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

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

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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 $\text{pH}_{24\text{h}}$ 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 $\text{pH}_{24\text{h}}$ were the main predictors of HWLFS in the regression model. Results from this study indicated that higher ham fat coverage and $\text{pH}_{24\text{h}}$ 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 ($\pm 10\%$) 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

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

79 PDO dry cured ham production requires a processing time of at least 12 months (for PDO
80 Parma ham) or 13 months (for PDO San Daniele ham). The key changes that occur during the
81 processing period are related to water loss of the legs, salt intake, lipolysis and proteolysis (Čandek-
82 Potokar & Škrlep, 2012). When the curing process is standardized, the final quality of dry cured
83 hams (i.e. typical texture and flavour sensorial characteristics and processing yield) is primarily
84 determined by the intrinsic characteristics of the fresh hams (Bosi & Russo, 2004). Sensory
85 properties developed by the hams are mainly due to physical, and biochemical changes caused by
86 endogenous proteolytic and lipolytic muscle enzymes that work during the drying and
87 ripening/maturation phases (Toldrá, Aristoy, & Flores, 2000; Toldrá & Flores, 1998; Zhou, et al.,
88 2019c,b).

90 To produce suitable legs to maximize dry-cured ham processing yield, breeding programmes
91 for Italian heavy pigs, beside performance traits (average daily gain and feed:gain ratio), carcass
92 traits (backfat thickness, weight of neck and loin and weight of hams), and meat quality (visible
93 intermuscular fat of trimmed hams), includes ham weight loss after the first salting step (HWLFS).
94 This latter characteristic, that is determined just 8-9 days after slaughter, is a parameter used in the
95 pig selection programmes with the aim to minimize the total weight loss of the hams up to the end
96

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

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

106 107 108 **2. Material and methods**

109
110 Animal care and slaughter of the animals used in this study were performed in compliance with the
111 European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009]
112 on the protection of animals during transport and related operations and at the time of the killing.
113 All slaughter procedures were monitored by the veterinary team appointed by the Italian Ministry of
114 Health and sampling occurred with the permission of the National Pig Breeder Association (ANAS;
115 <http://www.anas.it>).

116 117 118 **2.1. Animals**

119
120 A total of 260 Italian Large White pigs (172 entire gilts and 88 castrated males obtained from
121 79 sires) were involved in this study. These pigs were from triplets of siblings of the same litter
122 (two females and one castrated male) individually performance tested at the central station of
123 ANAS for the genetic evaluation of a candidate boar from the same litter (sib-testing). The test
124 period began when piglets were approximately 30 kg live weight and it ended when the pigs were
125 slaughtered at about 8 months following the rules of the test station.
126

127 All pigs were genotyped for the ryanodine receptor 1 (*RYR1*) g.1843C>T mutation (Fujii et
128 al., 1991), causing the Pale Soft and Exudative meat defect and for the p.R200Q mutation of the
129 protein kinase AMP-activated non-catalytic subunit gamma 3 (*PRKAG3*) gene (Milan et al., 2000),
130 causing the acid meat defect. Genotyping was carried out as previously described by Fontanesi et al.
131 (2008).

132 133 **2.2. In vivo performances of the animals**

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

141 142 **2.3. Slaughtering and carcass traits**

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

153 154 **2.4. Ham technological traits**

156 At a single processing plant, all raw hams were chilled for 24 h and then trimmed. The two
157 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.

161
162
163 **2.5. Raw ham quality traits measurements**

164
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 ($\mu\text{mol/g}$). 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 $2[\text{glycogen} +$
184 $\text{glucose} + \text{glucose-6-phosphate}] + [\text{lactate}]$ and expressed as $\mu\text{mol/g}$ (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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2.6. Statistical analyses

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.

221 222 **3. Results and discussion**

223 **3.1. Description of the *in vivo* performance, carcass and technological traits**

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

231
232 Descriptive statistics of the measured traits are reported in Table 1. The variability in HWLFS
233 as measured by the coefficient of variation ($CV = 17.4\%$) was high. This ham quality parameter
234 was on average equal to 220 ± 38 g, corresponding to a loss percentage in this phase of $1.75 \pm$
235 0.28% . Similar mean values ($1.93 \pm 0.59\%$), but with higher variability ($CV = 30.6\%$), were
236 previously reported by Schivazappa et al. (2002) in three Italian heavy pig breeds, Italian Duroc,
237 Italian Landrace and Italian Large White, with higher losses in this latter breed (2.16%).
238

