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Effect of protein-restricted diet during growing period on performance and carcass quality traits of Duroc x Iberian crossbred barrows under different management conditions

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HIGHLIGHTS

- Overall growth of Duroc x Iberian pigs was not affected by dietary protein level.
- Reducing protein in growing diets had minor effects on meat quality.
- Low-protein diets may increase intramuscular fat depending on production system and level of protein reduction.
- Production system should be consider when using low protein diets in crossbred pigs.

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ABSTRACT

This study investigated the effects of feeding protein-restricted diets during the growing period on performance and carcass quality traits of Duroc (boars) x Iberian (sows) crossbred pigs slaughtered at commercial weight for local pig production. Two trials were conducted in different management conditions, with 28 and 33 barrows, respectively. In each trial, pigs were divided into two dietary groups, one group received a control diet (CD, 16.5 % crude protein, CP) and the other one was fed a protein restricted diet (PRD_1 for Trial 1: 12.4 % CP; and PRD_2 for Trial 2: 10.0 % CP). At 100 kg of body weight (BW), five pigs per group and trial were slaughtered, while the remaining pigs were fattened on a standard finishing diet until reaching 160 kg BW before slaughter. No significant changes in growth, carcass and ham composition or backfat thickness due to decreased protein supply during the growing period were observed. However, the intramuscular fat content increased in *Longissimus thoracis et lumborum* (LTL) and ham muscles of PDR_2 pigs. Furthermore, the backfat fatty acid (FA) profile presented higher proportion of oleic and monounsaturated FAs in pigs fed PRD diets, while polyunsaturated FA decreased in PDR_2 pigs. The PRD_1 group exhibited higher redness and thawing water loss in LTL, while PDR_2 group showed greater redness, yellowness and thawing water loss values compared to the CD group. Overall, the results indicate that feeding protein-restricted diets during growing period did not have a substantial impact on growth or meat quality. The success of implementing such diets may be influenced by factors such as the protein restriction level and the animal management conditions.

1. Introduction

The production of the Iberian breed has traditionally focused on the production of high-quality dry-cured products. However, there has been an increase in the consumption of fresh meat in recent years, targeting the high-quality market as well. The intramuscular fat (IMF) content is a

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critical attribute that determines the organoleptic quality of meat. It is positively correlated with juiciness, tenderness, and overall acceptability of pork (Wood et al., 2008; Font-i-Furnols et al., 2012). The IMF content is influenced by several intrinsic factors such as muscle type and location within the muscle, age, gender and breed of the animals (Rosenvold and Andersen, 2003; Font-i-Furnols et al., 2019). However, one of the main factors affecting this trait is feeding (Lebret, 2008).

The production system based on the crossbreeding of Duroc boars with Iberian sows was developed to improve certain characteristics of the Iberian breed, such as its slower growth rate and higher fat deposition compared to most commercial breeds (Barea et al., 2011; Palma-Granados et al., 2017a). However, several studies have noted that crossbred pig meat tends to have lower IMF content compared to purebred Iberian meat (Ventanas et al., 2006). Furthermore, the primary regulation governing Iberian pig products (Standard Quality, BOE 2014) mandates specific requirements for Duroc x Iberian pigs reared in intensive management systems. These requirements include a minimum slaughter age of 10 months and a hot carcass weight of no less than 115 kg. However, the utilization of highly selected Duroc lines for improved growth rate, coupled with advancements in livestock management and feeding practices, often results in pigs reaching the commercial slaughter weight (~160 kg body weight, BW) several weeks prior to the mandatory age. To address these challenges, the consideration of specific Duroc lines selected for higher IMF content and slower growth rate for crossbreeding with Iberian sows is currently being explored. However, alternative approaches such as implementing nutritional strategies, including diets with reduced protein/lysine content, could also contribute to achieving these objectives.

Reducing the levels of dietary crude protein (CP) has been shown to result in several effects in pigs, including lower growth rate, higher fattening of carcasses and IMF content, and improve tenderness and juiciness of the meat (Teye et al., 2006; Alonso et al., 2010). However, these effects were not observed in other local heavy breeds such as Alentejano (Madeira et al., 2013), a breed which shares a close genetic relationship with Iberian breed (Muñoz et al., 2019). The effects of dietary protein content on growth, body composition and protein metabolism in Iberian pigs have been extensively studied at different growth stages (Nieto et al., 2012). This study has shown that pigs fed diets containing less protein than their ideal requirements tended to increase body and muscular fat deposition but decreased growth rate. Although other authors have examined the impact of low-protein diets on IMF and other meat quality traits in purebred Iberian pigs (Tejeda et al., 2020), to our knowledge, there are no similar studies in crossbred commercial Iberian pigs.

