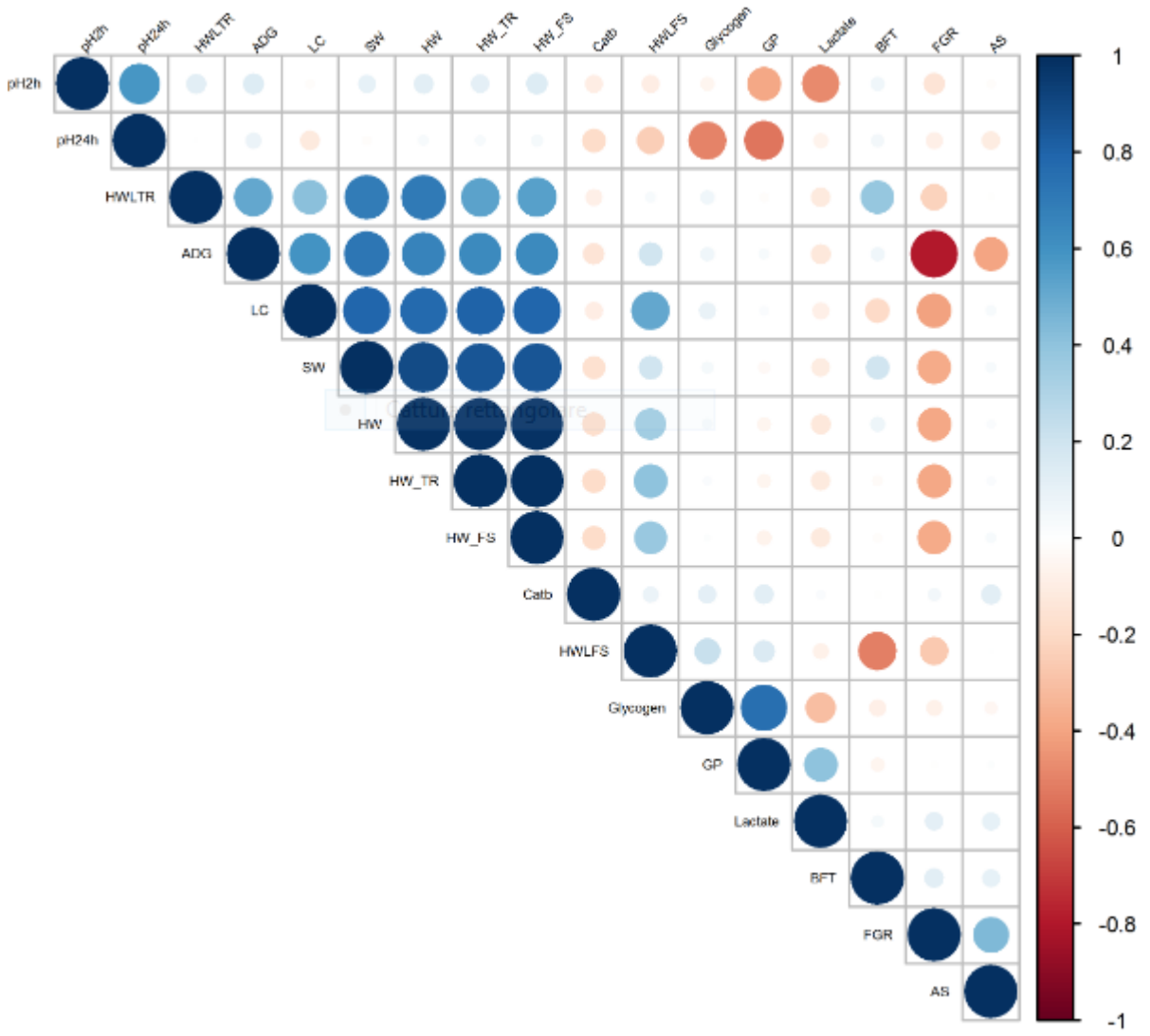

Table 3

The influence of carcass and ham quality traits on HWLFS using stepwise regression.

