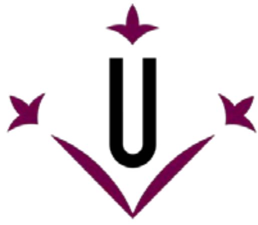

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1 **Carcass lean-yield effects on the fatty acid and amino acid composition of Duroc pork and its**
2 **technological quality after vacuum-ageing**

3 ***Running head: Carcass leanness effects on pork meat quality***

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13 **Abstract**

14 Eighty purebred Duroc castrated male pigs slaughtered at 210 days of age were used to evaluate the
15 effect of lean-yield (European Union carcass grading based on lean content; or R, O and P classes) on the
16 fatty acid and amino acid composition of raw pork (day 1 post-mortem), and technological meat quality
17 after vacuum ageing up to 4, 6 and 8 days.

18 A strong relationship between slaughter weight and carcass lean-yield was observed. Carcasses graded
19 as having a lower lean yield were fatter with higher intramuscular fat (IMF) concentration, and
20 differences in proportions of fatty acids with increased MUFA and decreased PUFA percentage, but
21 without adverse effect on ultimate pH, drip loss or colour attributes. There were no effects of carcass
22 lean-yield on amino acid composition of raw pork, with valine being the limiting amino acid relative to
23 lysine by about 30-35%. Vacuum ageing did not reduce the shear force of raw pork which may not be
24 indicative of cooked pork response. The lipid oxidation had an inverse relationship with the PUFA
25 content of each pork class, and it did not increase due to vacuum ageing up to 8 days. Meat fatness did
26 not affect its amino acid balance and technological quality (colour, drip loss, shear force and lipid
27 stability) but modified IMF composition.

28 **Additional keywords:** nutritive evaluation; pigs; amino acids; intramuscular fat.

Introduction

The Duroc swine breed has been largely used as a purebred or in sire lines to produce pigs intended for dry-cured and premium pork products, since it has a greater ability to accumulate intramuscular fat (IMF) than other common breeds in European Union such as Pietrain (Latorre *et al.* 2009).

A higher content of IMF improves the organoleptic characteristics of pork products (Fonseca *et al.* 2015). High levels of subcutaneous fat involve higher production costs and worsen the commercial grading of carcasses (Čandek-Potokar & Škrlep 2012). Thus, backfat content should be controlled without penalizing excessively the IMF characteristics. Therefore, certain breeding programmes tend to standardize carcass characteristics by reducing backfat thickness while maintaining steady IMF and oleic acid contents (Ros-Freixedes et al. 2013). Due to variation in these traits, carcasses in a given population are not equally classified from a commercial view. It has to be taken into account that only some carcass parts (ham and shoulder) are intended for the production of dry cured products and, therefore, selection on primal cuts aiming at production of dry cured products could modify correlated characteristics of the fresh meat from the rest of retail cuts (i.e. loin). In addition, it is now possible to incorporate the use of genetic markers that complement traditional methods of selection of the quality of fat (Estany et al. 2014).

After carcass processing, fresh pork may be normally aged for a short period of time to improve meat tenderness, but this practice should not affect negatively other meat attributes such as colour or lipid oxidation. Pork ageing is conducted usually by exposing meat cuts in air or by vacuum packaging. Earlier studies observed 50% reduction of pork meat hardness during the first 2 days and 80-90% reduction of hardness after 4-6 days of vacuum ageing (Dransfield et al. 1981; Rees et al. 2002). However, in these cases the meat came from purebred or hybrid genotypes commonly used in maternal lines (Landrace and Large-White), with usual IMF levels below 30 g/kg.

This study investigated the effect of carcass grade (R, O and P classes, where R has the greatest and P the lowest lean content) on the chemical composition (fatty acids and amino acids) of pork with IMF above 30 g/kg, and their colour parameters, drip loss, Warner-Bratzler shear force, and lipid stability after vacuum ageing for one, three, five and seven days.

Material and methods

Study design

A total of 80 barrows with 210 days of age (standard deviation ± 5 days) were selected at random from a purebred Duroc population (Selección Batallé, Riudarenes, Girona). The pigs were raised in two batches

60 (spring-summer 2013 and winter-spring 2014, n=40 per batch, 8 pigs per pen), and they were fed the
61 same diet *ad libitum*. The ingredients and chemical composition of diet from 180 to 210 days of age are
62 shown in Table 1.

63 ***On-farm and abattoir measurements***

64 The pigs were weighed individually at 180 and 210 days of age. The average daily gain during the last
65 month of the finishing period was calculated by difference between live-weights against time. On the
66 day before slaughter, feed was withdrawn for 12-24 h and the pigs were transported 30 km to a
67 commercial abattoir, where they were kept in lairage for 3 h with full access to water but not to feed.
68 The pigs were stunned using CO_2 (88%) (Butina ApS, Holbaek, Denmark), exsanguinated, scalded,
69 skinned, eviscerated, and split down the midline according to standard commercial procedures. The
70 animal management was in compliance with European welfare guidelines (Directive 2010/63/EU).

71 The hot carcasses were individually weighed to calculate the dressing out proportion. Backfat thickness
72 between ribs 12th and 13th assuming rib 15th was the last rib and fat depth at *Gluteus medius* muscle
73 on the carcass midline (skin included) were measured using an on-line ultrasound automatic scanner
74 (Autofom®, SFK-Technology, Herlev, Denmark). The carcass lean content was estimated using the
75 Spanish officially approved equation based on ultrasound measured variables (BOE, 2011) and carcasses
76 were then graded into standard categories based on European Union scale: R (45-50% lean content,
77 n=18), O (40-45% lean content, n=28) and P (<40% lean content, n=34). The carcasses were genotyped
78 according to Estany et al. (2014) to assess segregation of the single nucleotide polymorphism
79 AY487830:g.2228T>C in the promoter region of the stearoyl-CoA desaturase (SCD) gene, of which the T
80 allele enhances 18:1/18:0 and, consequently, the proportion of monounsaturated to saturated fat
81 without affecting total fat content.