In this context, this study aims to assess the impact of dietary protein restriction during the growing period (from 30 to 100 kg BW) on growth, carcass and ham compositions, and meat quality traits of Duroc x Iberian crossbred pigs at the end of the growing period and at commercial slaughtering weight for heavy local pig production.

2. Material and methods

2.1. Animals, diets and experimental design

Animal welfare and management were carried out according to Spanish Ministry of Agriculture guidelines (RD1221/2009; RD159/2013) and the regulations of the Spanish Policy for the protection of animals used in research (RD 53/2013).

The barrows included in this study were the offspring of Duroc boars crossed with Iberian sows from the Torbiscal strain. These animals were born in an intensive farm specialized in the production of crossbred piglets and they were transferred for the growing and fattening phases to two farms with different housing conditions. Two separate trials were conducted over consecutive years. The Trial 1 was performed in the same intensive farm where pigs were born, equipped with modern facilities (similar to white pig production), including automated feeding, animal health monitoring and qualified personnel. The pigs were allocated to each dietary group, avoiding full siblings within the same group, and ensuring a balanced body weight across the groups. The pigs from each group were housed in 45 m^2 pens with a concrete floor, with half of the area allocated for indoor use and the other half for outdoor access. This trial was carried out between June and November. In Trial 2, the pigs were transferred to a traditional farm with a semi-intensive management and rustic facilities, which included outdated facilities and equipment, lacking automation in food supply systems, minimum health requirements and low skill employees). In this farm, the indoor area consisted of pens with concrete floors covering 20 m^2, while the outdoor area provided over 100 m^2 of dust floor. This trial was carried out between February and July.

A total of 28 pigs, with an average age of 82 days and a BW of 28.9 ± 3.4 kg started the experiment in Trial 1. In Trial 2, 33 pigs with an average age of 113 days and 38.9 ± 3.7 kg BW were included. In each trial, pigs were divided into two dietary groups. Each group was housed in pens where group feeding was controlled. One group was fed a control diet (CD), which provided an adequate level of protein to meet the growth requirements of Duroc x Iberian crossbred pigs (16.5 % CP and 0.80 % Lys; De Blas et al., 2013). The other group was fed a protein restricted diet: in Trial 1, the protein restricted diet (PRD_1) contained a 12.4 % CP and 0.53 % Lys, and in Trial 2, its equivalent diet (PRD_2) contained a 10.0 % CP and 0.43 % Lys. Animals were fed ad libitum. The

Table 1
Ingredients, nutrient and fatty acid composition of experimental diets.

Item	Growing diets[1]			Finishing diet
	CD	PRD_1	PRD_2	
Ingredients, g/kg as-fed basis				
Barley	300	443	470	405
Corn	200	200	200	200
Wheat	182	182	200	180
Wheat bran (23 % starch)	50	50	50	50
Soybean meal, 44 % CP	212	65	20	120
Animal fat	16	16	16	10
L-Lys 50	0.5	2.4	2.5	2.5
Calcium carbonate	12.8	12.2	12.2	8.0
Bicalcium phosphate	10.0	12.0	12.3	7.5
Sodium chloride	4	4	4	4
Binder	10	10	10	10
Vitamin and mineral premix[2]	3	3	3	3
Nutrient composition, g/100 g as-fed basis				
Dry matter	91.4	91.6	91.3	90.3
Crude protein	16.5	12.4	10.0	13.7
Crude fat	4.02	3.95	3.98	3.20
Crude fiber[3]	4.03	3.84	3.75	3,94
Lysine	0.80	0.53	0.43	0.70
Methionine[3]	0.25	0.19	0.17	0.21
ME, MJ/kg[3]	12.98	12.96	12.96	12.94
Fatty acid, g/100 g of total fatty acid [4]				
C16:0	18.5	19.2	19.0	17.8
C16:1	1.01	1.05	1.08	0.82
C18:0	5.71	5.72	5.82	4.67
C18:1	31.0	31.2	30.2	30.4
C18:2	38.3	38.3	38.5	40.9
C18:3	2.94	1.91	2.89	3.14
C20:0	0.34	0.32	0.32	0.34
C20:1	0.78	0.84	0.79	0.75

[1] CD = diet adequate in crude protein; PRD_1 and PRD_2 = protein-restricted diet in Trial 1 and Trial 2, respectively.