Group, number	Explanatory variables [1]	Parameter estimate ± SE[2]	Partial R^2, %	P	Cumulative R^2, %	RMSE[3]
All pigs, N. = 260						
	Intercept	0.4034 ± 0.0503		<0.001		
	BFT	-0.0030 ± 0.0004	33.0	<0.001		
	LC	0.0042 ± 0.0012	13.8	<0.001		
	pH_{24h}	-0.0417 ± 0.0078	3.6	<0.001		
	SW	-0.0015 ± 0.0004	3.0	<0.001		
	HW	0.0152 ± 0.0027	2.5	0.003		
					55.9	0.0256
Absence of visible intermuscular fat (VIF = 0), N. = 106						
	Intercept	0.3759 ± 0.0989		<0.001		
	BFT	-0.0040 ± 0.0005	27.4	<0.001		
	HW_TR	0.0118 ± 0.0029	20.2	<0.001		
	pH_{24h}	-0.0405 ± 0.0165	2.9	0.003		
					50.5	0.0281
Presence of visible intermuscular fat >0 (VIF> 0), N. = 154						
	Intercept	0.3693 ± 0.0607		<0.001		
	BFT	-0.0033 ± 0.0004	34.2	<0.001		
	LC	0.0044 ± 0.0008	11.8	<0.001		
	pH_{24h}	-0.0333 ± 0.0089	4.6	0.003		
					50.6	0.0256

[1]Traits are defined in Table 1.

[2] Standard error.

[3] Root mean square error.

Figure 2

Distribution (%) of hams into weight loss at first salting (HWLFS) groups according to visible intermuscular fat (VIF) classes. Hams were grouped into two classes of HWLFS (low = 190 ± 21 g, ranging from 135 g to 221 g; high = 252 ± 24 g, ranging from 224 g to 317 g) based on the FASTCLUS procedure of SAS. VIF: subjective assessment of absence (0) or presence (> 0) of visual intermuscular fat of trimmed hams.