82 The carcasses were refrigerated by gradual reduction of their internal temperature: 16 ºC (1st hour post-
83 mortem), 8 ºC (2nd hour), 4 ºC (3rd hour), 2 ºC (4th hour) and 2 ºC (5th hour), and kept at approximately 4
84 ºC until 24 h post-mortem. At 24 h post-mortem, carcass length from the posterior edge of the
85 symphysis pubis to the anterior edge of the first rib was measured. In addition, ultimate pH of meat was
86 measured between ribs 12th and 13th (assuming rib 15th was the last rib) with a pH-meter equipped
87 with a spear-tipped probe (Testo 205, Testo AG, Lenzkirch, Germany). The carcasses were subsequently
88 processed and ham weight was recorded. A section of 5-cm of length (approximately 200 g) of
89 *Longissimus thoracis* muscle was excised at the 3th-4th last rib area and placed in individual vacuum-
90 packaged plastic bags at 4°C for transportation to laboratory.

91 Three slices were cut from each *L. thoracis* muscle sample to evaluate fatty acid and amino acid
92 composition of raw pork (1 cm-long), drip loss at 24 h post-mortem (1 cm-long), tenderness and lipid

93 oxidation during ageing for 8 days (3 cm-long). This last thickest slice was divided into 4 homogeneous
94 pieces; the first was used to evaluate colour at day 1 (T1) while the remaining three pieces were
95 vacuum-packaged and stored in the dark at 4 ºC for 4, 6 or 8 days (T1; T4; T6; and T8, respectively). The
96 four piece samples were used for colour measurement before subsequent freezing at -20 ºC until
97 concurrent analyses of tenderness and lipid oxidation. When required, the sliced *L. thoracis* samples
98 were thawed in the vacuum-packaged bags for 24 h at 4°C, removed from packages, blotted dry for 15
99 min, and weighed. Thawing loss was calculated by dividing the difference in weight between the fresh
100 and thawed samples by the initial fresh weight.

101 ***Instrumental meat quality analyses***

102 The *Longissimus thoracis* muscle colour was measured on polystyrene white trays after 1 h of blooming
103 the inner surface with a Konica Minolta CM-700d spectrophotometer (Konica Minolta Sensing Inc.,
104 Osaka, Japan) in the CIELAB space (CIE 1986) with a measured area diameter of 8 mm, including specular
105 component and a 0% ultraviolet, standard illuminant D65, which simulates daylight (colour temperature
106 6504 K), observer angle 10º and white calibration. The Commission Internationale de l'Éclairage (CIE)
107 lightness (L*), redness (a*), and yellowness (b*) colour-space values were reported as the average of
108 three randomly selected readings taken on each slice without any covering film, and mean values were
109 used for statistical analysis. Hue angle (H*) was calculated as: H*= tan-1(b*/a*) × 57.29, expressed in
110 degrees, whereas chroma (C*) (colour intensity, also known as saturation index) was calculated as: C*=
111 $\sqrt{a^{*2} + b^{*2}}$. In addition to these parameters, the reflectance spectra were collected from 400 nm to
112 700 nm at every 10 nm. The relative metmyoglobin (MMb) content was estimated through Kubelka-
113 Munk ratio $K/S_{572/525}$ (Hunt et al. 1991), which decreases when pigment content increases. The K/S
114 values at 572 nm and K/S at 525 nm were calculated by linear interpolation.

115 Drip loss at 24 h post-mortem was determined by centrifugation, according to a modification of the
116 method used by Kristensen & Purslow (2001). Briefly, raw meat samples were weighed, cut carefully
117 with a scalpel to avoid slight water losses, and transferred to centrifugation tubes which allow
118 separating meat from exudate during centrifugation. Centrifugation losses were calculated as the
119 percentage of initial sample weight (approximately 0.1 g).

120 ***Fatty acid analyses***

121 Feed lipids were extracted using a chloroform/methanol/water mixture (2/2/1.8 v/v/v) (Hanson & Olley
122 1963). Feed and meat fatty acid (FA) methyl esters were directly obtained by transesterification using a
123 solution of boron trifluoride 20% in methanol (Rule 1997), followed by 2 h heating at 80ºC,
124 centrifugation at 2,500 rpm during 5 min and collection of the final supernatant. Analysis of FA methyl
125 esters were performed in duplicate by GC with a 30 m x 0.25 mm capillary column (Agilent DB-23,

126 Agilent Technologies, Santa Clara, United States) and a flame ionization detector with helium as the
127 carrier gas at 2 mL/min. The oven temperature program increased from 150-220 °C at 8 °C per min, and
128 the injector and detector temperatures were both 250 °C.

129 The quantification was carried out through area normalization after adding into each sample 1,2,3-
130 tripentadecanoylglycerol as internal standard. IMF was calculated as the sum of each individual FA
131 expressed as triglyceride equivalents (AOAC 2000), following the methodology described in Bosch et al.
132 (2009). Fatty acid composition was calculated as the percentage of each individual acid relative to total
133 FA and expressed as g per 100 g FA. The proportion of polyunsaturated (PUFA) (C18:2n-6; C18:3n-3;
134 C20:2n-6; C20:3n-6; C20:4n-6; and C22:6n-3), monounsaturated (MUFA) (C16:1n-7; C17:1n-7; C18:1n-9;
135 and C20:1n-9) and saturated (SFA) (C10:0; C12:0; C14:0; C16:0; C17:0; C18:0; and C20:0) fatty acid
136 contents were calculated.