[2] Provided (per kg of premix): 1665,000 IU retinol as retinyl acetate, 333,000 IU cholecalciferol, 8 g DL-α-tocopherol as DL- α-tocopheryl acetate, 0.4 g menadione as menadione sodium bisulfite, 32 mg thiamine, 0.17 g riboflavin, 0.4 g pyridoxine, 10 g biotin, 0.7 g cyanocobalamin, 5 mg folic acid, 7 g nicotinic acid, 4 g D-pantothenic acid as calcium pantothenate, 7 g Cu as CuSO4·5H2O, 34 g Fe as FeSO4·7H2O, 5 g Mn as MnSO4·4H2O, 22 g Zn as ZnO, 0.15 g I as KI, and 0.1 g Se as Na2SeO3.

[3] Calculated (De Blas et al., 2013).

[4] Major FA.

ingredients and nutritional details are shown in Table 1. At the end of the growing period (approximately at 100 kg BW), 5 and 6–7 animals per diet from Trial 1 and Trial 2, respectively, were slaughtered. The remaining pigs continued the experiment and both dietary groups were fattened with a standard finishing diet (13.7 % CP and 0.70 % Lys, Table 1) in both trials, until they reached the commercial BW for local pig production (~160 kg). Subsequently, they were slaughtered. The feed intake cannot be individually measured and it was measured by each dietary group, instead. The average daily feed intake (ADFI) per group was calculated after the period was finished, dividing the feed amount provided during each period between number of pigs in each experimental group.

All the animals were slaughtered in the same slaughterhouse, with approximately the same age. Table 2 provides information on the number of animals, age and BW at the beginning and at the end of the growing and fattening periods of each experimental diet and trial. The recorded weights for each production phase (growing and fattening) were used to calculate the average daily gain during both phases (ADG_{GR} and ADG_{FT}, respectively).

2.2. Slaughter procedures, carcass measurements and tissue sample collection

Pigs were stunned with CO_2 and slaughtered by exsanguination at a commercial slaughterhouse and then scalded, skinned, eviscerated and the weight of the hot carcasses without renal pelvic fat was recorded. Each carcass was handled and processed according to standard commercial procedures by the Iberian pig industry. The weights of the untrimmed hams, shoulders and one fat-cleaned *Longissimus thoracis et lumborum* (LTL) muscle were also recorded. Several measurements were performed and samples taken for further analysis. Backfat thickness at the last rib (BFT), carcass length, and pH levels at 45 min and 24 h after slaughter on LTL (pH_{45} and pH_{24}) were measured. Samples of subcutaneous backfat at the level of the tailbone and half LTL without fat were vacuum-packed in nylon/polyethylene bags and frozen at −20 °C, 24 h after slaughter, for subsequent meat quality analyses. Besides, a piece of LTL was briefly exposed to liquid N_2 during approximately 20 s and then stored at −20 °C for myoglobin content and water loss determinations by centrifugal force (CFL). Furthermore, the left ham was frozen at −20 °C for computed tomography scanning and muscles dissection analyses.

Table 2
Number of animals (n), initial and final ages (day) and weights (kg), and ADFI (kg/day per pig) per dietary group and productive phase (growing or fattening) in each trial.

	Trial 1			
	Growing		Fattening	
	CD[1] (SD)	PRD_1 (SD)	CD (SD)	PRD_1 (SD)
n	13	15	8	10
Initial age	82 (2.4)	82 (2.4)	163 (2.4)	171 (2.4)
Initial weight	28.6 (2.66)	29.3 (3.33)	96.0 (5.17)	100 (11.3)
Final age	163 (2.4)	172 (2.4)	226 (11.0)	240 (2.4)
Final weight	97.0 (5.40)	99.2 (9.90)	151 (11.0)	157 (11.7)
ADFI	2.64	2.69	4.10	3.86
	Trial 2			
	Growing		Fattening	
	CD (SD)	PRD_2 (SD)	CD (SD)	PRD_2 (SD)
n	17	16	10	10
Initial age	113 (2.6)	113 (2.6)	188 (2.6)	202 (2.6)
Initial weight	38.8 (3.98)	38.9 (3.55)	99.0 (5.93)	106 (14.4)
Final age	188 (2.6)	202 (2.6)	273 (2.6)	266 (2.6)
Final weight	96.9 (8.8)	105 (13.5)	158 (7.0)	161 (12.4)
ADFI[2]	2.95	3.34	3.96	4.00

[1] CD = Control diet (16.5 % CP); PRD_1 and PRD_2 = Protein-restricted diet, respectively, in Trial 1 (12.4 % CP) and Trial 2 (10.0 % CP); SD = Standard deviation.
[2] ADFI = Average daily feed intake (kg/day per pig).