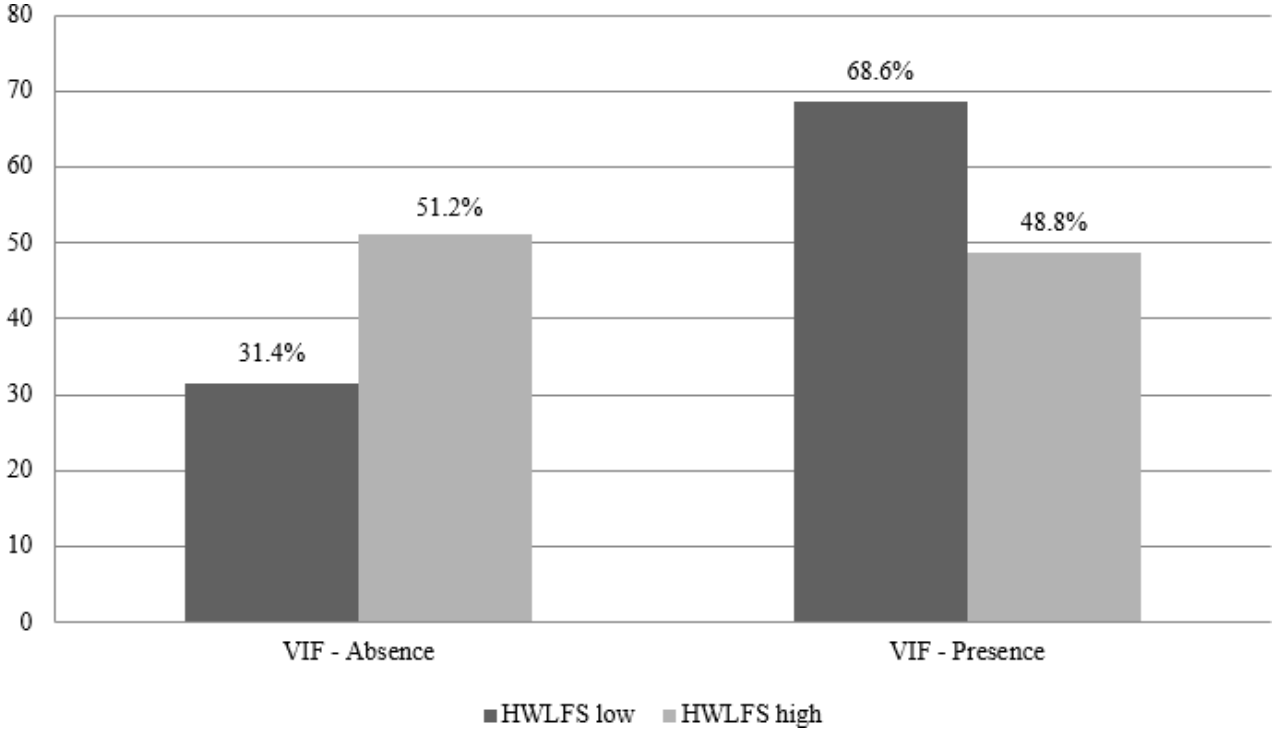

Table S1

Least square means (LSM) ± standard errors (SE) of investigated traits in castrated males and gilts of Italian Large White pigs.

Traits[1]	Castrated males	Gilts	Gender
	LSM ± SE	LSM ± SE	p-values[2]
Average daily gain, ADG; kg/d	0.783 ± 0.009	0.774 ± 0.006	0.393
Feed:gain ratio, FGR	4.00 ± 0.04	3.95 ± 0.03	0.262
Slaughter weight, SW; kg	149.83 ± 1.26	148.35 ± 0.89	0.335
Slaughter age, SA; g	238.71 ± 0.86	240.09 ± 0.61	0.191
Backfat thickness of *gluteus medius* m., BFT; mm	27.95 ± 0.56	26.17 ± 0.40	**0.001**
Lean cuts, LC; kg	28.00 ± 0.30	28.76 ± 0.21	**0.041**
Ham weight			
Raw, HW; kg	15.43 ± 0.14	15.37 ± 0.10	0.724
Trimming, HW_TR; kg	12.47 ± 0.12	12.61 ± 0.09	0.358
Post first salting, HW_FS, kg	12.26 ± 0.12	12.39 ± 0.08	0.363
Ham weight loss			
Trimming, HWLTR; kg	2.96 ± 0.04	2.76 ± 0.03	**<0.001**
First salting, HWLFS; g	217 ± 4	220 ± 2	0.472
pH_{2h}	5.93 ± 0.03	5.94 ± 0.02	0.769
pH_{24h}	5.68 ± 0.02	5.67 ± 0.02	0.726
Glycogen	49.82 ± 2.38	44.08 ± 1.68	**0.049**
Lactate	56.25 ± 1.71	57.61 ± 1.21	0.518
Glycolytic potential, GP	106.08 ± 2.47	101.69 ± 1.75	0.147
Cathepsin B activity, Catb	1.14 ± 0.02	1.16 ± 0.02	0.569

[1] Traits are defined in Table 1.

[2] The significant P values are indicated in bold.

Table S2

Least square means (LSM) ± standard errors (SE) of traits investigated in different classes of visible intermuscular fat (VIF) of Italian Large White pigs.