137 ***Nitrogen and amino acids analysis***

138 After colour measurement, the sliced muscle sample was defatted, freeze-dried and pulverized using an
139 electric grinder and used for protein (N x 6.25) analysis by automated Dumas method (AOAC 2000).

140 The amino acids content of the muscle was determined by hydrolysis of samples (50 mg) which was
141 performed by incubating them under nitrogen in 5 mL 6N HCl for 12 h at 110ºC (Colgrave et al. 2008).
142 Hydrolysis tubes were cooled and centrifuged at 3,000 g for 30 min to remove particulate matter.
143 Aliquots of 25 µL of hydrolysate were evaporated under nitrogen steam and re-diluted in 500 µL of
144 water/acetonitrile (20/80 v/v) containing 250 ng/mL of Trans-4-Hydroxy-L-proline-2,3,5-d3 (Hpro-d3)
145 (CDN Isotopes, Sainte Foy La Grande, France).

146 Quantitation of individual amino acids was performed using a method described by Guo et al. (2013)
147 with modifications, as follows. An Ultra-High-Performance Liquid Chromatography (UPLC) Acquity
148 system (Waters, Milford, MA) holding a BEH Amide column (2.1 x 150 mm; 1.7 µm) was used. Solvent A
149 was 10mM ammonium formate in water with 0.15% formic acid; solvent B was ammonium formate-
150 saturated acetonitrile with 0.15% formic acid. The gradient included five steps. Initial conditions were
151 15% A and 85% B maintained for 3 min at 0.5 mL/min. Then, from 15% to 20% A in 3 min; from 20% to
152 24% A in 1.5 min; from 24% to 60% A at 0.6 mL/min in 1.5 min and maintained for 3 min. Then, initial
153 conditions were regained in 2 min. Weak and strong washing solvents were 80% acetonitrile and 20%
154 acetonitrile, respectively. Samples were filtered through a 0.20 µm hydrophilic PTFE membrane prior to
155 injection. The injection volume was 5 µL.

156 Quantitation of amino acids in the hydrolysate was performed by using a Multiple Reaction Monitoring
157 method (MRM) in a Waters TQD mass spectrometer (Micromass MS Technologies, Manchester, UK).

158 Briefly, the system was equipped with an ESI source operated in positive ion mode. The parameters in
159 the source were set as in Guo et al. (2013). Moreover, their MRM transitions were tested successfully in
160 our conditions for Phenylalanine, Leucine, Isoleucine, Methionine, Valine, Proline, Tyrosine, Alanine,
161 Threonine, Glycine, Glutamic acid, Serine, Aspartic acid, Histidine, Arginine, Lysine and Cistine. Cone
162 voltage and collision energy were optimized for each individual amino acid. An additional MRM
163 transition was determined for internal standard, $Hpro^{d3}$, obtaining the following values: 135.1/89;
164 135.1/70.9; precursor/product for quantitation and verification, respectively. Calibration curves were
165 constructed from a commercial amino acid standard mixture (Ref.: AAS19, Sigma-Aldrich, St. Louis, MO)
166 and diluted to a series of appropriate concentrations with water/acetonitrile (20/80 v/v) containing 250
167 ng/mL of $Hpro^{d3}$. Triptophan concentration was not determined because of under these conditions is
168 completely degraded (Fontoulakis & Lahm 1998). The results were processed using QuanLynx software
169 (MassLinx, Waters Corporation, USA). For hydroxyproline quantitation, $Hpro^{d3}$ was used as an internal
170 standard. The other amino acids were quantified from absolute response without internal standard. The
171 amino acids content of muscle are expressed as a proportion of crude protein content. The sums of
172 essential amino acids (EAA) and non-essential amino acids (NEAA) and their ratios out of the total amino
173 acid content were calculated according to the NRC (2012) classification. The following EAA were
174 accounted for: Lysine, Methionine, Threonine, Isoleucine, Valine, Phenylalanine, Leucine and Histidine.
175 The remaining analysed amino acids were considered as NEAA: Cistine, Arginine, Hydroxyproline,
176 Tyrosine, Alanine, Glycine, Glutamic acid, Serine, Proline, and Aspartic acid. Tryptophan concentration
177 was not determined as this would have required alkaline hydrolysis before quantitation.

178 ***Tenderness and lipid oxidation***

179 The *L. thoracis* muscle samples analysed for tenderness and lipid oxidation were thawed in vacuum-
180 packaged bags for 24 h at 4°C, removed from packages, blotted dry for 20 min, and weighed. Samples
181 were then cut parallel to the long axis of the muscle fibres into rectangular cross-section slices of 10 × 10
182 mm and 30 mm length without cooking. Four slices per *L.thoracis* muscle sample were sheared
183 perpendicular to the fibre orientation, with a Warner-Bratzler device attached to a texture analyser TA-
184 TX2 (Stable Micro Systems Ltd, Surrey, UK) attached to a PC, and equipped with a 5-kg load cell and a
185 crosshead speed of 2.5 mm/s.

186 Lipid oxidation was assessed by thiobarbituric acid reactive substances (TBARS) analysis, following a
187 modification of the method of Buege & Aust (1978). Meat samples (5 g) were homogenised with 15 ml
188 of distilled water in falcon tubes at 13500 rpm during 15 seconds. Afterwards, 1 ml of the resultant
189 solution was transferred in duplicate to pyrex glass tubes and it was mixed with 2 ml of a solution
190 containing 15% trichloroacetic acid, 0.375% thiobarbituric acid and 1 N HCl.

