

1 Lysine restriction in growing phase for heavy barrows and gilts

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7 **The effect of protein restriction during growing period on carcass, meat**
8 **and fat quality of heavy barrows and gilts**

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21 **Abstract**

22 Nutritional strategies are being researched in pigs to increase fatness and then
23 to improve quality of dry-cured products. A total of 160 Duroc x (Landrace x
24 Large White) pigs, 50% barrows and 50% gilts, were used in a trial. During the
25 growing period (73-118 d of age), four feeds were formulated with decreasing
26 levels of crude protein (CP; 21.6, 17.7, 14.7 and 13.5%) to achieve 1.10, 0.91,
27 0.78 and 0.52% of total Lysine, respectively. From 118 d until slaughter, at 123
28 kg (183, 181, 178 or 192 d of age, respectively), a common diet was provided
29 (17.7% CP and 0.91% Lysine). Barrows had fatter carcasses than gilts but
30 intramuscular fat (IMF) proportion was similar for both. Dietary CP restriction
31 promoted wider backfat depth and pork with higher IMF percentage which was
32 more monounsaturated and less polyunsaturated. We conclude that CP
33 restriction during the grower period improves desirable carcass and meat traits
34 in barrows and gilts intended for dry-cured products.

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36 **Keywords:** protein restriction; carcass; meat; sex; heavy pigs.

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38 **1. Introduction**

39 Spain is one of the world leaders in the elaboration of dry-cured meat
40 products from pigs, such as ham or shoulder. To optimize of processing,
41 minimum carcass fatness is required (Ruíz, García, Muriel, Andrés, &
42 Ventanas, 2002) and then heavy market body weights (BW) are needed (120-
43 130 kg) and males have to be castrated (Latorre, García-Belenguer, & Ariño,
44 2008). However, even in that case, a notable proportion of carcasses show lack

45 of backfat depth because, during decades, pig breeds were improved for lean
46 deposition due to the benefits on performances. This problem is especially
47 important in gilts which are more efficient in converting feed into weight gain
48 and are leaner than barrows (Latorre, Ripoll, García-Belenguer, & Ariño,
49 2009a). In addition, currently, higher intramuscular fat (IMF) content is desirable
50 in pork because it has been related to eating quality (Ruíz et al., 2002).

51 Decreasing, during the finishing period, the dietary crude protein (CP) or
52 Lysine (Lys) level, as the first amino acid which limits the growth, could be
53 strategies to get it (Rodríguez-Sánchez et al., 2011; Wood et al., 2013). In a
54 recent work (Suárez-Belloch, Guada, & Latorre, 2015), it was observed that
55 total Lys restriction from 1.10 to 0.52% (21.6 to 13.5% CP) during the grower
56 phase produced an incomplete compensatory growth in the subsequent phase,
57 which confirm previous results from other researchers (Campbell, Taverner, &
58 Curie, 1988; Fabian et al., 2002). The compensatory growth does not seem to
59 be due to better utilization of nutrients (Lovatto, Sauvant, Noblet, Dubois, &
60 Milgen, 2006) but to variations in tissues accretion decreasing the rate of
61 protein synthesis and increasing the proportion of energy retained as fat
62 (Friesen et al., 1994; Deng et al., 2007). Kamalakar et al. (2009) also related
63 the compensatory growths with higher carcass fatness and IMF content and
64 tenderness in pork and Doran et al. (2006) justified it by the increase in activity
65 of stearyl-CoA desaturase. However, the impact seems to depend on several
66 factors such as sex (Stolzenbach et al., 2009) or realimentation period length
67 (Skiba, 2010).

68 Therefore, the reduction of the dietary **protein or Lys content** during the
69 growing period can be useful and practical ways to manipulate carcass and
70 meat traits of pigs which also would contribute to reduce environmental
71 problems by minimizing of the nitrogen excretion (Gallo et al., 2015). The aim of
72 this study was to quantify the impact of different dietary **CP** levels in the grower
73 feed on carcass, meat and fat quality at slaughter in heavy barrows and gilts.

74

75 **2. Material and methods**

76

77 *2.1. Pigs, diets and experimental design*

78 Readers are referred to Suárez-Belloch et al. (2015) for details regarding
79 the pig husbandry, diet characteristics, growth performances and serum
80 metabolites. All the experimental procedures used in this study were in
81 compliance with the Spanish guidelines for the care and use of animals in
82 research (Boletín Oficial del Estado, 2007).

83 A total of 200 Duroc x (Landrace x Large White) pigs, 50% barrows and
84 50% gilts, of 26.3 ± 0.55 kg BW and 73 ± 3 d of age were allotted at farm by
85 sex, in 40 pens of five, to blocks of increasing BW and distributed within each
86 block to the experimental diets. The last day of the grower period (118 ± 3 d of
87 age), a total of 40 animals (one per pen, randomly chosen) were slaughtered to
88 evaluate some characteristics related to lipid metabolism whose results were
89 recently published (Suárez-Belloch et al., 2015). The remaining pigs (160) were
90 slaughtered at the end of the finishing period, when achieved around 123.0 kg
91 BW, and were used for the study of carcass and pork quality.

92 During the growing period (45 d), four diets with decreasing levels of CP
93 (21.6, 17.7, 14.7 and 13.5%) were formulated to achieve 1.10, 0.91, 0.78 and
94 0.52% of total Lys and the feeds were offered to barrows and gilts. Therefore,
95 there were 8 experimental treatments following a 2 x 4 factorial arrangement (2
96 sexes and 4 diets) with 20 pigs per combination of treatments. Feeds were
97 formulated to be isoenergetic and that diet with 1.10% total Lys (21.6% CP),
98 intended for meeting the Lys recommendations for 25-50 kg BW pigs (National
99 Research Council, 2012), was considered as the control. The other diets were
100 formulated by the progressive replacement of soybean meal by barley and
101 synthetic amino acids were not added in any case.