Traits[1]	VIF = 0[2] LSM ± SE [3]	VIF = 1[2] LSM ± SE [3]	VIF = 2[2] LSM ± SE [3]	VIF p-values[4]
Average daily gain, ADG; kg/d	0.778 ± 0.009	0.785 ± 0.011	0.773 ± 0.009	0.691
Feed:gain ratio, FGR	3.93 ± 0.04	4.01 ± 0.05	3.98 ± 0.04	0.466
Slaughter weight, SW; kg	149.83 ± 1.26	148.35 ± 0.89	148.35 ± 0.89	0.312
Slaughter age, SA; g	238.71 ± 0.86	240.09 ± 0.61	240.09 ± 0.61	0.571
Backfat thickness of *gluteus medius* m., BFT; mm	24.97 ± 0.55 $^{B, D}$	28.37 ± 0.66^{C}	27.84 ± 0.57^{A}	**0.001**
Lean cuts, LC; kg	29.03 ± 0.30^{A}	28.10 ± 0.36^{B}	28.01 ± 0.31^{B}	**0.049**
Ham weight				
Raw, HW; kg	15.69 ± 0.14 ^{a}	15.34 ± 0.17	15.17 ± 0.15 ^{b}	0.054
Trimming, HW_TR; kg	12.84 ± 0.12$^{a, c}$	12.45 ± 0.14^{b}	12.34 ± 0.12^{d}	**0.017**
Post first salting, HW_FS, kg	12.60 ± 0.12^{a}	12.24 ± 0.14$^{b, c}$	12.13 ± 0.12^{d}	**0.026**
Ham weight loss				
Trimming, HWLTR; kg	2.85 ± 0.04	2.89 ± 0.04	2.84 ± 0.04	0.553
First salting, HWLFS; g	238 ± 03 ^{C}	210 ± 4 ^{D}	207 ± 4 ^{D}	**<0.001**
pH_{2h}	5.94 ± 0.03	5.96 ± 0.03	5.91 ± 0.03	0.446
pH_{24h}	5.63 ± 0.02^{b}	5.70 ± 0.03^{a}	5.70 ± 0.02^{a}	**0.031**
Glycogen	54.00 ± 2.35 $^{a, A}$	46.33 ± 2.82 ^{b}	40.52 ± 2.43^{B}	**0.001**
Lactate	55.01 ± 1.69	56.48 ± 2.03	59.30 ± 1.75	0.238
Glycolytic potential, GP	109.01 ± 2.44 ^{a}	102.81 ± 2.93	99.83 ± 2.52 ^{b}	**0.044**
Cathepsin B activity, Catb	1.16 ± 0.02	1.14 ± 0.03	1.15 ± 0.03	0.803

[1] Traits are defined in Table 1.

[2] VIF: subjective assessment of visual intermuscular fat of trimmed hams (0 = absence in both hams, 1 = presence in one ham, 2 = presence in both hams).

[3] Different lower case superscript letters in the same line indicate statistically significant differences of classes (a,b: P<0.05; c,d: P<0.01; A,B: P< 0.001; C,D: P<0.0001).

[4] The significant P values are indicated in bold.

Table S3

Residual correlations[1] of ham weight loss at first salting (HWLFS) with production, carcass and ham technological traits of Italian Large white pigs.