191 The tubes were homogenized and incubated at 90 °C for 60 min in a water bath to induce colour
192 development. The absorbance at 540 nm was measured with a iEMS Reader MF spectrophotometer
193 (Labsystems Oy, Helsinki, Finland). A standard calibration curve was created with increasing
194 concentrations (from 0 to 100 µl) of malonaldehyde (MDA), which was obtained by hydrolysis of 1,3,3-
195 tetraethoxypropane (TEP) in 100 ml of 1 N HCl. The tubes used for the calibrations, including the blank
196 and samples to be analysed, were put through the TBA procedure at the same time. TBARS values are
197 expressed as milligrams of MDA per gram of IMF.

198 ***Statistical analyses***

199 The data were analysed with the Jmp Pro 11 statistical software (SAS Institute, Cary, NC, USA). Growth
200 performance, carcass traits, pH of *L. thoracis* muscle, centrifugation drip loss, IMF composition, protein
201 and amino acid contents were analysed with a standard least squares linear model including carcass
202 category (R, O and P) and batch (2 levels) as fixed effects. Colour attributes, thawing losses, Warner-
203 Bratzler shear force and TBARS data were analysed with a linear mixed model including, in addition to
204 the afore-mentioned effects of carcass category and batch, the effect of ageing time (T1, T4, T6 and T8)
205 and its interaction with carcass category as fixed effects using a repeated-measures analysis of variance.
206 The pig was considered as the random effect. The rest of first order interactions were removed from the
207 final models because they result not significant (P>0.05). To assess the potential role of genotype on
208 chemical composition and technological quality of carcass and meat traits, the *SCD* genotype effect was
209 also accounted for as a fixed effect in preliminary statistical analyses. . The level of significance was set
210 at 0.05. Differences (P<0.05) between least square means were assessed using the Tukey test. Values
211 are presented as least square means and their standard errors (S.E.). Pearson correlation coefficients
212 between adipose tissue traits and amino acid composition were obtained by using REML estimation
213 method.

214 **Results**

215 Three SCD genotypes were obtained: CC (n=12), CT (n=42), and TT (n=26). The most frequent SCD
216 genotype in all carcass grades was CT (13/18 in R; 13/28 in O and 16/34 in P carcasses). As expected, the
217 SCD genotype only affected the MUFA and SFA content of the meat (P<0.001) and did not have any
218 interaction with carcass grade or batch (P>0.05). The loin MUFA content increased linearly in CC, CT and
219 TT genotypes (492±4, 502±4 and 516±4 g/kg of fatty acids, respectively; P<0.05) whereas the SFA
220 content was greater in CC and CT compared to TT genotype (406±4 and 399±3 vs. 381±5 g/kg of fatty
221 acids, respectively; P<0.05).

222 ***Growth performance and carcass traits***

Growth performance and carcass traits are shown in Table 2. Although all the pigs had similar age at slaughter, the realized carcass groups came from pigs with different productive traits. The average daily gain was lower in pigs leading to R (45-50% lean) and O (40-45% lean) carcasses than to P (<40% lean) carcasses ($P<0.05$), but dressing out did not differ among groups ($P>0.05$). The ham weight was lower in R and O compared to P carcasses ($P<0.05$). Backfat thickness at different anatomical locations (*Gluteus medius* and 3rd-4th last ribs) was the lowest in R carcasses ($P<0.05$).

Technological traits and chemical composition of fresh meat

The technological traits and chemical composition of fresh pork (24 h post-mortem) according to carcass lean-yield is detailed in Table 3. No detectable differences were observed in ultimate pH or centrifugation drip losses among carcass groups ($P>0.05$). The crude protein content of pork was higher while the IMF content was lower in R carcasses than in the rest ($P<0.05$). The fatty acid composition differed among carcass groups, with R carcasses showing lower MUFA and greater PUFA content than the rest of groups ($P<0.05$) and with similar SFA content across groups ($P>0.05$).

The amino acid composition of fresh pork according to carcass lean-yield is shown in Table 4. The individual amino acid content was mostly similar among groups ($P>0.05$) except for a tendency for isoleucine and glycine to be lower in P and R carcasses, respectively ($P<0.1$). Studied correlations between pork content of isoleucine and glycine with subcutaneous and IMF adipose tissues traits were not significant ($P>0.05$) except between glycine and pork IMF fat content ($r=0.23$; $P=0.039$). The balance of amino acids in the pork was compared with the recommended balance of indispensable amino acids for adults (WHO/FAO/UNU 2007) by expressing the content of relevant amino acids relative to lysine and then calculating the proportion of the recommended amount of each amino acid that was provided when a sample containing the recommended amount of lysine was consumed (Table 5). The balance of indispensable amino acids was less than ideal, with valine being the most limiting by about 30-35%.

Colour attributes at different vacuum ageing times

None of the CIELab colour attributes differed among carcass groups ($P>0.05$) (data not shown). Redness index (a*) increased from day 1 (T1) to day 4 of vacuum ageing (T4) (1.72 vs. 2.97 ± 0.20, respectively; $P<0.05$) and it was kept steady at T6 and T8 (2.87 and 2.73 ± 0.20, respectively; $P>0.05$). The yellowness index (b*) also increased from T1 to T4 (7.02 vs. 8.64 ± 0.38, respectively; $P<0.05$) but it decreased slightly at T6 and T8 (8.10 and 7.40 ± 0.38, respectively; $P>0.05$).

Overall lightness (L*) increased at T6 ($P<0.05$), but hue angle (H*) did not differ among ageing times ($P>0.05$), and chroma (C*) increased at T4 ($P<0.05$) and slightly decreased at T8 (Figure 1). The estimated relative content of metmyoglobin based on reflectance spectra did not differ between T1 and

255 T6 (K/S=1.30±0.01; P>0.05), but it increased significantly at T8 (K/S=1.28±0.01, P<0.05). There was no
256 interaction between carcass grade and ageing time on the CIELab colour attributes (lightness, redness,
257 yellowness, hue angle and chroma) (P>0.05).