2.3. Computed tomography scanning and image analysis

The hams were thawed at 4 °C for one week before being scanned with the GE HiSpeed/Zx/I device (GE HealthCare, Madrid, ES). The scanning protocol used was 140 kV, 145 mA, with a slice thickness of 10 mm, a matrix size of 512×512, and field of view of 350 mm. The scanned images were analyzed using VisualPork software (Bardera et al., 2012), and the information obtained was consistent with the description provided by Font-i-Furnols et al. (2021). In summary, the volume associated with each Hounsfield (values of X-ray attenuation based on the type of tissue) value was calculated from the images. Tissues with Hounsfield values between 0 and +140 were considered lean, values between −149 and −1 as fat, and values between +141 and +1400 were classified as bones (Font-i-Furnols et al., 2015). The proportion of volume (%) of each tissue type in the ham was determined. The length and width of the hams were measured from the scout image. From the axial image, where the joint between the femur and the pelvic bones were visible, the fat thickness was measured perpendicular to the skin at the junction of *biceps femoris* and *tensor fasciae latae* muscles. In the same image, the subcutaneous fat area and the total area of the slice were also determined.

2.4. Meat and fat quality analysis

2.4.1. LTL samples

The instrumental colour of the fresh LTL muscle was measured at 24 h after slaughter (L^*_{24}, a^*_{24} and b^*_{24}) using a reflectance colorimeter (Konika Minolta CR-400, New Jersey, USA). A small steak of 1.5–2 cm of thickness was separated and exposed to oxygen through contact with air during 15 min. The light source chosen was Illuminant D65, with an 8 mm diameter aperture, and a 2° standard observer. Thawing water loss (TL) was calculated by taking the difference between the initial weight of the fresh LTL sample and the final weight after thawing. The thawed samples were then cooked in vacuum bags in a water bath at 70 °C for 1 h (Combes et al., 2004). The difference in weight before and after cooking was used to calculate the cooking losses (CL). For CFL, 1.5 g of minced sample, previously thawed, was wrapped in pre-weighed filter paper and centrifuged in tubes at 4000 rpm for 20 min at 16 °C in a Thermo Fisher Scientific centrifuge (Sorvall ST16R, Waltham, Massachusetts, USA), according to Tejerina et al. (2012). After centrifugation, the sample was removed and the filter paper was re-weighed. The CFL was calculated as the difference between the initial weight of the filter papers and the final weight of the wet filters. All measurements were performed in duplicates and expressed as a percentage.

The myoglobin content was determined following Alberti et al. (2005). A total of 2.5 g of minced sample was ground and homogenized with 10 mL of acetone and 0.25 mL of 37 % HCl. After 24 h, the mixtures were filtered, and the absorbance of the supernatant was detected at 512 nm against a blank using a spectrophotometer (Thermo Scientific, Sorvall, ST16R). The analyses were performed in duplicates and the results were presented as mg of myoglobin/g of LTL. The IMF was extracted and quantified according to the method described by Bligh and Dyer (1959). Shear force (SF) was measured in cooked meat using the Warner-Bratzler test with a blade and a Stable Microsystems TA.XT Plus instrument (Godalming, U.K.) following Honikel (1997). Eight pieces of meat measuring 3 × 1 × 1 cm (length, width and thickness) were cut perpendicular to the direction of muscle fibres. The average of the eight repeated measures was recorded. The shear force was expressed as *N*.

2.4.2. Ham muscles

The IMF percentages of the ham muscles including the *biceps femoris, semimembranosus* and *gluteus medius* (*gluteus medius* weight was not recorded in Trial 1) were determined using the near infrared FoodScan equipment (Foss Analytical, Hillerød, Denmark). Prior to analysis, muscles were removed from the ham after computed tomography scanning. The muscles were minced and homogenized and a frozen sample was retained for analysis.

2.4.3. Subcutaneous fatty acid profile

Lipids from subcutaneous fat samples were extracted using a microwave oven following the method described by De Pedro et al. (1997). Then, fatty acids (FA) were methylated by acid transesterification and analyzed by gas chromatography, using a Hewlett-Packard HP-4890 Series II gas chromatograph equipped with a split/splitless injector and a flame ionization detector. Separation was carried out on a Carbowax™ fused silica capillary column (30 m × 0.25 mm id; 0.25 µm film thickness; Ohio Valley) maintained at 200 °C for 25 min. Injector and detector temperatures were 250 °C. The carrier gas was nitrogen at 1.8 mL/min. Individual FAs were identified by comparison of their retention times with a standard mixture of 37 Component FAME Mix (Sigma–Aldrich, Supelco 37 Component FAME Mix- CRM47885, St. Louis, MO, USA) and expressed as g FA/100 g tissue.