	HWLFS g	ADG kg/d	FGR	SW Kg	AS d	BFT mm	LC kg	HW kg	HW_TR kg	HW_FS kg	HWLTR kg
HWLFS g	1	**+0.19** **0.0020**	**-0.27** **0.0001**	**+0.19** **0.0021**	0.00 0.9931	**-0.51** **<0.0001**	**+0.51** **<0.0001**	**+0.34** **<0.0001**	**+0.40** **<0.0001**	**+0.37** **<0.0001**	0.03 0.5870
ADG kg/d		1	**-0.79** **<0.0001**	**0.72** **<0.0001**	**-0.40** **<0.0001**	0.06 0.3741	**+0.59** **<0.0001**	**+0.66** **<0.0001**	**+0.63** **0.0001**	**+0.63** <**0.0001**	**+0.51** <**0.0001**
FGR			1	**-0.38** **<0.0001**	**+0.44** **<0.0001**	+0.12 0.0516	**-0.41** <**0.0001**	**-0.39** **<0.0001**	**-0.39** **<0.0001**	**-0.38** **<0.0001**	**-0.23** **0.0002**
SW kg				1	+0.03 0.6551	**+0.19** **0.0031**	**+0.79** **<0.0001**	**+0.89** **<0.0001**	**+0.85** **<0.0001**	**+0.85** **<0.0001**	**+0.69** **<0.0001**
AS d					1	+0.10 0.1226	+0.03 0.6098	+0.02 0.7767	+0.02 0.6950	+0.03 0.6915	-0.01 0.8572
BFT mm						1	**-0.20** **0.0011**	+0.07 0.2669	-0.03 0.6219	-0.02 0.7956	**+0.38** **<0.0001**
LC kg							1	**+0.77** **<0.0001**	**+0.80** **<0.0001**	**+0.79** **<0.0001**	**+0.41** **<0.0001**
HW kg								1	**+0.98** **<0.0001**	**+0.98** **<0.0001**	**+0.70** **<0.0001**
HW_TR kg									1	**+0.99** **<0.0001**	**+0.53** **<0.0001**
HW_FS kg										1	**+0.54** **<0.0001**
HWLTR kg											1

[1] The values for the phenotypic correlations are shown in the upper rows, the lower rows report the P values, significant correlations (at least $P < 0.05$) are indicated in bold. Traits are defined in Table 1.

Table S4

Residual correlations[1] of ham weight loss at first salting (HWLFS) with raw ham quality traits of Italian Large White pigs.

	HWLFS g	pH_{2h}	pH_{24h}	Glycogen µmol/g	Lactate µmol/g	GP µmol/g	Catb nmol AMC/min/g
HWLFS g	1	-0.10 0.1303	**-0.25** **<0.0001**	**+0.22** **0.0004**	-0.08 0.2117	**+0.16** **0.0113**	+0.08 0.1815
pH_{2h}		1	**+0.58** **<0.0001**	-0.06 0.3383	**-0.48** **<0.0001**	**-0.39** **<0.0001**	-0.10 0.1048
pH_{24h}			1	**-0.50** **<0.0001**	-0.07 0.2534	**-0.54** **<0.0001**	**-0.19** **0.0024**
Glycogen µmol/g				1	**-0.31** **<0.0001**	**+0.75** **<0.0001**	+0.11 0.0800
Lactate µmol/g					1	**+0.40** **<0.0001**	+0.02 0.7001
GP µmol/g						1	+0.12 0.0504
Catb nmol AMC/min/							1

[1] The values for the phenotypic correlations are shown in the upper rows, the lower rows report the P values, significant correlations (at least $P < 0.05$) are indicated in bold. Traits are defined in Table 1.

Table S5

Distribution of hams into weight loss at first salting (HWLFS)[1] groups according to visible intermuscular fat (VIF) [2]

Traits	HWLFS clusters[1]		P-value
	Low Number (%)	High Number (%)	
Visible intermuscular fat, VIF[2]	137	123	0.0012
0 = absence in both hams	43 (31.4)	63 (51.2)	
> 0= presence in one or both hams	94 (69.6)	60 (48.8)	

[1] Hams were grouped into two classes of HWLFS (low = 190 ± 21 g, ranging from 135 g to 221 g; high = 252 ± 24 g, ranging from 224 g to 317 g) based on FASTCLUS procedure of SAS.

[2] VIF: subjective assessment of visual intermuscular fat of trimmed hams.