258 ***Thawing losses, tenderness and lipid oxidation at different vacuum ageing times***

259 Thawing losses during vacuum ageing of pork did not differ significantly among carcass groups
260 (228±10.5, 208±8.5 and 204±8.0 g/kg, in R, O and P, respectively; P>0.05). However, thawing losses
261 were the lowest in T1 and the highest in T8 (Figure 2; P<0.05), whereas the thawing losses in pork aged
262 for T4 and T6 were intermediate (P>0.05).

263 The shear force on thawed raw pork did not differ significantly either among carcass groups (2.45±0.13,
264 2.30±0.11, 2.18±0.10 kg, in R, O and P, respectively; P>0.05) or among ageing times (P>0.05, Figure 2).
265 There was no interaction between carcass grade and ageing time on thawing losses or Warner-Bratzler
266 shear force (P>0.05).

267 The TBARS assay in pork from the different carcass groups according to vacuum ageing times is shown in
268 Figure 3. There was an interaction between carcass grade and ageing time on lipid oxidation (expressed
269 on a pork fat content basis), which was higher in R (45-50% lean) than in the rest of group carcasses
270 from T4 onwards (P<0.05). The lean-yield did not affect lipid oxidation during ageing, except for P group
271 (<40% lean), which showed greatest TBARS values at T4 and T6 (P<0.05).

272 **Discussion**

273 A normal practice to increase the IMF content is to slaughter pigs at heavier live weights. In this study,
274 purebred Duroc barrows from a selected population were slaughtered at the same age (210 ±5 days) but
275 they were classed into three carcass groups: R (45-50% lean), O (40-45% lean) and P (<40% lean), which
276 derived from the following approximate live weight intervals: 125-130 kg, 130-135 kg and 135-140 kg. A
277 strong relationship between slaughter weight and carcass lean-yield was observed. Since carcass fatness
278 and IMF are correlated, the carcass classes differed also in their IMF content. Carcass class R had an
279 average of 32 g of IMF/kg, carcass class O had an average of 40 g of IMF/kg and carcass class P had an
280 average of 43 g of IMF/kg. Although major differences were observed in carcass traits among groups,
281 the dressing out, ultimate pH and drip loss was not affected by carcass grade.

282 In earlier studies, increasing the slaughter live-weight from 100-105 kg to 125-130 kg in purebred Duroc
283 pigs increased dressing out and back-fat at slaughter whereas drip loss and thawing loss were reduced
284 (Candek-Potokar et al. 1998; Maignel et al. 2007). Similar to the present results, ultimate pH and the
285 value of colour reflectance (L*) were not affected by carcass grade. In those studies, the average daily

286 gain of the pigs did not differ, since greater live weight was achieved by increasing age at slaughter. In
287 the present study, the pigs with the best growth rate (achieving 140 kg at slaughter), did not have
288 increased dressing out, but greatest carcass lipid content (back-fat thickness and IMF) without
289 detrimental effects on drip loss or colour attributes.

290 The highest value for essential amino acid contents was observed for leucine followed by lysine, while
291 the highest value in non-essential amino acid contents was observed for glutamic acid followed by
292 aspartic acid. Hydroxyproline was the amino acid with lowest content. In a previous experiment using
293 carcasses from lean genotypes (all with >50% lean) (Okrouhlá et al. 2008), lysine and secondly leucine
294 represented the greatest content of the essential amino acids, while the proportion of non-essential
295 amino acids were similar to the present results. In agreement with this study, the amino acid contents of
296 the lean pork did not differ among carcass groups, but they found consistent differences in the
297 *Semimembranosus* muscle.

298 In the present experiment, there were no differences among carcass grades in the amino acid contents
299 of pork. However, a significant positive correlation between the content of total glycine and the IMF of
300 pork was detected. A decrease of free glycine content with carcass weight has been reported in the *L.*
301 *thoracis* muscle in previous studies (Usborne et al. 1968). Because glycine produces a sweet taste (Solms
302 1969), these results suggest the hypothesis that the most marbled pork showed a correlated change of
303 taste, especially in meat products where protein hydrolysis occurs.

304 The pork from the studied Duroc barrows had low connective tissue content, based on the association
305 between hydroxyproline content and henceforth collagen content (Colgrave et al. 2008). In fact, the
306 rather low shear force values measured in this study were in agreement with reduced hydroxyproline
307 content. Although the proportion of hydroxyproline in pork was low, earlier studies detected differences
308 in this amino acid content in *Longissimus dorsi* muscle between clearly contrasting pork (extensive and
309 intensive feeding systems in New Zealand and between light and heavy Singaporean pigs) (Purchas et al.
310 2009). Different concentrations were also observed among genotypes (Duroc, Iberian x Duroc, Landrace
311 x Large-White) in hydroxyproline content in *Gluteus medius* muscle (Tor et al. 2012).

312 Relative to the amino acid balance recommendations for humans our results showed that valine was the
313 most limiting aminoacid at 66-70% of requirement, indicating that 144-151 g of pork would need to be
314 consumed in order to match 100 g of a sample with the recommended balance of the indispensable
315 amino acids. The next most limiting amino acids were isoleucine and leucine at 75-85%. Our results
316 differ slightly from those of Wilkinson et al. (2014), who found that the first limiting amino acid in pork
317 was leucine followed by valine. However, in that case the samples derived from crossbred genetic lines
318 which were probably leaner than the present genotype. The amount of Duroc pork meat that would

need to be consumed to get a satisfactory balance of amino acids to meet human requirements for all amino acids was higher in the P (<40% lean) group, although the amino acid composition did not differ significantly among carcass grades.

