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

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Abstract

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

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

1. Introduction

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

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

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

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

2. Material and methods

2.1. Pigs, diets and experimental design

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

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

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

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

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

2.2. Slaughtering, carcass measures and meat sampling

The slaughter was planned at a fixed BW (around 123.0 kg BW) and then pigs were 183, 181, 178 and 192 d of age depending on the experimental feed received (21.6, 17.7, 14.7 and 13.5% CP, respectively). The day before slaughter, animals were individually weighed and transported 100 km to a commercial abattoir (Turolense Ganadera SA, Teruel, Spain), where they were kept in lairage for 8 h with full access to water but not to feed. Pigs were stunned by exposure to CO_2 (83% mean atmosphere concentration of gas; CO_2 cycle of 90 s, with 60 s interval between discharges and sticking). After stunning, they were exsanguinated, scalded, dehaired, eviscerated and split down the midline according to standard commercial procedures. The following measures were individually taken from all carcasses (160) at the end of the slaughter line. Hot carcass weight was recorded and used to calculate dressing percentage. At 45 min *postmortem*, carcass length from the posterior edge of the *symphysis pubis* to the anterior edge of the first rib, ham length from the anterior edge of the *symphysis pubis* to the hock joint, and ham perimeter at its widest were measured on the left side of each carcass using a flexible ruler with a precision of 0.5 cm. In addition, fat depth at level of *gluteus medius* (GM) muscle was measured by a ruler with a precision of 1 mm. The head was removed at the atlanto-occipital junction and carcasses were suspended in the air and refrigerated at 2ºC (1 m/s; 90% relative humidity) for 12 h. Then, carcasses were processed, and the left ham, shoulder and loin were trimmed (removing part of the covering fat) to fit commercial requirements. Then they 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 4ºC 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 -20ºC until subsequent
158 analyses.
159 When it was required, the LT samples were thawed for 24 h at 4ºC,
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

determined by the method described by Honikel (1998) considering the pre- and post-cooked weights. Briefly, a slice (140 ± 20 g) was taken from each chop, weighed, placed in a plastic bag, and cooked to an internal temperature of 70ºC in a 75ºC water bath. Internal temperature was monitored during cooking with a handheld temperature probe (model HI 9063, Hanna Instruments, Woonsocket, RI). Cooked samples were allowed to cool at 15ºC for 30 min, blotted dry and weighed. In addition, the texture was determined by a Warner Bratzler device attached to an Instron Universal testing machine model 4301 (Massachusetts, USA). Slices (6 per chop) were cut with a cross-section of 100 mm^2 (10 mm x 10 mm) with the fibre direction parallel to a long dimension of at least 30 mm length.

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

In feeds, the following analyses were carried out. The dry matter content was determined by oven drying (# 930.15; AOAC, 2005), organic matter and total ash by muffle furnace (# 942.05; AOAC, 2005), CP by the Kjeldahl method (# 984.13; AOAC, 2005) and ether extract by Soxhlet analysis (# 920.39; AOAC, 2005). The neutral detergent fiber was determined with an ANKOM 220 Fiber Analyser on dried samples (608ºC for 48 h), as described by Mertens (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 contanining 100
201 μgl 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 100ºC for 20 min. The rest was evaporated
207 in a sand bath under N gas at 50ºC. 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 130ºC, then raised at a rate of 8ºC/min to 145ºC and kept constant for 26
214 min, and after raised at 2ºC/min to 240ºC and kept constant for 20 min. The
215 temperature of injector was 260ºC and for the detector 280ºC. 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**

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

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

3.1. Carcass characteristics

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

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

3.2. Meat characteristics

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

3.3. Fatty acid profile of fat

In the IMF of LT muscle (Table 4), barrows had higher SFA (P<0.05) and 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.

4. Discussion

4.1. Carcass characteristics

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

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

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

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-Sanchez 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^{o}). 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

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

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

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

5. Conclusions

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

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Table 1

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)
% Crude protein	21.6	17.7	14.7	13.5	
% 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
Standarized 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

[1] According to Fundación Española Desarrollo Nutrición Animal (2010).
[2] According to National Research Council (2012).

Table 2

The effect of sex and diet during the growing period on carcass characteristics 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	Diet
% 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**

[1] Residual Standard Deviation.

[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).

[3] Measured at *gluteus medius* muscle.

[4] Ham + shoulder + loin (from half a carcass).

Table 3

The effect of sex and diet during the growing period on meat characteristics of heavy pigs[1].

Sex	Barrows				Gilts				RSD[2]	*P*-value[3]	
% Crude protein	21.6	17.7	14.7	13.5	21.6	17.7	14.7	13.5		Sex	Diet
% total Lysine	1.10	0.91	0.78	0.52	1.10	0.91	0.78	0.52			
Longissimus thoracis 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, Hº	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	$L^{0.07}$
Gluteus medius 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	$L^{0.06}$

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

[2] Residual Standard Deviation.

[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).

Table 4

The effect of sex and diet during the growing period on fatty acid profile (%) of intramuscular fat from *longissimus thoracis* 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	Diet
% 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*

[1] Residual Standard Deviation.

[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).

[3] The sum of some minor fatty acids.

[4] ∑ Saturated fatty acids.
[5] ∑ Monounsaturated fatty acids.
[6] ∑ Polyunsaturated fatty acids.

Table 5

The effect of sex and diet during the growing period on fatty acid profile (as % total fatty acids) of intramuscular fat from *gluteus medius* 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	Diet
% 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*

[1] Residual Standard Deviation.

[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).

[3] The sum of some minor fatty acids.

[4] ∑ Saturated fatty acids.
[5] ∑ Monounsaturated fatty acids.
[6] ∑ Polyunsaturated fatty acids.

Table 6

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	Diet
% 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

[1] Residual Standard Deviation.

[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).

[3] The sum of some minor fatty acids.

[4] ∑ Saturated fatty acids.

[5] ∑ Monounsaturated fatty acids.

[6] ∑ Polyunsaturated fatty acids.