2.5. Statistical analyses

To determine the significance of the differences ($P < 0.05$) observed between dietary treatments, analysis of variance (ANOVA) was performed with the individual phenotypes (data) of each animal for each trial, using the anova function and emmeans library in R studio software. The lineal model included as fixed effects the productive stage (after growing or after fattening), dietary protein content and their interactions. The BW was included as a covariate for carcass traits analyses. If there was a significant interaction between the main factors, means from each of the four weight-diet combinations were compared using one-way ANOVA. The results were expressed as least squares means. Statistical significance was assessed using Tukeýs t-test.

3. Results

The effects of the protein restricted diets supplied during the growing period on ADG are represented in Fig. 1. In Trial 1, there was a lower ADG_{GR} in PRD_1 compared to the CD group (776 vs 845 g/d, $P = 0.047$) but no significant differences were observed between diets in the fattening period (~850 g/d). Otherwise, in Trial 2, ADG_{FT} was significantly higher in the PRD_2 group compared to the CD group (852 vs 692 g/d, $P = 0.016$), while no significant differences were observed for ADG_{GR} (~760 g/d). However, the significant differences observed in ADG_{GR} in Trial 1 and ADG_{FT} in Trial 2 were not reflected in total ADG (from ~35–160 kg) in either of the trials (824 and 769 g/d for Trial 1 and 2, respectively; $P > 0.05$).

Table 3 shows the results for carcass and ham traits. There were no significant effects of the diets on carcass and premium cuts yield. However, some differences were observed in ham composition. The PRD_1 animals had a greater width of ham during the fattening period, while the PRD_2 pigs had a longer ham in both the growing and fattening periods.

Table 4 displays the effects of protein restriction on meat quality of the LTL and IMF content in ham muscles. Only minor differences between diets were observed. In the growing period of Trial 1, a slightly higher pH_{24} value was observed in the PRD_1 diet. In terms of colour traits measures at 24 h, here were also small differences, with higher redness observed in the PRD_1 pigs. However, no significant effects of diet on myoglobin content were detected. Concerning IMF of LTL, there were no diet-related differences in Trial 1, whereas in Trial 2, an increase in IMF was only found in the PRD_2 group after fattening. In terms of water losses, only the TL showed significant higher values in the restricted diets of both trials. Regarding cooking loss, an interaction effect was observed for Trial 1, where the PRD_1 LTL during growing period exhibited greater water losses compared to the control diet. Regarding the IMF of ham muscles, in Trial 1 there were no differences between experimental diets but in Trial 2 the PRD_2 pigs showed a higher percentage of IMF compared to the CD diet for all three muscles studied.

Table 5 presents the results of backfat fatty acids (FA) composition. In both trials, a significant increase in oleic acid (C18:1) and monounsaturated FA (MUFA) percentages was observed when the protein-restricted diets were supplied. Additionally, a decrease in arachidic acid (C20:0) proportion in PRD_1 group was observed only in growing phase and PRD_2 pigs presented lower proportions in linoleic (C18:2), linolenic (C18:3), C20:0 and polyunsaturated FA (PUFA).

4. Discussion

The reduction of protein in the diet has been evaluated as nutritional strategy to modify productive and meat quality traits of Duroc x Iberian crossbred pigs during the growing period (up to 100 kg), which appears to be a phase more sensitive to diet changes in CP than the fattening phase (Wang et al., 2019). Two consecutive trials were designed, in Trial 1 the pigs reached their slaughter weight under an intensive production system similar to the usual one currently applied for this type of pig, known as *cebo* (Horrillo et al., 2023). In Trial 2 the available space per pig was much larger, and therefore the production system was closer to the semi-intensive free-range system known as *cebo de campo* (Horrillo et al., 2023). The significant differences in management between the two trials are reflected in the standard error of the final weight after the