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

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

250 251 **3.2 Description of the ham quality traits**

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

256 The pH values were much less variable ($\text{pH}_{2\text{h}}$: CV = 4.1% and $\text{pH}_{24\text{h}}$: CV = 3.7%), in
257 agreement with what was previously reported by other authors in both light and heavy pigs (Ramos,
258 Serenius, Stalder, & Rothschild, 2007; Sturaro, Gallo, Noventa, & Carnier, 2008; Ventura et al.,
259 2011). Several samples were not in the range of 5.6 - 6.2 recommended for $\text{pH}_{24\text{h}}$ in fresh hams
260 intended for curing (Arnau, 2004). Although the pigs did not carry the *PRKAG3* p.200Q allele,
261 known to decrease the ultimate pH and pork quality characteristics (Cherel et al., 2010), eight
262 samples exhibited low $\text{pH}_{24\text{h}}$ values (<5.4) compatible with the acid meat defect. This indicated that
263 low muscle $\text{pH}_{24\text{h}}$ can occur in pigs that do not carry the negative allele of the *PRKAG3* gene.
264 Conversely, nine samples had an insufficient decrease in the final pH ($\text{pH}_{24\text{h}}$ above 6.2).

265 Among the investigated traits, the highest variability of the fresh hams was observed for glycogen
266 (CV = 47.7 %) lactate (CV = 28.2 %) and GP (CV = 22.1%). In pigs that do not carry the negative
267 allele at the *RN* locus (i.e. animals with the recessive genotype at the *PRKAG3* p.R200Q
268

270 polymorphic site), the muscle glycogen level is mainly determined by husbandry and feeding
271 practices of the animals, transport conditions, climatic and environmental factors, handling
272 procedures at slaughter and muscle type (England et al., 2016; Larzul, Monin, Sellier & Le Roy,
273 1998; Scheffler & Gerrard, 2007). Lactate was the main component (56.4 ± 15.9 %) of GP, as
274 already reported in other studies (Maribo, Støier, & Jørgensen, 1999; Przybylski, Sionek, Jaworska,
275 & Santé-Lhoutellier, 2016). The contribution of lactate in GP reported in this study was lower than
276 those in other investigations. This difference could probably be due to the difference in the
277 sampling time (Maribo et al., 1999; Przybylski et al., 2016). However, according to Maribo et al.
278 (1999) the determination of GP is not affected by the different *post mortem* sampling time.

279 In our samples, Catb activity (1.16 ± 0.23 nmol AMC min⁻¹ g⁻¹ meat; CV = 19.6%) was lower
280 than what was on average obtained in other Italian heavy pigs (i.e. 1.36 nmol AMC min⁻¹ g⁻¹ and
281 CV = 22.8%, Sturaro et al., 2008; 1.55 nmol AMC min⁻¹ g⁻¹ and CV = 18%, Virgili, Schivazappa,
282 Parolari, Bordini, & Degni, 1998). Catb activity has been found to exhibit variability depending on
283 genetic type, breeding, age at slaughter, type of muscle, season and, during the processing period of
284 the legs, by several other factors such as salt, temperature, humidity and time of ripening (Arnau,
285 Guerrero, & Sárraga, 1998; Mora, Escudero, & Toldrá, 2016; Sárraga, Gil, & García-Regueiro,
286 1993; Sturaro, Gallo, Noventa, & Carnier, 2008; Virgili, Parolari, Schivazappa, Bordini, & Borri,
287 1995; Zhou, et al., 2019a). Sturaro et al. (2008) reported a slight decrease in Catb activity when the
288 carcass weight increased in crossbred of Large White boar line x crossbred sows (1.38 nmol
289 AMC/min/g for lighter carcasses and 1.33 nmol AMC/min/g for heavier carcasses; $P < 0.05$). In
290 addition, Sárraga et al. (1993) identified lower Catb activity in green hams from heavy pigs than
291 light pigs, and they suggested that lower levels in heavy pigs could be attributed to their slower
292 protein turnover.

293 **32. Effects of sex, slaughter days and visible intermuscular fat**

294
295

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 ($P = 0.044$).