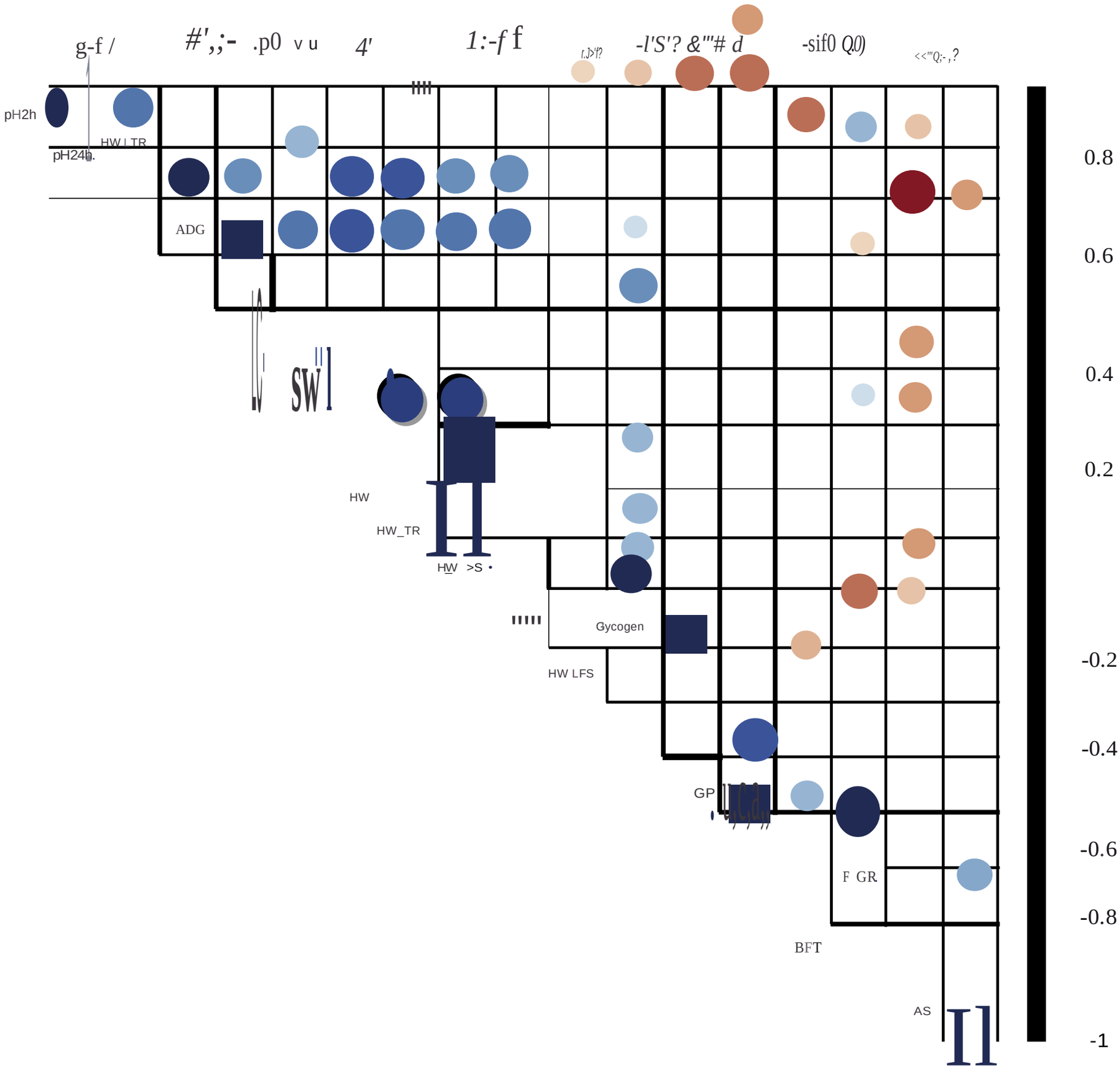
pH2h
HW LTR
pH24h.
ADG
HW
HW_TR
HW >S
Glycogen
HW LFS
GP
F GR
BFT
AS
0.8
0.6
0.4
0.2
-0.2
-0.4
-0.6
-0.8
-1