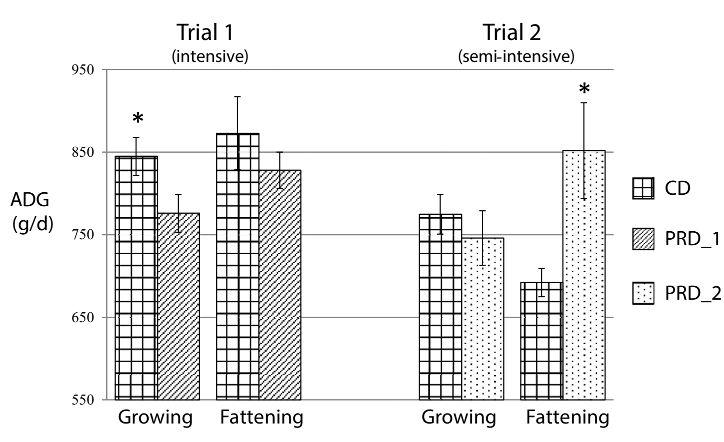

Fig. 1. Effects of dietary protein content on average daily gain (ADG) of Duroc x Iberian crossbred barrows in growing and fattening periods. CD = Control diet (16.5 % CP); PRD_1 and PRD_2 = Protein-restricted diet, respectively, in trial 1 (12.4 % CP) and trial 2 (10.0 % CP); BW = Body weight * $P < 0.05$.

Table 3
Effects of dietary protein content and productive phase on carcass and ham traits of Duroc x Iberian crossbred barrows[1].

Trial 1

Traits[2]	Growing		Fattening		*P*-value[4]	
	CD[3] (SE)	PRD_1 (SE)	CD (SE)	PRD_1 (SE)	Diet	Period
Carcass Traits						
Carcass, %	75.1 (0.71)	75.3 (0.71)	79.6 (0.56)	79.8 (0.50)	NS	***
Ham, %	26.8 (0.47)	26.2 (0.47)	24.8 (0.37)	24.4 (0.33)	NS	***
Shoulder, %	17.7 (0.44)	17.6 (0.44)	16.8 (0.34)	17.2 (0.31)	NS	NS
LTL, %	5.14 (0.225)	4.70 (0.225)	4.34 (0.178)	4.48 (0.159)	NS	*
Carcass Length, cm	81.5 (1.79)	82.4 (1.79)	91.0 (1.42)	92.3 (1.27)	NS	***
BFT, cm	–	–	5.03 (0.228)	4.58 (0.203)	NS	–
Ham traits						
Lenght, cm	72.6 (0.99)	74.9 (0.99)	81.9 (0.81)	81.7 (0.72)	NS	***
$Width_{max}$, cm	26.9 (0.53)[a]	26.1 (0.50)[a]	29.4 (0.40)[b]	32.1 (0.40)[c]	*	***
Fat thickness, mm	13.8 (1.60)	16.0 (1.60)	23.6 (1.26)	25.0 (1.13)	NS	***
SubFat area, cm^2	46.6 (6.74)	53.6 (6.74)	90.7 (5.32)	104 (4.8)	NS	***
Total area, cm^2	262 (8.3)[a]	244 (8.3)[a]	343 (6.5)[b]	361 (5.8)[b]	NS	***
Fat, %	22.7 (1.70)	24.8 (1.70)	31.0 (1.34)	33.3 (1.20)	NS	***
Muscle, %	63.5 (2.12)	59.5 (2.12)	52.4 (1.67)	53.3 (1.50)	NS	***
Bone, %	7.72 (0.275)	7.85 (0.275)	6.39 (0.217)	6.28 (0.194)	NS	***

Trial 2

Traits[2]	Growing		Fattening		*P*-value[4]	
	CD[3] (SE)	PRD_2 (SE)	CD (SE)	PRD_2 (SE)	Diet	Period
Carcass Traits						
Carcass, %	75.6 (0.58)	77.0 (0.63)	79.0 (0.49)	79.0 (0.49)	NS	***
Ham, %	25.6 (0.31)[c]	24.7 (0.33)[bc]	23.2 (0.26)[a]	23.8 (0.26)[ab]	NS	***
Shoulder, %	18.2 (0.28)	18.2 (0.30)	17.2 (0.23)	17.3 (0.23)	NS	**
LTL, %	4.49 (0.146)	4.48 (0.158)	4.13 (0.122)	3.90 (0.122)	NS	**
Carcass Length, cm	79.0 (1.21)	80.8 (1.31)	90.1 (1.02)	92.4 (1.02)	NS	***
BFT, cm	3.16 (0.243)	3.35 (0.263)	5.20 (0.204)	4.61 (0.204)	NS	***
Ham traits						
Lenght, cm	72.7 (0.09)	75.6 (0.09)	84.1 (0.69)	86.0 (0.70)	**	***
$Width_{max}$, cm	24.6 (0.59)	25.8 (0.59)	30.9 (0.49)	30.2 (0.48)	NS	***
Fat thickness, mm	14.5 (1.82)	19.5 (1.97)	26.0 (1.52)	24.7 (1.52)	NS	***
SubFat area, cm^2	46.3 (6.24)	52.6 (6.74)	100 (5.2)	99.1 (5.22)	NS	***
Total area, cm^2	238 (9.1)	246 (9.8)	351 (7.6)	358 (7.6)	NS	***
Fat, %	23.2 (1.66)	26.6 (1.79)	33.6 (1.39)	34.7 (1.39)	NS	***
Muscle, %	62.5 (1.50)	60.4 (1.62)	53.5 (1.25)	53.2 (1.39)	NS	***
Bone, %	8.26 (0.240)	8.26 (0.260)	6.64 (0.201)	6.56 (0.201)	NS	***