102 During the finisher period (until slaughter at 123.0 ± 2.35 kg BW), pigs
103 received a common diet with 0.91% Lys (17.7% CP) according to National
104 Research Council (2012) recommendations for >50 kg BW-pigs. The change of
105 diet was carried out at a fixed age, rather than constant BW, to simulate
106 commercial management conditions. The estimated energy content (Fundación
107 Española Desarrollo Nutrición Animal, 2010) and the determined nutrient
108 composition of diets is shown in Table 1.

109 Feeds, in mash form, and water were provided *ad libitum* throughout the
110 trial. Weekly samples of each feed were pooled at the end of each experimental
111 period and the composite samples preserved at room temperature to be
112 analysed.

113

114 2.2. Slaughtering, carcass measures and meat sampling

115 The slaughter was planned at a fixed BW (around 123.0 kg BW) and then
116 pigs were 183, 181, 178 and 192 d of age depending on the experimental feed
117 received (21.6, 17.7, 14.7 and 13.5% CP, respectively). The day before
118 slaughter, animals were individually weighed and transported 100 km to a
119 commercial abattoir (Turolense Ganadera SA, Teruel, Spain), where they were
120 kept in lairage for 8 h with full access to water but not to feed. Pigs were
121 stunned by exposure to CO₂ (83% mean atmosphere concentration of gas; CO₂
122 cycle of 90 s, with 60 s interval between discharges and sticking). After
123 stunning, they were exsanguinated, scalded, dehaired, eviscerated and split
124 down the midline according to standard commercial procedures. The following
125 measures were individually taken from all carcasses (160) at the end of the
126 slaughter line. Hot carcass weight was recorded and used to calculate dressing
127 percentage. At 45 min *postmortem*, carcass length from the posterior edge of
128 the *symphysis pubis* to the anterior edge of the first rib, ham length from the
129 anterior edge of the *symphysis pubis* to the hock joint, and ham perimeter at its
130 widest were measured on the left side of each carcass using a flexible ruler with
131 a precision of 0.5 cm. In addition, fat depth at level of *gluteus medius* (GM)
132 muscle was measured by a ruler with a precision of 1 mm. The head was
133 removed at the atlanto-occipital junction and carcasses were suspended in the
134 air and refrigerated at 2°C (1 m/s; 90% relative humidity) for 12 h. Then,
135 carcasses were processed, and the left ham, shoulder and loin were trimmed
136 (removing part of the covering fat) to fit commercial requirements. Then they
137 were weighed to calculate their yield in the carcass.

138 After collection of carcass data, from a total of 80 pigs (2 pigs per pen,
139 randomly chosen), three samples were taken; 400 ± 20 g of loin (*longissimus*
140 *thoracis* (LT) muscle) at the last rib level, 200 ± 25 g of ham (GM muscle) and
141 50 ± 5 g of subcutaneous fat including fat layers, skin and lean at the tail
142 insertion in the coxal region. All samples were stored in individual plastic bags
143 and vacuum-packaged for 24 h at 4°C until subsequent analyses.

144

145 2.3. Meat measures and analyses

146 The day after slaughter, the LT samples were removed from packages
147 and after blooming for 15 min, colour was evaluated with a chromameter (CM
148 2002, Minolta Camera, Osaka, Japan) using objective measurements
149 (Commission International de l'Eclairage, 1976). Previously, the chromameter
150 was calibrated with a white tile, according to manufacturer's recommendations.
151 The average of three random readings was used to measure lightness (L^* , a
152 greater value is indicative of lighter colour), redness (a^* , a greater value is
153 indicative of redder colour) and yellowness (b^* , a greater value is indicative of
154 more intense yellow colour). Additionally, chroma (C^*) and hue angle (H°) were
155 calculated as $C^* = \sqrt{a^{*2} + b^{*2}}$ and $H^\circ = \tan^{-1}(b^*/a^*) \times 57.29$, respectively
156 (Wyszcecki & Stiles, 1982). After, all samples (LT, GM and subcutaneous fat)
157 were individually vacuum-packaged and stored at -20°C until subsequent
158 analyses.

159 When it was required, the LT samples were thawed for 24 h at 4°C,
160 removed from packages, blotted dry for 20 min, and weighed. Thawing loss was
161 calculated taking into account the fresh and thawed weight. Cooking loss was

162 determined by the method described by Honikel (1998) considering the pre- and
163 post-cooked weights. Briefly, a slice (140 ± 20 g) was taken from each chop,
164 weighed, placed in a plastic bag, and cooked to an internal temperature of 70°C
165 in a 75°C water bath. Internal temperature was monitored during cooking with a
166 handheld temperature probe (model HI 9063, Hanna Instruments, Woonsocket,
167 RI). Cooked samples were allowed to cool at 15°C for 30 min, blotted dry and
168 weighed. In addition, the texture was determined by a Warner Bratzler device
169 attached to an Instron Universal testing machine model 4301 (Massachusetts,
170 USA). Slices (6 per chop) were cut with a cross-section of 100 mm² (10 mm x
171 10 mm) with the fibre direction parallel to a long dimension of at least 30 mm
172 length.

173 Chemical components analyzed were moisture by the oven drying
174 method for 48 h at 105°C, protein using a Kjeldahl MT 2300 analyser (Höganäs,
175 Switzerland) and IMF by an ANKOM XT15 equipment (New York, USA)
176 according to Boletín Oficial del Estado (1979). In the samples of GM muscle,
177 the thawing process and the chemical composition analyses were also done as
178 described above.