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

325 326 **3.3 Correlations between HWLFS and *in vivo* performance, carcass and ham technological** 327 **traits**

328
329 HWLFS is a quality trait used to assess the aptitude of the meat for salting to obtain legs
330 with optimal yield. Almost all correlations of HWLFS with *in vivo* performance, carcass and
331 technological ham traits were significant (Figure 1. Table S3). As expected, HWLFS was negatively
332 correlated with BFT ($r = -0.51$, $P < 0.0001$), confirming that an adequate level of BFT is beneficial
333 to minimizing HW loss (Bosi & Russo, 2004). This is due to lower water content of adipose tissue
334 than muscular tissue (5-15% vs 70-75%) and to the positive barrier effect of fat on water which
335 drips out from the hams through diffusion and evaporation during the processing period (Bosi &
336 Russo, 2004; Ramos et al., 2007). Conversely, HWLFS was positively correlated with LC ($r =$
337 $+0.51$; $P < 0.0001$), indicating that the selection for carcass conformation (muscularity) tends to
338 worsen this quality characteristic of the ham. The relationships between HWLFS and HW at
339 different stages were positives and significant ($r = +0.34/+0.40$; $P < 0.0001$). Čandek-Potokar &
340 Škrlep (2011) observed higher daily moisture losses in hams with heavier weight due to their larger
341 contact area for exchange. Other studies showed no or very low correlations between HW and
342 seasoning losses or yield (Čandek-Potokar & Škrlep, 2012; Ramos et al., 2007).

343 344 **3.4 Correlations between HWLFS and ham quality traits** 345

346
347 The correlations between HWLFS and fresh meat quality traits were generally low (Figure 1.
348 Table S4). HWLFS was correlated negatively with pH_{24h} ($r = -0.25$, $P < 0.0001$). This result is in
349 agreement with previous studies reporting higher weight losses during the initial phase of curing in
350 hams exhibiting low pH_{24h} values (Čandek-Potokar & Škrlep, 2011; Schivazappa et al., 2002). The

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

359 360 361 **3.5 Correlations between raw ham quality traits**

362
363 Initial pH measured at 2 h was moderately correlated with pH_{24h} ($r = +0.58$. Table S4). The
364 correlation between pH_{2h} and lactate was significant and negative ($r = -0.48$), no correlation was
365 found between pH_{24h} and lactate. The correlation between glycogen and pH_{24h} ($r = -0.50$) suggested
366 that glycogen, responsible for *post mortem* glycolysis, lead to hams with low pH_{24h} . The correlation
367 between GP and pH_{24h} resulted negative ($r = -0.54$; $P < 0.0001$), confirming that an increased GP
368 indicates a high level of final lactate that can be accumulated in the post mortem muscles (Hamilton
369 et al., 2003; Maribo et al., 1999; Monin & Sellier, 1985; Przybylski et al., 2016). However, other
370 authors found that GP was only weakly associated with pH_{24h} in a synthetic Duroc line (England et
371 al., 2016).

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

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

3.6 Cluster analysis and relationships between HWLFS and VIF

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386 The distribution (count and frequency) of HWLFS clusters (low and high) by fatness classes
387 of visible intermuscular fat ($VIF = 0$ and $VIF > 0$) was significant ($P = 0.0012$; Table S5). About
388 51% of the hams clustered in the group with high HWLFS were scored to have no VIF on both legs
389 (Fig. 2). In the cluster based on low HWLFS, only 31.4% of samples were scored to have no VIF.

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

3.7 Stepwise multiple regression analyses predicting HWLFS

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

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

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

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

418 419 **4. Conclusions**

420 This study identified significant phenotypic residual correlations between HWLFS and 17
421 parameters, including performance, carcass and ham quality traits.
422
423

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

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

433 434 **Conflict of interest**

435

436 The authors declare that they do not have competing interests.
437

438 439 **Acknowledgments**

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443

444 445 **References**

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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 (%)
<i>Production (in vivo performances) and carcass traits</i>					
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 <i>gluteus medius</i> 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
<i>Ham technological traits¹</i>					
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
<i>Raw ham quality traits²</i>					
pH at 2 h <i>post mortem</i> , pH _{2h}	5.94	0.24	5.41	6.80	4.1
pH at 24 h <i>post mortem</i> , 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 ($\mu\text{mol/g}$).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 <i>Gluteus medius</i> 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; $\mu\text{mol/g}$	0.049	0.002	0.001	11.5
Lactate; $\mu\text{mol/g}$	0.518	0.001	0.238	8.9
Glycolytic potential, GP; $\mu\text{mol/g}$	0.147	0.018	0.044	7.7
Cathepsin B activity, Catb; nmol AMC/min/g	0.569	0.031	0.803	5.0

¹Traits are defined in Table 1.