Figure 1

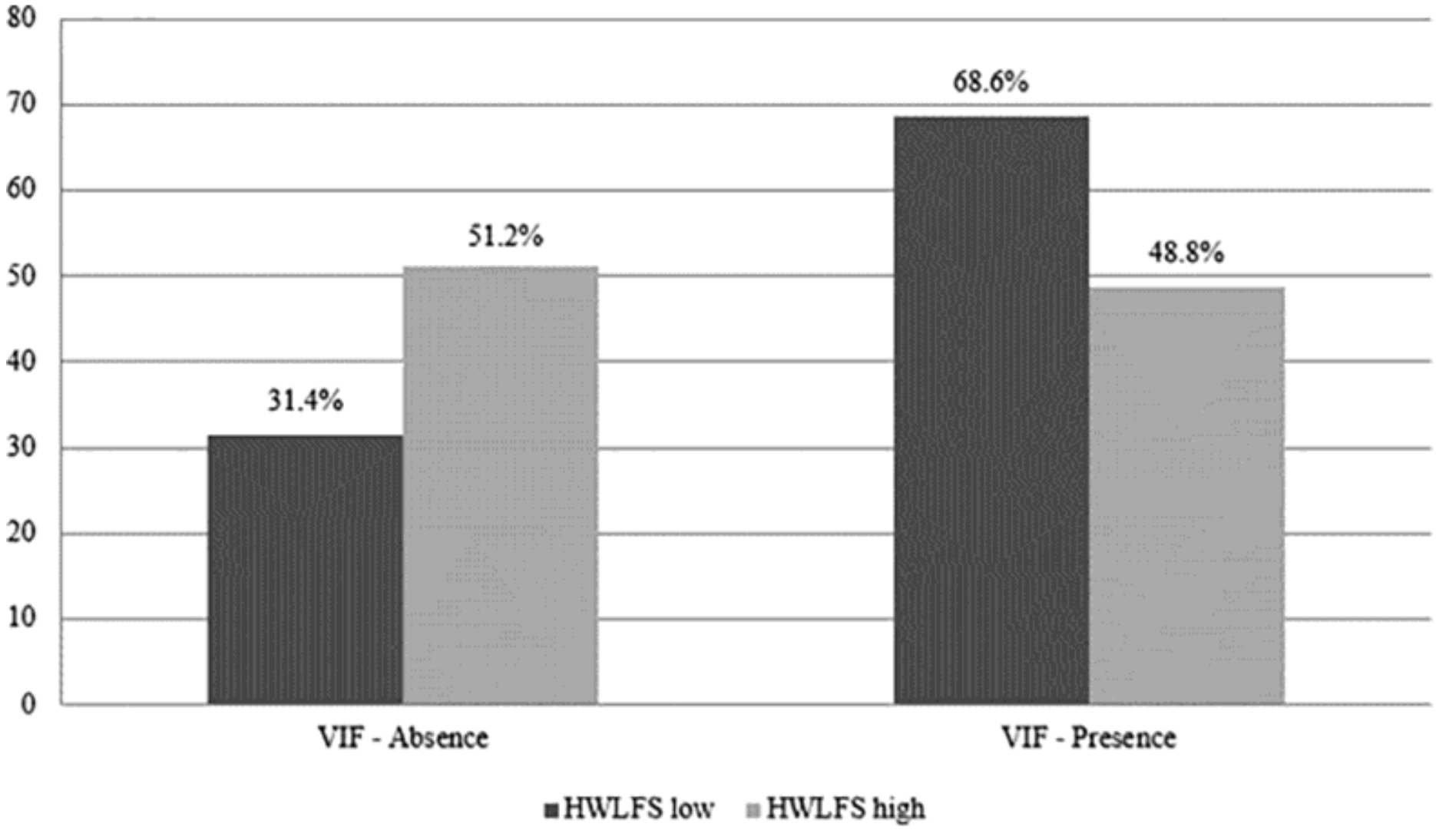
80
70
60
50
40
30
20
10
0
68.6%
51.2%
48.8%
31.4%
VIF - Absence
VIF - Presence
HWLFS low
HWLFS high

Figure 2