179 In feeds, the following analyses were carried out. The dry matter content
180 was determined by oven drying (# 930.15; AOAC, 2005), organic matter and
181 total ash by muffle furnace (# 942.05; AOAC, 2005), CP by the Kjeldahl method
182 (# 984.13; AOAC, 2005) and ether extract by Soxhlet analysis (# 920.39;
183 AOAC, 2005). The neutral detergent fiber was determined with an ANKOM 220
184 Fiber Analyser on dried samples (608°C for 48 h), as described by Mertens
185 (2002), and results were expressed as ash-free residues. The starch was

186 analyzed by polarimetry after hydrolysis with ethanol and HCl (Commission of
187 the European Communities, 1999). The amino acid composition was
188 determined by HPLC-Fluorescence (PNT-M-109), with the exception of
189 tryptophan and cystine that were analyzed by HPLC-UV and gas
190 chromatography, respectively. The fatty acids of diets were extracted and
191 quantified by the one-step procedure described by Jenkins (2010) in freeze dry
192 samples. After, methylated fatty acids samples were identified according to Rey,
193 López-Bote, & Arias (1997) by gas chromatography as will be described in the
194 section of fatty acid analyses.

195

196 *2.4. Analyses of fatty acid profile of fat*

197 Lipids from the LT, GM and subcutaneous fat samples were extracted in
198 chloroform methanol, according to Bligh & Dyer (1959) and 2,6-di-ter-butyl-4-
199 methyphenol (BHT) from Panreac[®] (1 g/10 ml methanol) was used as
200 antioxidant. Briefly, a 1:2 (v/v) mixture of chloroform:methanol containing 100
201 µg/l of BHT solution was added to 10 g of muscle, homogenizing in a blender
202 (Ultraturrax) chilled with ice to prevent heat generation. Afterwards, chloroform
203 and 0.88% (w/v) KCl in water was added to each sample up to a ratio of
204 2:2:2:2:2 (muscle:chloroform:methanol:water), homogenizing again. After
205 centrifugation at 4000 rpm, the organic phase was obtained, and 1 ml was used
206 to assess IMF content by drying at 100°C for 20 min. The rest was evaporated
207 in a sand bath under N gas at 50°C. Fatty acid methyl esters were generated by
208 trans-esterification of 30 mg of lipids extracts, dissolved in n-hexane with 2N
209 KOH in methanol, and collected in hexane. After, their composition was

210 determined using a Hewlett-Packard 6890 II gas chromatograph (Agilent,
211 Pennsylvania, USA) with a capillary column SP2380 (100 m x 0.25 mm x 0.20
212 µm) and oven temperature programming as follows: column temperature was
213 set at 130°C, then raised at a rate of 8°C/min to 145°C and kept constant for 26
214 min, and after raised at 2°C/min to 240°C and kept constant for 20 min. The
215 temperature of injector was 260°C and for the detector 280°C. Helium was used
216 as gas carrier at a constant flow rate of 2 ml/min with an injected volume of 0.5
217 µl. The methyl esters were identified using retention times of Sigma chemical
218 Co. standards. The proportions of total saturated (SFA), monounsaturated
219 (MUFA) and polyunsaturated fatty acids (PUFA) were calculated from individual
220 fatty acid proportions.

221

222 *2.4. Statistical analyses*

223 Data were analysed as a randomized block factorial design (2 x 4) using
224 the GLM procedure of the SAS package (version 9.2). The model included
225 block, sex (barrows and gilts) and diet (21.6, 17.7, 14.7 and 13.5% CP, and
226 1.10, 0.91, 0.78 and 0.52% total Lys, respectively) as main effects, as well as
227 the interaction sex by diet. Linear and quadratic responses were studied by
228 orthogonal polynomials for equally spaced treatments. The experimental unit
229 was the animal (n=20 per treatment for carcass traits and n=10 per treatment
230 for meat and fat traits). A *P*-value <0.05 was considered as a significant
231 difference and a *P*-value between 0.05 and 0.10 as a trend.

232

233 **3. Results**

234 Although it was not the goal of the present work, a summary is included
235 (data not shown in tables) about the effect of sex and diet on growth
236 performances of the 160 pigs used. During the grower period, the CP restriction
237 affected quadratically all production traits, reducing the average daily gain
238 ($P<0.001$) and the feed intake ($P<0.05$) and increasing the feed conversion ratio
239 ($P<0.01$). During the finisher period, when all experimental animals received a
240 common diet, a compensatory increase of BW gain was observed ($P<0.001$).
241 The effect was linear and associated to a higher daily feed intake ($P=0.097$)
242 giving finally a linear decrease of feed conversion ratio ($P<0.01$). For the overall
243 trial (grower + finisher), the daily gains showed a quadratic reduction ($P=0.05$)
244 whereas feed conversion ratio increased linearly ($P=0.001$), especially for pigs
245 fed diets with 14.7 and 13.5% CP. Also, barrows ate more and grew faster than
246 gilts ($P<0.001$) with no difference in feed conversion ratio.

247 No significant interaction sex by diet was detected for any of the
248 variables studied related to carcass and pork quality ($P>0.10$) and therefore
249 results are described as main effects.

250

251 3.1. Carcass characteristics

252 Carcass weight and yield and also ham size (length and perimeter) were
253 not affected by sex ($P>0.10$) (Table 2). However, barrows had shorter
254 carcasses ($P<0.001$), wider fat depth at GM muscle ($P<0.001$) and lower yield
255 of ham ($P<0.05$) and loin ($P<0.001$) than gilts resulting in a lower proportion of
256 total trimmed lean cuts ($P<0.01$).

257 The **protein** restriction during the grower period tended to vary
258 quadratically the ham length ($P=0.07$), providing the highest size the diet with
259 **13.5% CP**, and increased linearly fat thickness at GM muscle ($P<0.05$) and
260 shoulder yield ($P<0.05$).