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

³Significant 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.

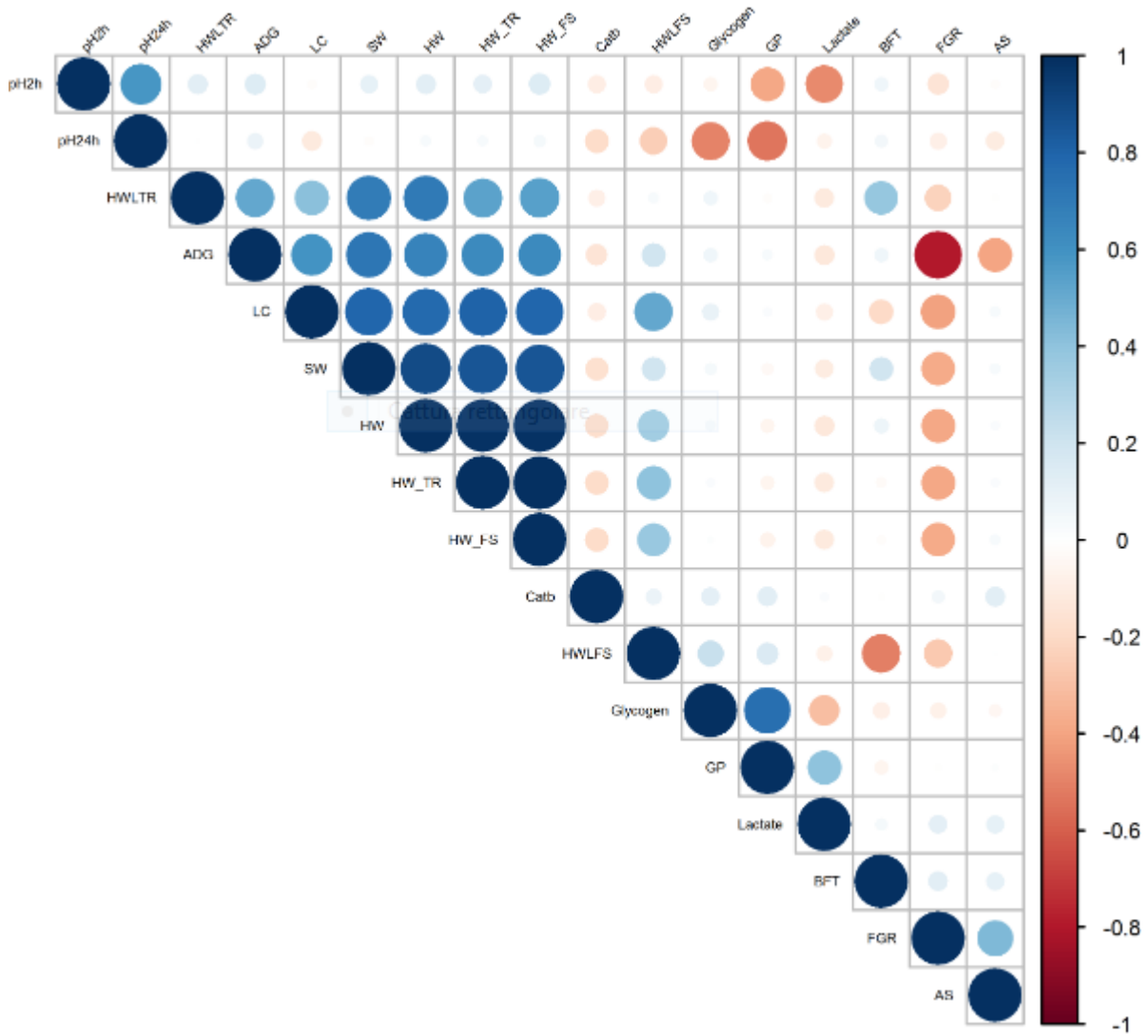


Table 3

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

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

¹Traits are defined in Table 1.

²Standard error.

³Root 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.