The objective colour attributes did not differ between lean-yield classes. In addition, vacuum-ageing up to 8 days had not clear deleterious effects on meat colour traits, although the best colour appeal (low hue angle, high redness index and low calculated metmyoglobin content) was shown in vacuum-aged pork for 4 days, in line with results of film-packaged refrigerated pork (Alvarez-Rodriguez et al. 2015).Although IMF content has been positively related to tenderness (Jeleníková et al. 2008) our results showed that neither carcass grade nor ageing affected tenderness measured by Warner Bratzler. Similar results were reported by Candek-Potokar et al. (1998) though they did report a reduction in sensory tenderness in heavier Duroc pigs (mostly P graded), which was related to increase in fibre diameter. Using cooked pork loins, Channon et al. (2004) and Juárez et al. (2009) reported that vacuum ageing for 7-14 days significantly improved tenderness based on shear force. These pigs however had lower IMF contents (18-25 g/kg of IMF) than those used in the present experiment (over 30 g/kg of IMF) and this may have attenuated any improvement in Warner Bratzler shear force over the seven days investigated (overall 2.3±0.1 kg of shear force). Based on the currents results it would be difficult to justify ageing to improve tenderness in the Duroc line investigated. The results might however differ for sensory tenderness. In another experiment using the current texture meter device with lean Pietrain-sired pigs (below 30 g/ kg of IMF), the mean shear force of raw loins was 3.3±0.1 kg (García-Hernández et al. 2017). Thus, the values recorded for this Duroc raw pork are indicative of the loin being tender, which may mask the potential effects of ageing on this attribute. It must be pointed out that Warner Bratzler shear measurements are normally made on cooked samples, to reflect the most common form of meat when eaten. In the afore-mentioned study by García-Hernández et al. (2017), cooking at 70ºC another sub-sample from the same lean loins increased shear force up to 5.7±0.1 kg. However, it has been suggested that relationships between shear force readings on raw and aged cooked samples are low, probably due to the fact that the effects of ageing on cooked meat tenderness are not apparent on the tenderness of raw meat (Purchas 1973).

Lipid oxidation was greater in pork loins from the leanest carcasses (R group, 45-50% lean), especially from 4 days of vacuum storage onwards. This was likely associated with the higher PUFA content in the IMF of this group. Bosch et al. (2012) previously reported for the same Duroc line a decline in PUFA content of the loin with increasing live-weight. Nevertheless, the mean TBARS of all groups after seven days ageing were considerably below sensory detection of rancid flavours of 1 mg MDA/kg of meat (Rossi et al. 2013).

Conclusions

353 • A strong relationship between slaughter weight and carcass lean-yield was observed.
354 • Carcass grading based on lean content had a major effect on IMF composition, since increased
355 fatness was associated with increased MUFA and decreased PUFA content.
356 • Carcass adiposity did not affect the amino acid balance and the technological quality (colour,
357 drip loss, shear force and lipid stability) of raw pork.
358 • Ageing for 8 days was unlikely to increase the tenderness of pork with intramuscular fat levels
359 over 30 g/kg, as was the case for pigs in this study.
360 • The lipid oxidation had an inverse relationship with the PUFA content of each pork class, and it
361 did not increase due to vacuum ageing for 8 days.

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367 **Conflict of interest statement**

368 The authors declare no conflicts of interest.

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460

461 Table 1. *Chemical composition of the diet fed to pigs from 180 to 210 days of age (g/kg of feed, unless*

462 *otherwise stated) (Means)*

Gross energy, MJ/kg	17.0
Dry matter	886
Crude protein (CP)	142
Lysine	8.6
Methionine	1.2
Methionine+Cysteine	3.6
Threonine	4.7
Valine	3.5
Isoleucine	9.9
Leucine	2.9
Histidine	2.8
Phenylalanine	5.8
Sum of EAA	60.4
Ether extract	64
SFA, g/kg fatty acids	100
MUFA, g/kg fatty acids	588
PUFA, g/kg fatty acids	312
Crude fibre	36

463 Ingredients (g/kg of feed, in descending order): barley (372), wheat (224), rye (126), canola meal 00 (70),

464 pea (47), soyabean meal 46% CP (31), animal-vegetable blended fat (42), sugar-beet pulp (30),

465 sugarcane molasses (18), calcium carbonate (13), vitamin-mineral premix (7), sodium chloride (5), L-

466 Lysine 50% CP (5), L-Tryptophan 85% CP (5), monocalcium phosphate (3), L-Threonine 72.5% CP (1),

467 Choline chloride (1).

468 EAA= sum of essential amino acids (Lysine, Methionine, Threonine, Isoleucine, Valine, Phenylalanine,

469 Leucine and Histidine).

470 Table 2. *Growth performance and carcass traits of Duroc barrows slaughtered at 210 days of age*
471 *according to carcass commercial grading (Least square means ± standard error)*

	Carcass grade			
	R (45-50% lean)	O (40-45% lean)	P (<40% lean)	P-value
N	18	28	34	-
Weight at 180 days old, kg	105.8±1.8[b]	106.7±1.5[b]	113.4±1.4[a]	0.002
Weight at 210 days old, kg	126.9±2.3[b]	130.8±1.8[b]	138.9±1.7[a]	<0.001
Average daily gain, g	698±45[b]	806±36ab	839±34[a]	0.05
Dressing out, %	75.9±0.4	75.6±0.3	76.3±0.3	0.33
Carcass weight, kg	96.3±1.9[b]	98.9±1.5[b]	106.0±1.4[a]	<0.001
Carcass length,cm	86.0±0.6	86.8±0.5	87.8±0.5	0.07
Ham weight, kg	12.47±0.25[b]	12.44±0.20[b]	13.29±0.19[a]	0.005
Fat depth at *Gluteus medius* muscle, mm	21.6±0.4[c]	25.1±0.3[b]	29.2±0.3[a]	<0.001
Backfat thickness between ribs 12th and 13th assuming rib 15th was the last rib, mm	21.3±0.3[c]	24.4±0.3[b]	28.1±0.3[a]	<0.001
Carcass lean content, %	46.6±0.5[a]	42.5±0.4[b]	35.7±0.4[c]	<0.001