261

262 3.2. Meat characteristics

263 No differences between barrows and gilts were detected in meat quality
264 except for protein content of GM muscle that was lower in barrows than in gilts
265 ($P<0.05$) (Table 3). However, dietary **CP** restriction at early age had influence
266 on colour, increasing linearly redness ($P=0.001$) and chroma ($P<0.001$) and
267 varying quadratically lightness ($P<0.01$), yellowness ($P<0.01$) and Hue angle
268 ($P<0.05$) achieving the highest values with the diet containing **14.7% CP**. In
269 addition, cooking loss increased linearly ($P=0.01$) and hardness varied
270 quadratically ($P<0.05$) with the **protein** restriction providing the highest
271 tenderness the diet with **14.7% CP**. Also, diet affected the chemical composition
272 of LT muscle, varying the moisture content (quadratic, given the lowest humidity
273 the diet with **14.7% CP**; $P<0.001$) and tending to increase the IMF proportion
274 (linear, $P=0.07$) as **CP** level decreased. The effects of experimental feeds on
275 chemical composition of GM muscle were similar ($P<0.01$ for moisture and
276 $P=0.06$ for IMF proportion).

277

278 3.3. Fatty acid profile of fat

279 In the IMF of LT muscle (Table 4), barrows had higher SFA ($P<0.05$) and
280 lower MUFA ($P<0.05$) proportions than gilts due to the higher percentage of

281 C16:0 ($P<0.05$) and lower of C18:1n-9 ($P<0.05$). Also, the CP restriction during
282 the grower period affected some fatty acids, increasing the C18:1n-9 ($P<0.05$)
283 and decreasing the C18:2n-6 ($P<0.01$) and C18:3n-6 and n-9 ($P<0.001$) which
284 resulted in quadratic variations of total MUFA ($P<0.05$) and total PUFA ($P<0.05$)
285 proportions achieving the highest value of MUFA and the lowest of PUFA with
286 the 13.5% CP-diet.

287 Similarly, in the IMF of GM muscle (Table 5), barrows had higher SFA
288 ($P<0.05$) and lower PUFA ($P<0.01$) proportions than gilts due to the higher
289 percentage of C16:0 ($P<0.01$) and lower of C18:2n-6 ($P<0.01$) observed in
290 barrows. In addition, the CP restriction affected some fatty acids, increasing the
291 C18:1n-9 ($P<0.01$) and decreasing the C14:0 ($P<0.01$), C17:0 ($P<0.05$) and
292 C18:2n-6 ($P<0.01$) which resulted in a quadratic variation of total MUFA
293 ($P<0.01$) and total PUFA ($P<0.05$) percentages achieving the highest value of
294 MUFA and the lowest of PUFA with 14.7% CP.

295 In the subcutaneous fat (Table 6), gilts had lower percentages of C14:0
296 ($P<0.01$), C16:0 ($P<0.001$) and C16:1 ($P<0.05$) and higher of C18:2n-6
297 ($P<0.001$) and C18:3n-6 and n-9 ($P<0.001$) than barrows giving, the female fat,
298 a lower total SFA ($P<0.01$) and total MUFA ($P<0.05$) and higher total PUFA
299 ($P<0.001$) proportions. The CP restriction decreased linearly the total SFA
300 ($P<0.01$), due to the reduction in C16:0 ($P<0.01$) and C20:0 ($P<0.01$), and
301 increased linearly the total MUFA proportion ($P<0.01$) because of the increment
302 in C18:1n-9 ($P<0.01$) and C20:1n-9 ($P<0.01$). No effect of diet was observed in
303 total PUFA ($P>0.10$) in spite of the linear increase in C18:3n-6 and n-9 ($P<0.05$)
304 found as CP decreased in diet.

305

306 **4. Discussion**

307

308 *4.1. Carcass characteristics*

309 Carcasses from gilts were longer than those from barrows. Rodríguez-
310 Sánchez et al. (2011) found similar carcass size for both sexes when animals
311 were slaughtered at the same age (196 d old) but different BW, being gilts
312 lighter than barrows (126 vs 132 kg, respectively). Therefore, the BW could be
313 responsible, at least in part, for these differences. Also, across CP levels,
314 barrows showed higher fat depth at GM muscle, indicating higher potential for
315 fattening than females in agreement with previous reports (Cisneros et al.,
316 1996; Latorre et al., 2008) which is likely related to the higher voluntary feed
317 intake of barrows. So, the lower yield of total trimmed cuts detected in barrows
318 would suggest that carcasses from gilts were leaner, because these pieces are
319 the main contributors to lean tissue. In fact, loin was the most affected piece
320 and is the leanest of those studied here.

321 The experiment was intended for testing how different patterns of growth
322 might affect fattening of pigs using the CP restriction during early period as a
323 practical strategy in commercial conditions. The response of carcass fat depth
324 to dietary CP (or Lys) concentration was clear and confirms that the aim was
325 achieved. In the literature, there is an agreement on the effect of protein
326 restriction enhancing body fatness at the end of the depletion period (Chiba,
327 1995; Fabian et al., 2002; Kamalakar et al., 2009). Also, Gallo et al. (2015)
328 found that restrictive diets in CP and indispensable amino acids did not have