[1] In Trial 1, 5 pigs were slaughtered after the growing and 8–10 pigs after fattening periods, with an average final BW of 98.1 and 154.2 kg, respectively; in Trial 2, 6–7 were slaughtered after the growing and 10 pigs after fattening periods, with an average final BW of 101.1 and 159.4 kg, respectively.

[2] BFT = Backfat thickness at last rib; LTL = *Longissimus thoracis et lumborum* muscle; Fat thickness = Thickness measured perpendicularly to the skin at the junction between the femur and the pelvic bones; SubFat = Subcutaneous fat area measured in the same image as fat thickness; Total area = area of the whole ham in the same image as fat thickness.

[3] CD = Control diet (16.5 % CP); PRD_1 and PRD_2 = Protein-restricted diet, respectively, in Trial 1 (12.4 % CP) and Trial 2 (10.0 % CP); SE = Standard error.

[4] NS = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.[a–c]Means within a row with different superscripts differ (Diet x Period < 0.05).

growing period (Table 2) which was greater by 3.4 kg and 3.6 kg in Trial 2 for the control and restricted protein diets, respectively, highlighting the greater influence of environmental conditions on production in intensive and semi-intensive systems (García Casco et al., 2014). Additionally, the mean ADG_{GR} and ADG_{FT} values were 70 g/d and 181 g/d lower, respectively, for the Control group in Trial 2 compared with Trial 1.

The slaughter batches after the growing period were conducted approximately at 100 kg BW, which is not the commercial weight for this breed due to the low weight and scarce fat for proper dry curing of the premium cuts. Therefore, the number of slaughtered animals in this period was low (5–7 pigs). The slaughter weight (in growing and fattening period) was included as a fixed effect in the statistical analysis models. Since the objective of this experiment was to evaluate whether the effect of dietary protein restriction is maintained until the animal reaches commercial BW for heavy local pig production (~160 kg), this discussion focuses primarily on the effects of the diet in the fattening phase, with some comments on the few interactions found between the two production periods.

In this study, the reduced growth of pigs fed under protein restriction conditions, as reported by other authors in commercial breeds (Teye et al., 2006; Alonso et al., 2010), was partially observed. The growth of PRD_1 animals was reduced in the growing phase but not in the fattening phase. However, this reduction in growth was not sufficient to meet the minimum age requirements established by law for this crossbreed (10 months; Horrillo et al., 2023). In contrast, ADG_{FT} was clearly higher in PRD_2 pigs compared to the CD group, possibly due to compensatory growth. The effects of low protein diets on growth in crosses between cosmopolitan breeds are inconsistent. For instance, Teye et al. (2006) found lower growth in Duroc x (Large-White x Landrace) pigs fed 18 % CP diets compared to those fed 21 % CP. Similarly, the same crossbreed showed slower growth as dietary protein level decreased from 21.6 to 13.5 % (Suárez-Belloch et al., 2016). While Fabro et al. (2013) found no differences in ADG, total feed intake or total feed conversion when offering diets with 13 % CP to Duroc x Large White pigs compared to those fed 15 % CP. Shifting our focus to heavy local pig breeds, Rojas-Cano et al. (2014) found no differences in ADG when diets with 18, 14.3, and 11.4 % CP were provided to lower weight Duroc x Iberian pigs (60 to 76 kg). Barea et al. (2006) conducted a study on the effects of protein content and feeding level on the growth of Iberian pigs from 50 to 100 kg BW, and observed an increase in ADG with a low protein content in the diet until reaching the ideal CP content (95 g/kg dry matter), after which their growth was reduced. In an experimental design with diets containing lower protein but similar lysine content in Alentejano pigs, Madeira et al. (2013) did not find any differences on growth at 93 kg BW. This could be attributed to the lower protein requirements of these pigs, so the applied protein restriction might not have been enough to affect growth, as was observed in Iberian pigs within a similar weight range (Barea et al., 2006). In our study, the lack of significant effects on the ADG_{GR} of Trial 2 could be attributed to the higher feed intake by PRD_2 animals, which may have compensated for the lower protein content. At the same time, the greater protein restriction in this trial, compared to Trial 1, may have caused a compensatory growth effect in the fattening phase. Conversely, the lower protein restriction in trial 1

Table 4

Effects of dietary protein content and productive phase on meat quality of *Longissimus thoracis et lumborum* (LTL) muscle and intramuscular fat (IMF) content in ham muscles of Duroc x Iberian crossbred barrows[1].