472 Means in the same row with no superscript letters after them or with a common superscript letter
473 following them are not significantly different (P < 0.05)

Table 3. *Technological traits and chemical composition of fresh L. thoracis muscle (24 h post-mortem) from Duroc barrows according to carcass commercial grading (Least square means ± standard error)*

	Carcass grade			
	R (45-50% lean)	O (40-45% lean)	P (<40% lean)	P-value
Ultimate pH	5.88±0.04	5.92±0.03	5.93±0.03	0.56
Centrifugation drip loss, g/kg	21.2±2.7	16.4±2.1	17.5±2.0	0.37
Dry matter, g/kg	282.7±2.7	287.2±2.2	286.5±2.0	0.40
Crude protein, g/kg	217.0±2.0[a]	214.3±1.6[ab]	210.9±1.5[b]	0.04
Intramuscular fat, g/kg	31.8±2.5[b]	40.7±2.0[a]	43.2±1.9[a]	0.003
∑MUFA, g/kg fatty acids	492.0±4.[b]	501.7±3.2[ab]	508.8±3.0[a]	0.006
∑SFA, g/kg fatty acids	391.4±4.5	403.2±3.6	402.1±3.4	0.10
∑PUFA, g/ kg fatty acids	116.5±3.6[a]	95.1±2.9[b]	89.1±2.7[b]	<0.001

MUFA= monounsaturated fatty acids (C16:1n-7; C17:1n-7; C18:1n-9; and C20:1n-9); SFA= saturated fatty acids (C10:0; C12:0; C14:0; C16:0; C17:0; C18:0; and C20:0); PUFA=polyunsaturated fatty acids (C18:2n-6; C18:3n-3; C20:2n-6; C20:3n-6; C20:4n-6; C20:4n-6 and C22:6n-3).

Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$)

481 Table 4. *Essential and non-essential amino acid composition of fresh L. thoracis muscle (24 h post-*
482 *mortem) from Duroc barrows according to carcass commercial grading (Least square means ± standard*
483 *error)*

	Carcass grade			
	R (45-50% lean)	O (40-45% lean)	P (<40% lean)	P-value
Amino acids, g/g CP				
Lysine	8.48±0.29	8.43±0.23	8.62±0.22	0.83
Methionine	1.95±0.08	2.06±0.07	2.08±0.06	0.46
Threonine	4.68±1.00	4.80±0.08	4.85±0.07	0.45
Valine	4.96±0.11	5.09±0.09	4.94±0.08	0.45
Isoleucine	4.54±0.09	4.56±0.07	4.34±0.07	0.09
Leucine	9.29±0.13	9.42±0.11	9.20±0.10	0.34
Histidine	5.16±0.17	5.16±0.14	5.15±0.13	0.99
Phenylalanine	3.82±0.04	3.89±0.04	3.83±0.03	0.39
∑EAA*	42.9±0.7	43.4±0.6	43.0±0.5	0.80
Cistine	0.97±0.04	0.97±0.03	0.96±0.03	0.99
Hydroxyproline	0.27±0.02	0.28±0.01	0.26±0.01	0.63
Proline	3.76±0.06	3.84±0.05	3.80±0.04	0.53
Alanine	5.21±0.10	5.27±0.08	5.14±0.08	0.52
Arginine	5.97±0.07	6.01±0.06	5.90±0.05	0.35
Aspartic acid	10.34±0.26	10.79±0.21	10.73±0.20	0.37
Glutamic acid	15.47±0.30	15.89±0.25	15.48±0.23	0.42
Glycine	3.92±0.08	4.15±0.06	4.03±0.06	0.06
Serine	4.13±0.08	4.28±0.06	4.28±0.06	0.26
Tyrosine	3.34±0.10	3.22±0.08	3.33±0.08	0.53
∑NEAA	53.4±0.6	54.7±0.5	53.9±0.5	0.27
Total	96.25±1.22	98.09±0.99	96.90±0.92	0.47

484 EAA= essential amino acids (Lysine, Methionine, Threonine, Isoleucine, Valine, Phenylalanine, Leucine
485 and Histidine); NEAA= non-essential amino acids (Cistine, Arginine, Hydroxyproline, Tyrosine, Alanine,
486 Glycine, Glutamic acid, Serine, Proline, and Aspartic acid).
487 *Tryptophan (essential amino acid) was not measured in the current samples and therefore it was not
488 included.
489

490 Table 5. *The amino acids (AA) balance relative to lysine in raw pork according to carcass grade compared*

491 *with the recommended balance of indispensable amino acids for adults (WHO/FAO/UNU, 2007)**

	Target balance of AA for adults expressed relative to lysine at 100 (WHO/FAO/UNU, 2007)	Balance of AA in pork relative to lysine			Proportion of the recommended amount of each AA in a sample containing the target amount of lysine		
		R	O	P	R	O	P
Lysine	100.0	100.0	100.0	100.0	1.00	1.00	1.00
Methionine + Cysteine†	48.9	45.9	47.4	46.4	0.94	0.97	0.95
Threonine	55.6	55.2	56.9	56.3	0.99	1.02	1.01
Valine	86.7	58.5	60.4	57.3	0.67	0.70	0.66
Isoleucine	66.7	53.5	54.1	50.3	0.80	0.81	0.75
Leucine	131.1	109.6	111.7	106.7	0.84	0.85	0.81
Histidine	33.3	60.8	61.2	59.7	1.83	1.84	1.79
Phenylalanine + Tyrosine	84.4	84.4	84.3	83.1	1.00	1.00	0.98

492 Carcass grades based on lean content (European Union standards): R (45-50% lean), O (40-45% lean) and

493 P (<40% lean).

494 *Tryptophan (essential amino acid) was not measured in the current samples and therefore it was not

495 included.