329 effects on the uniformity of the carcasses and commercial cuts. When CP or
330 Lys restriction is removed during the finishing period, pigs previously restricted
331 have higher daily BW gains and are more efficient in converting feed into gain,
332 confirming the existence of compensatory growth (Fabian et al., 2002;
333 O'Connell, Lynch, & O'Doherty, 2006). This meant in the current trial a delay to
334 reach the slaughter BW of 1.50 ± 0.292 d per each percentage unit of dietary
335 CP restriction which was associated with increasing thickness of fat at the GM
336 muscle by 0.35 ± 0.103 mm. In the present trial, the compensatory growth was
337 not complete (Suárez-Belloch et al., 2015) and differences in subcutaneous fat
338 thickness were also recorded at slaughter. It agrees with the results of de Greef,
339 Kemp, & Verstegen (1992) who found, at 105 kg BW, that the previously
340 restricted-protein pigs were still older and fatter than controls. Also, Heyer &
341 Lebret (2007) observed higher deposition rate of subcutaneous fat in ham
342 during realimentation after a period of feed restriction from 30 to 70 kg BW,
343 which is a desirable trait for ham producers to favour the salting and to avoid an
344 excessive dried (Ruíz et al., 2002). This response would be associated to an
345 increased feeding intake during realimentation and the subsequent response of
346 fat and protein deposition rates to energy intake (Bikker, Verstegen, &
347 Campbell, 1996). In fact, the feed consumption during finishing phase in the
348 present trial increased from 2.69 to 2.83 kg/d when CP content had been
349 previously decreased from 21.6 to 13.5% (1.10 to 0.52% Lys) in the growing
350 diet (Suárez-Belloch et al., 2015).

351

352 *4.2. Meat characteristics*

353 The differences between sexes (barrows and gilts) in meat traits were
354 limited. Several researchers have indicated that pork color, determined by
355 visual score, objective measurements, or myoglobin content is independent of
356 sex (Barton-Gade, 1987; Leach, Ellis, Sutton, McKeith, & Wilson, 1996), which
357 is confirmed by the present results. Also, water holding capacity and tenderness
358 neither were influenced by sex, which agree with previous observations of
359 Weatherup, Veattie, Moss, Kilpatrick, & Walker (1998) and Maiorano et al.
360 (2007). Some authors, such as Latorre, Ripoll, García-Belenguer, & Ariño
361 (2009b), have found lower hardness in pork from barrows indicating that
362 probably it was due to the higher IMF content. To this respect, in the present
363 trial, ham from barrows had lower protein content than ham from gilts with no
364 significant difference on IMF proportion. A higher IMF percentage was expected
365 in pork from barrows, taking into account their fatter carcasses and in
366 accordance with several authors (Ellis et al., 1996; Rodríguez-Sánchez et al.,
367 2011). However, in this case, the differences were limited and the reason could
368 be that, in most of reports, animals are usually slaughtered at the same age and
369 then gilts were lighter than barrows whereas in this trial, both sexes had similar
370 BW which reduced the differences.

371 The CP restriction affected meat colour increasing all variables studied
372 (L^* , a^* , b^* , C^* and H^0). Human perception of pork colour is strictly linked with
373 lightness and hue angle, which usually are affected by pH and water holding
374 capacity, whereas redness and chroma appear to be less important (Zanardi,
375 Novelli, Ghiretti, Dorigoni, & Chizzolini, 1999). In this sense, the increase in
376 cooking loss promoted by CP restriction is an index of lower water holding

377 capacity. An excess of water losses impair some testing aspects, such as
378 juiciness, but if it is moderate can improve some visual attributes providing
379 higher lightness. Therefore, the lowest level of protein content in diet might give
380 more attractive meat to consumers due to the high L* values. On the other
381 hand, the yellowness value has been related to marbling which would be
382 confirmed by the increase of both parameters (b* and IMF) with the CP
383 restriction in the present experiment. Whereas there is a notable agreement on
384 the increase of IMF in response to long term protein restriction (Karlsson et al.,
385 1993; da Costa et al., 2004; Jin et al., 2010) there is much controversy on the
386 effect of protein or Lys restriction at early age followed by compensatory growth.
387 Kamalakar et al. (2009) observed higher marbling in response to Lys restriction
388 from 0.95 to 0.57% (17.7 to 12.6% CP) suggesting that the cause might be the
389 activation of protein expression whereas Fabian et al. (2002) did not detect any
390 effect with similar levels of restriction indicating that it could depend on the
391 length of the realimentation period. The evolution observed in IMF was
392 comparable to that in backfat measured at the GM muscle. A greater IMF
393 concentration has been associated with more juiciness and greater acceptability
394 of the meat (Madeira et al., 2013) although the effect of other factors, such as
395 tenderness, cannot be disregarded (Barton-Gade, 1987). In the current trial, the
396 value of Warner-Bratzler shear force, which is indicative of hardness, was
397 reduced quadratically with CP restriction. Some authors have related the
398 tenderness to compensatory growth (Kristensen, Therkildsen, Aaslyng,
399 Oksbjerg, & Ertbjerg, 2004) mediated by increased protein turnover rate of
400 some muscles (Therkildsen et al., 2004) or changes in *post-mortem* proteolysis

401 (Chaosap, Parr, & Wiseman, 2011). However, it seems that the effect on
402 tenderness also depends on crossbred and sex, having more effect in lean than
403 in autochthonous genotypes (Madeira et al., 2013). Stolzenbach et al. (2009)
404 recorded more tender pork in gilts but not in entire males, concluding that the
405 reason could be that males did not achieve full compensation during the
406 realimentation period. Recent papers suggest a tendency towards greater fat
407 deposition in pigs fed diets restricted in CP but with regular levels of essential
408 amino acids from 40 to 115 kg BW; whereas the most tender and juiciest steaks
409 were obtained with diets restricted in both CP and amino acids. It was observed
410 in trials where carcass composition was measured by dissection (Wood et al.,
411 2013) or by computed tomography (Lambe et al., 2013).