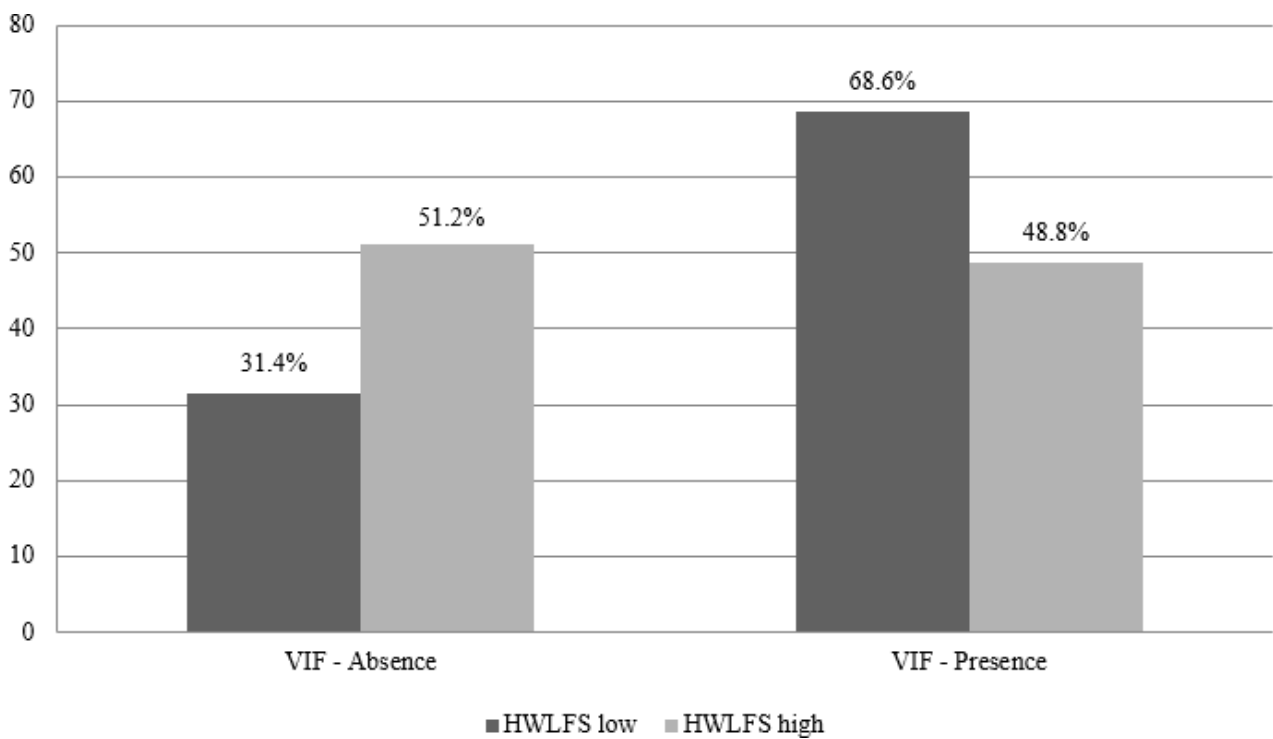


Table S1

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

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

¹Traits are defined in Table 1.

²The significant P values are indicated in bold.

Table S2

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

Traits ¹	VIF = 0 ²	VIF = 1 ²	VIF = 2 ²	VIF p-values ⁴
	LSM \pm SE ³	LSM \pm SE ³	LSM \pm SE ³	
Average daily gain, ADG; kg/d	0.778 \pm 0.009	0.785 \pm 0.011	0.773 \pm 0.009	0.691
Feed:gain ratio, FGR	3.93 \pm 0.04	4.01 \pm 0.05	3.98 \pm 0.04	0.466
Slaughter weight, SW; kg	149.83 \pm 1.26	148.35 \pm 0.89	148.35 \pm 0.89	0.312
Slaughter age, SA; g	238.71 \pm 0.86	240.09 \pm 0.61	240.09 \pm 0.61	0.571
Backfat thickness of <i>gluteus medius</i> m., BFT; mm	24.97 \pm 0.55 ^{B, D}	28.37 \pm 0.66 ^C	27.84 \pm 0.57 ^A	0.001
Lean cuts, LC; kg	29.03 \pm 0.30 ^A	28.10 \pm 0.36 ^B	28.01 \pm 0.31 ^B	0.049
Ham weight				
Raw, HW; kg	15.69 \pm 0.14 ^a	15.34 \pm 0.17	15.17 \pm 0.15 ^b	0.054
Trimming, HW_TR; kg	12.84 \pm 0.12 ^{a, c}	12.45 \pm 0.14 ^b	12.34 \pm 0.12 ^d	0.017
Post first salting, HW_FS, kg	12.60 \pm 0.12 ^a	12.24 \pm 0.14 ^{b, c}	12.13 \pm 0.12 ^d	0.026
Ham weight loss				
Trimming, HWLTR; kg	2.85 \pm 0.04	2.89 \pm 0.04	2.84 \pm 0.04	0.553
First salting, HWLFS; g	238 \pm 03 ^C	210 \pm 4 ^D	207 \pm 4 ^D	<0.001
pH _{2h}	5.94 \pm 0.03	5.96 \pm 0.03	5.91 \pm 0.03	0.446
pH _{24h}	5.63 \pm 0.02 ^b	5.70 \pm 0.03 ^a	5.70 \pm 0.02 ^a	0.031
Glycogen	54.00 \pm 2.35 ^{a, A}	46.33 \pm 2.82 ^b	40.52 \pm 2.43 ^B	0.001
Lactate	55.01 \pm 1.69	56.48 \pm 2.03	59.30 \pm 1.75	0.238
Glycolytic potential, GP	109.01 \pm 2.44 ^a	102.81 \pm 2.93	99.83 \pm 2.52 ^b	0.044
Cathepsin B activity, Catb	1.16 \pm 0.02	1.14 \pm 0.03	1.15 \pm 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 S3Residual correlations¹ 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 S4Residual correlations¹ 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.