496 † Cysteine supply was calculated as twice the analysed Cys content.

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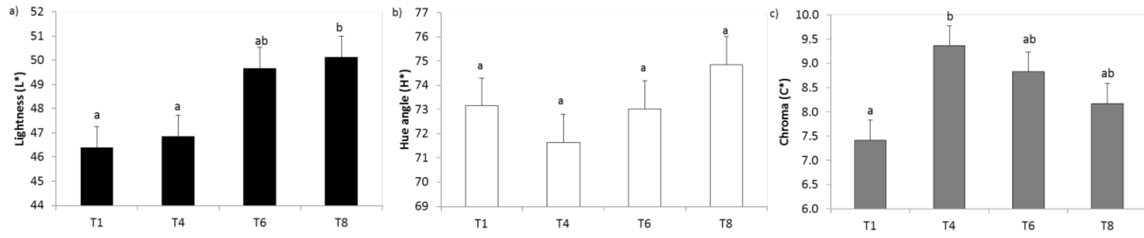

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500 Figure 1. *Lightness (a), hue angle (b) and chroma (c) colour attributes (CIELab scale) of L. thoracis muscle*
501 *from purebred Duroc barrows at day 1 post-mortem or subsequently vacuum aged up to 4, 6 or 8 days.*
502 *Different letter within each colour attribute indicates significant differences (P<0.05) between ageing*
503 *times (Least square means ± standard error).*
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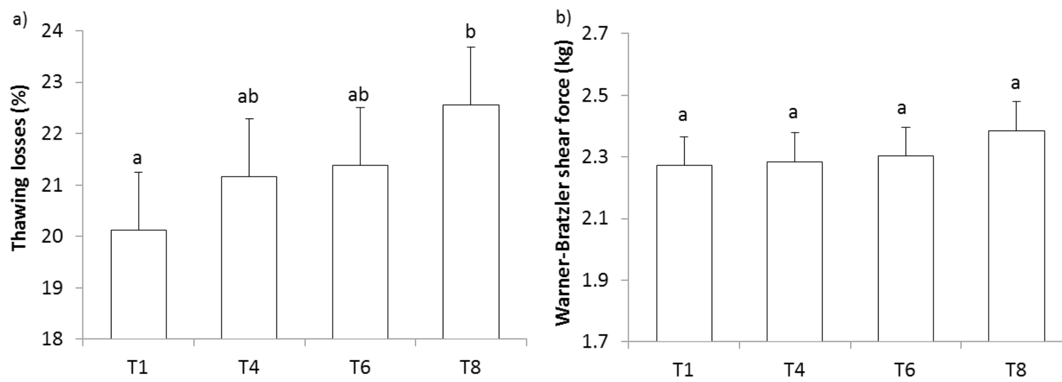

509 Figure 2. *Thawing losses (a) and Warner-Bratzler shear force (b) in raw, uncooked meat (L. thoracis*
510 *muscle) from purebred Duroc barrows at day 1 post-mortem or subsequently vacuum aged up to 4, 6 or*
511 *8 days. Different letter within each variable indicates significant differences (P<0.05) between ageing*
512 *times (Least square means ± standard error).*
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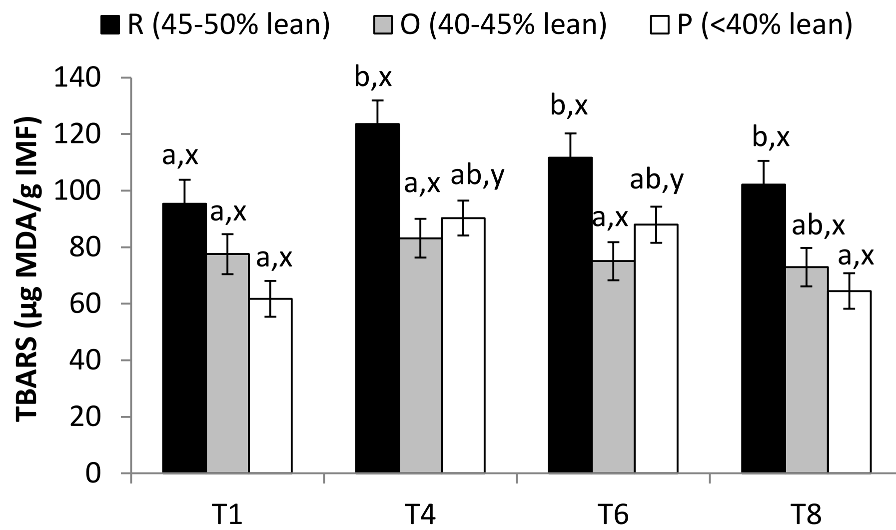

515
516
517 Figure 3. *Lipid oxidation (TBARS, µg malonaldehyde-MDA/g of intramuscular fat -IMF) of raw loins from*
518 *purebred Duroc barrows according to carcass grade (R, O or P category) at day 1 post-mortem or*
519 *subsequently vacuum aged up to 4, 6 or 8 days. Different letter within an ageing time (a, b) indicates*
520 *significant differences (P<0.05) between carcass categories. Different letter within carcass group (x, y)*
521 *indicates significant differences (P<0.05) between ageing times (Least square means ± standard error).*
522 *The mean TBARS values was 0.59±0.02 µg MDA/g of meat (R group=0.62 µg MDA/g of meat; O*
523 *group=0.53 µg MDA/g of meat and P group=0.61 µg MDA/g of meat).*
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525