412

413 *4.3. Fatty acid profile of fat*

414 The IMF from barrows was more saturated and less polyunsaturated
415 than that from gilts which agrees with results of Warnants, Van Oeckel, &
416 Boucque (1999) and Mas et al. (2010). It is related to differences in fatness
417 since SFA and MUFA proportions increase faster than PUFA with increasing
418 fatness. The greater SFA proportion in barrows was mainly due to C16:0
419 concentration, and the greater PUFA proportion in gilts was mainly explained by
420 the higher level of C18:2. The linoleic acid retained in the carcass is completely
421 derived from the diet and its proportion has been negatively associated with
422 fatness and positively to lean weight (Cameron, 1990). The C18:2 plays an
423 important role in human nutrition because it can reduce firmness and

424 cohesiveness of adipose tissue and increases the fat oxidation rate (Wood et
425 al., 2008).

426 The CP restriction during the grower period affected linearly some fatty
427 acids in the IMF of pork, resulting in an increase of MUFA and in a decrease of
428 PUFA. The higher level of PUFA is in agreement with results reported by Teye
429 et al. (2006). This effect is likely a consequence of the distinct distribution of
430 fatty acids between triacylglycerol (richer in SFA) and phospholipids (richer in
431 PUFA) and the increasing proportion of triacylglycerol with increasing IMF
432 content (Ntawubizi, Raes, Buys, & De Smet, 2009).

433 The changes in fatty acid composition, but mainly in IMF percentage,
434 detected in the present work are smaller than in the papers reviewed in the
435 literature. The reason could be the crossbred, the slaughter BW and/or the
436 length of restriction and realimentation. In any case, the results can be
437 considered successful.

438

439 **5. Conclusions**

440 The dietary crude protein (or Lys) restriction during the growing period
441 increased carcass fat depth, which is desirable for heavy pigs intended for dry-
442 cured products. Although moderately, it also improved some traits related to
443 meat quality, such as decreasing the hardness and tending to increase the IMF
444 content which was associated to higher proportion of monounsaturated and
445 lower of polyunsaturated fatty acids. The effects were similar for barrows and
446 gilts.

447

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457

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1 Table 1

2 Nutrient composition of the experimental diets (% , as-fed basis unless otherwise indicated).

	Growing diet (from 26 kg body weight, during 45 d)				Finishing diet (until 123 kg)
	21.6	17.7	14.7	13.5	
% Crude protein					
% total Lysine	1.10	0.91	0.78	0.52	
Calculated nutrients ¹					
Metabolizable energy (Kcal/kg)	3,260	3,260	3,260	3,260	3,260
Analysed nutrients					
Dry matter	90.0	91.4	91.2	91.1	91.4
Total ash	6.51	6.38	6.14	5.00	6.38
Crude protein	21.6	17.7	14.7	13.5	17.7
Ether extract	3.97	3.79	4.06	4.04	3.79
Neutral detergent fibre	11.1	11.1	11.7	12.5	11.1
Starch	34.8	36.8	40.5	43.8	36.8
Total amino acids					
Lysine	1.10	0.91	0.78	0.52	0.91
Methionine	0.34	0.26	0.26	0.19	0.26
Methionine + Cystine	0.72	0.64	0.56	0.39	0.64
Threonine	0.81	0.68	0.57	0.47	0.68
Tryptophan	0.23	0.23	0.17	0.14	0.23
Standardized ileal digestible amino acids ²					
Lysine	0.95	0.78	0.65	0.42	0.78
Methionine	0.35	0.23	0.17	0.16	0.23
Methionine + Cystine	0.74	0.74	0.36	0.33	0.74
Threonine	0.67	0.67	0.46	0.37	0.67
Tryptophan	0.20	0.20	0.15	0.12	0.20

Ideal protein (% of crude protein) ²	59.2	59.0	59.5	41.6	79.3
Fatty acids (% of total fatty acids)					
C16:0	21.2	21.4	21.7	21.9	21.4
C18:0	10.6	10.4	10.3	10.2	10.4
C18:1n-9	37.1	36.8	36.6	36.3	36.8
C18:2n-6	24.1	24.3	24.5	24.7	24.3
C18:3n-6 and n-3	2.04	2.03	2.01	1.99	2.03

3

4 ¹ According to Fundación Española Desarrollo Nutrición Animal (2010).

5 ² According to National Research Council (2012).

6 Table 2

7 The effect of sex and diet during the growing period on carcass characteristics of heavy pigs.

Sex	Barrows				Gilts				RSD ¹	P-value ²	
										Sex	Diet
% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7	13.5			
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
Slaughter weight (kg)	122	123	124	122	123	124	123	124	8.433	NS	NS
Carcass weight (kg)	93.9	94.3	94.7	93.9	94.7	95.3	94.9	95.9	6.878	NS	NS
Carcass yield (%)	77.1	77.0	76.5	76.7	76.9	76.8	77.2	77.4	1.407	NS	NS
Carcass length (cm)	84.6	85.0	84.9	85.2	87.1	87.0	86.5	87.2	2.448	***	NS
Ham length (cm)	39.3	39.0	39.1	39.3	39.1	39.0	39.3	39.9	1.004	NS	L* Q ^{0.07}
Ham perimeter (cm)	75.5	75.0	75.2	74.5	74.8	75.5	74.9	75.0	2.354	NS	NS
Fat depth (mm) ³	17.1	17.2	20.5	20.4	14.7	15.4	15.9	16.8	4.032	***	L*
Trimmed lean cuts (% carcass)											
Ham	13.6	13.5	13.4	13.6	13.7	13.6	13.7	13.7	0.416	*	NS
Shoulder	7.85	7.77	7.45	7.35	7.58	7.76	7.63	7.65	0.443	NS	L*
Loin	3.36	3.56	3.57	3.11	3.69	3.55	3.51	3.63	0.378	***	NS
Total ⁴	26.5	26.4	26.2	25.8	26.7	26.6	26.6	26.5	0.821	**	L**

8

9 ¹ Residual Standard Deviation.10 ² NS: P>0.10; *P<0.05; **P<0.01; ***P<0.001; L: lineal effect; Q: quadratic effect. No interaction sex x diet was detected (P>0.10).11 ³ Measured at *gluteus medius* muscle.12 ⁴ Ham + shoulder + loin (from half a carcass).