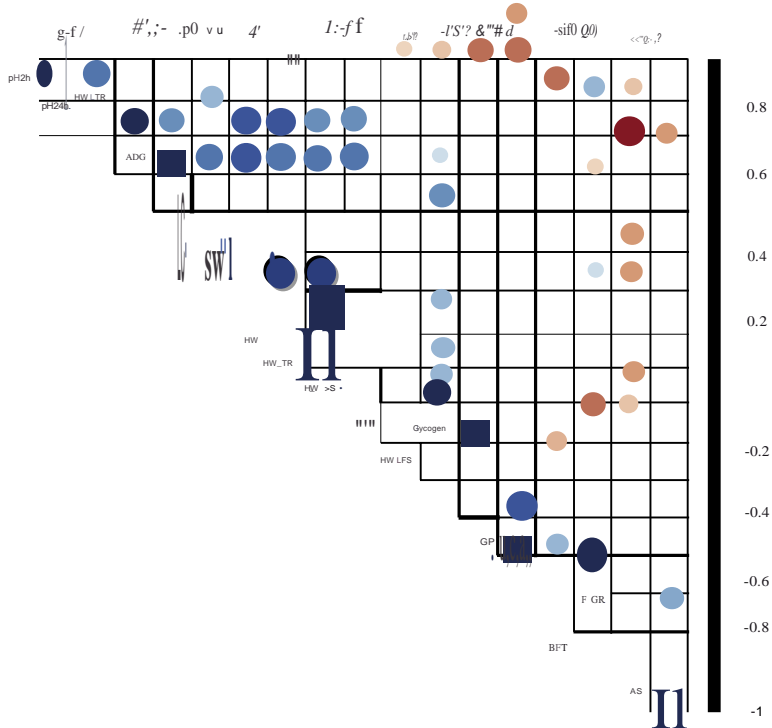


Figure 1

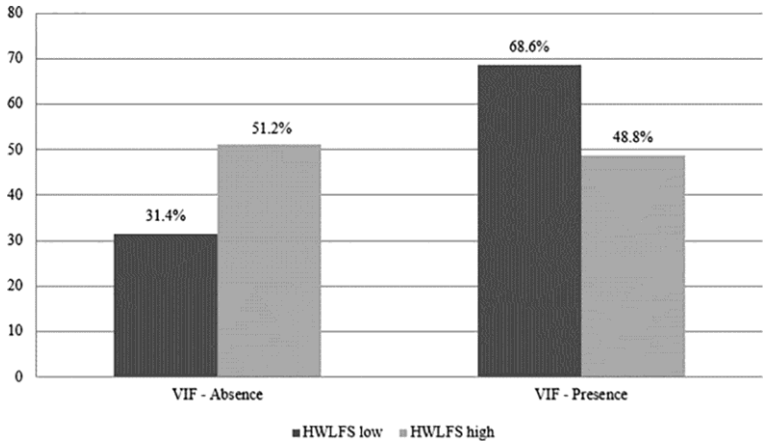


Figure 2