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16

17 Table 3

18 The effect of sex and diet during the growing period on meat characteristics of heavy pigs¹.

Sex	Barrows				Gilts				RSD ²	P-value ³	
										Sex	Diet
% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7	13.5			
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
<i>Longissimus thoracis</i> muscle											
Color traits											
Lightness, L*	54.9	55.8	58.3	57.4	54.3	57.2	56.9	56.7	3.074	NS	L*** Q**
Redness, a*	6.40	6.58	7.85	7.45	7.02	6.73	7.32	7.74	1.210	NS	L***
Yellowness, b*	3.74	4.12	4.90	4.34	3.72	4.31	4.23	4.29	0.944	NS	L** Q**
Chroma, C*	7.47	7.81	9.28	8.65	7.96	8.07	8.49	8.87	1.292	NS	L***
Hue angle, H ^o	30.6	32.6	31.8	29.9	27.8	33.3	30.1	28.8	6.354	NS	Q*
Water holding capacity indicators											
Thawing loss (%)	10.0	11.8	8.33	10.2	9.24	10.1	9.87	9.66	2.969	NS	NS
Cooking loss (%)	33.1	34.1	33.4	34.3	32.8	31.0	36.1	37.0	4.471	NS	L**
Warner-Bratzler shear force (kg)	2.60	2.35	2.21	2.36	2.68	2.45	2.40	2.55	0.480	NS	Q*
Chemical composition (%)											
Moisture	70.5	69.5	67.7	71.4	72.3	70.0	69.1	70.5	2.035	NS	Q***
Protein	23.6	23.3	23.3	23.1	24.1	23.8	23.5	23.1	1.269	NS	NS
Intramuscular fat	3.85	3.88	5.57	4.54	3.31	4.55	5.94	5.57	0.983	NS	L ^{0.07}
<i>Gluteus medius</i> muscle											
Chemical composition (%)											
Moisture	72.4	71.7	71.4	73.1	73.6	70.7	71.3	72.0	1.609	NS	Q**
Protein	23.7	22.7	22.9	23.0	23.1	24.0	23.9	23.5	1.005	*	NS
Intramuscular fat	2.94	2.72	3.99	3.10	2.37	3.08	3.44	3.63	1.208	NS	L ^{0.06}

19 ¹ Measured on thawed samples except for colour of *longissimus thoracis* muscle which was measured on fresh samples.

20 ² Residual Standard Deviation.

21 ³ NS: $P > 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; L: lineal effect; Q: quadratic effect. No interaction sex x diet was detected ($P > 0.10$).

22 Table 4

23 The effect of sex and diet during the growing period on fatty acid profile (%) of intramuscular fat from *longissimus thoracis* muscle of
 24 heavy pigs.

Sex	Barrows				Gilts				RSD ¹	P-value ²	
										Sex	Diet
% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7	13.5			
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
C12:0	0.10	0.10	0.12	0.08	0.11	0.10	0.09	0.08	0.041	NS	NS
C14:0	1.25	1.20	1.25	1.19	1.24	1.18	1.16	1.09	0.174	NS	NS
C16:0	24.8	24.4	24.4	24.5	23.9	23.5	23.9	24.0	1.084	*	NS
C16:1	3.17	3.55	3.60	3.44	3.39	3.60	3.47	3.33	0.482	NS	NS
C17:0	0.21	0.18	0.16	0.16	0.19	0.18	0.13	0.15	0.054	NS	NS
C17:1	0.22	0.21	0.20	0.20	0.20	0.22	0.14	0.18	0.051	NS	NS
C18:0	12.7	11.3	10.6	11.1	11.2	10.9	11.1	10.9	1.127	NS	L ^{0.07}
C18:1n-9	47.0	49.8	50.8	50.3	48.6	50.4	51.0	52.2	1.914	*	L*** Q*
C18:2n-6	6.40	5.66	4.92	5.11	6.67	5.49	5.01	4.79	0.931	NS	L*** Q**
C18:3n-6 and n-3	0.33	0.28	0.29	0.22	0.35	0.32	0.25	0.19	0.067	NS	L***
C20:0	0.18	0.18	0.19	0.17	0.18	0.17	0.16	0.15	0.053	NS	NS
C20:1n-9	0.91	0.82	0.81	0.94	0.86	0.88	0.97	0.83	0.156	NS	NS
C20:4n-6	0.12	0.07	0.19	0.12	0.17	0.22	0.14	0.09	0.136	NS	NS
C20:5n-3	0.16	0.17	0.21	0.21	0.28	0.22	0.23	0.13	0.140	NS	NS
Others ³	2.47	2.09	2.27	2.25	2.70	2.59	2.25	1.92	0.672	NS	NS
SFA ⁴	39.8	37.9	37.4	37.7	37.5	36.7	37.1	36.9	1.736	*	NS
MUFA ⁵	52.2	55.1	56.1	55.7	54.0	56.0	56.4	57.2	1.991	*	L*** Q*
PUFA ⁶	8.07	7.00	6.47	6.59	8.49	7.28	6.49	5.90	1.212	NS	L*** Q*

25 ¹ Residual Standard Deviation.

26 ² NS: P>0.10; *P<0.05; **P<0.01; ***P<0.001; L: lineal effect; Q: quadratic effect. No interaction sex x diet was detected (P>0.10).

27 ³ The sum of some minor fatty acids.

- 28 \sum^4 Saturated fatty acids.
29 \sum^5 Monounsaturated fatty acids.
30 \sum^6 Polyunsaturated fatty acids.
31

32 Table 5

33 The effect of sex and diet during the growing period on fatty acid profile (as % total fatty acids) of intramuscular fat from *gluteus medius*
 34 muscle of heavy pigs.

Sex	Barrows				Gilts				RSD ¹	P-value ²	
	% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7		13.5	Sex
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
C12:0	0.15	0.14	0.13	0.12	0.14	0.11	0.13	0.11	0.036	NS	NS
C14:0	1.36	1.37	1.19	1.26	1.33	1.28	1.28	1.24	0.106	NS	L**
C16:0	22.8	23.3	22.6	23.3	22.5	22.5	22.1	22.6	0.910	**	NS
C16:1	3.32	3.32	3.49	3.33	3.13	3.29	3.22	3.28	0.443	NS	NS
C17:0	0.38	0.33	0.27	0.29	0.37	0.28	0.32	0.30	0.093	NS	L*
C17:1	0.40	0.32	0.32	0.33	0.39	0.36	0.35	0.34	0.087	NS	NS
C18:0	11.5	11.1	10.5	11.1	11.5	11.0	10.8	10.6	1.014	NS	NS
C18:1n-9	47.0	47.6	50.9	49.1	46.7	48.6	49.4	50.0	1.784	NS	L*** Q**
C18:2n-6	9.15	8.72	7.43	7.62	10.6	8.69	8.56	8.23	1.066	**	L*** Q**
C18:3n-6 and n-3	0.31	0.25	0.23	0.24	0.27	0.32	0.31	0.28	0.096	NS	NS
C20:0	0.18	0.13	0.14	0.18	0.16	0.16	0.15	0.13	0.068	NS	NS
C20:1n-9	0.72	0.87	0.85	0.87	0.80	0.73	0.92	0.75	0.140	NS	NS
C20:4n-6	0.07	0.04	0.05	0.05	0.06	0.07	0.06	0.06	0.040	NS	NS
C20:5n-3	0.21	0.24	0.15	0.18	0.20	0.16	0.20	0.14	0.102	NS	NS
Others ³	2.45	2.28	1.73	2.07	2.51	2.40	2.25	1.97	0.608	NS	NS
SFA ⁴	36.8	36.8	35.2	36.6	36.2	35.8	35.2	35.3	1.555	*	NS
MUFA ⁵	52.3	52.9	56.2	54.3	51.6	53.8	54.6	55.1	1.894	NS	L*** Q**
PUFA ⁶	10.9	10.3	8.58	9.01	12.2	10.4	10.2	9.58	1.292	**	L*** Q*

35 ¹ Residual Standard Deviation.

36 ² NS: P>0.10; *P<0.05; **P<0.01; ***P<0.001; L: lineal effect; Q: quadratic effect. No interaction sex x diet was detected (P>0.10).

37 ³ The sum of some minor fatty acids.

- 38 $^4 \Sigma$ Saturated fatty acids.
- 39 $^5 \Sigma$ Monounsaturated fatty acids.
- 40 $^6 \Sigma$ Polyunsaturated fatty acids.
- 41
- 42
- 43

44 Table 6

45 The effect of sex and diet during growing period on fatty acid profile (as % total fatty acids) of subcutaneous fat of heavy pigs.

Sex	Barrows				Gilts				RSD ¹	P-value ²	
	% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7		13.5	Sex
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
C12:0	0.09	0.08	0.08	0.07	0.07	0.08	0.09	0.09	0.016	NS	NS
C14:0	1.21	1.18	1.13	1.19	1.16	1.11	1.11	1.10	0.082	**	NS
C16:0	23.1	23.1	22.1	22.5	22.4	22.0	21.3	21.3	0.775	***	L**
C16:1	1.92	1.94	1.89	1.87	1.74	1.85	1.67	1.83	0.239	*	NS
C17:0	0.41	0.37	0.35	0.42	0.38	0.38	0.39	0.41	0.069	NS	NS
C17:1	0.34	0.31	0.32	0.34	0.30	0.31	0.32	0.33	0.059	NS	NS
C18:0	13.5	13.4	12.6	13.0	13.8	12.8	13.0	12.4	1.141	NS	NS
C18:1n-9	43.3	44.1	45.6	44.6	43.2	43.9	43.7	44.5	1.284	NS	L**
C18:2n-6	11.2	11.0	11.2	11.2	12.0	12.5	13.3	13.1	1.024	***	NS
C18:3n-6 and n-3	0.75	0.71	0.74	0.73	0.78	0.82	0.89	0.88	0.068	***	L*
C20:0	0.23	0.22	0.21	0.21	0.23	0.20	0.19	0.18	0.036	0.06	L**
C20:1n-9	0.97	0.94	0.96	0.93	1.01	0.91	0.86	0.81	0.126	0.07	L**
C20:4n-6	0.03	0.03	0.04	0.05	0.03	0.04	0.03	0.03	0.026	NS	NS
C20:5n-3	0.25	0.24	0.28	0.25	0.25	0.31	0.30	0.28	0.073	0.09	NS
Others ³	2.71	2.45	2.58	2.59	2.69	2.77	2.87	2.79	0.247	**	NS
SFA ⁴	39.0	38.8	36.9	38.0	38.4	37.1	36.5	36.0	1.640	**	L**
MUFA ⁵	47.3	48.0	49.5	48.4	47.1	47.8	47.4	48.4	1.390	*	L**
PUFA ⁶	13.6	13.2	13.6	13.6	14.5	15.1	16.0	15.7	1.111	***	NS

46 ¹ Residual Standard Deviation.

47 ² NS: P>0.10; *P<0.05; **P<0.01; ***P<0.001; L: lineal effect; Q: quadratic effect. No interaction sex x diet was detected (P>0.10).

48 ³ The sum of some minor fatty acids.

49 ⁴ Σ Saturated fatty acids.

50 ⁵ Σ Monounsaturated fatty acids.

51 Σ^6 Polyunsaturated fatty acids.