Trial 1

Traits[2]	Growing		Fattening		P-value[4]	
	CD[3] (SE)	PRD_2 (SE)	CD (SE)	PRD_2 (SE)	Diet	Period
LTL muscle						
pH45	6.49 (0.091)	6.33 (0.091)	6.29 (0.069)	6.24 (0.064)	NS	NS
pH24	5.59 (0.035)[a]	5.73 (0.086)[b]	5.64 (0.034)[ab]	5.65 (0.024)[ab]	**	NS
L^*_{24}	–	–	45.8 (0.89)	45.6 (0.84)	NS	–
a^*_{24}	–	–	6.28 (0.291)	7.36 (0.261)	*	–
b^*_{24}	–	–	4.82 (0.252)	5.00 (0.222)	NS	–
Myoglobin, mg/g muscle	0.88 (0.089)	0.92 (0.089)	1.09 (0.070)	1.12 (0.063)	NS	*
IMF, %	4.24(0.831)	4.55 (0.831)	7.48 (0.598)	7.61 (0.594)	NS	***
Thawing Loss, %	4.75 (0.942)	7.77 (0.940)	5.52 (0.742)	8.07 (0.674)	**	NS
Cooking Loss, %	22.5 (1.04)[a]	25.9 (1.04)[b]	22.2 (0.82)[a]	22.1 (0.74)[a]	NS	*
CFL, %	34.5 (0.85)	34.9 (0.85)	33.1 (0.67)	33.8 (0.60)	NS	NS
Shear Force, N	55.0 (7.55)	65.8 (7.55)	53.1 (5.98)	45.5 (5.30)	NS	NS
IMF (%) of ham muscles						
Biceps femoris	4.05 (0.571)	4.08 (0.572)	5.70 (0.449)	5.92 (0.405)	NS	**
Semimembranous	3.32 (0.539)	3.64 (0.544)	4.34 (0.430)	4.60 (0.385)	NS	0.05
Gluteus medius	2.85 (0.425)	3.02 (0.469)	3.99 (0.331)	3.90 (0.304)	NS	NS

Trial 2

Traits[2]	Growing		Fattening		P-value[4]	
	CD[3] (SE)	PRD_2 (SE)	CD (SE)	PRD_2 (SE)	Diet	Period
LTL muscle						
pH45	6.32 (0.091)	6.32 (0.097)	6.02 (0.071)	6.17 (0.070)	NS	*
pH24	5.68 (0.035)	5.69 (0.031)	5.67 (0.030)	5.65 (0.030)	NS	NS
L^*_{24}	45.9 (0.89)	45.3 (0.96)	42.0 (0.71)	42.7 (0.70)	NS	***
a^*_{24}	7.08 (0.364)	7.60 (0.381)	8.10 (0.304)	8.19 (0.304)	NS	*
b^*_{24}	5.03 (0.333)	5.02 (0.363)	4.67 (0.278)	5.11 (0.281)	NS	NS
Myoglobin, mg/g muscle	1.26 (0.088)	1.25 (0.095)	1.26 (0.067)	1.35 (0.067)	NS	NS
IMF, %	3.86 (0.594)[a]	4.79 (0.639)[a]	5.40 (0.486)[a]	8.77 (0.490)[b]	***	***
Thawing Loss, %	7.75 (0.884)	10.48 (0.950)	7.55 (0.740)	9.81 (0.740)	**	NS
Cooking Loss, %	25.5 (0.85)[b]	23.4 (0.91)[ab]	22.3 (0.71)[a]	24.8 (0.71)[ab]	NS	NS
CFL, %	34.8 (1.15)	35.1 (1.24)	31.2 (0.96)[a]	33.2 (0.96)	NS	*
Shear Force, N	49.5 (3.73)[b]	34.0 (4.02)[a]	29.4 (3.14)[a]	35.4 (3.14)[a]	NS	*
IMF (%) of ham muscles						
Biceps femoris	3.83 (0.829)	4.99 (0.414)	4.23 (0.310)	4.52 (0.313)	*	NS
Semimembranous	3.69 (0.524)	4.88 (0.559)	3.28 (0.433)	4.65 (0.430)	*	NS
Gluteus medius	2.88 (0.351)	4.07 (0.381)	3.12 (0.292)	3.67 (0.289)	*	NS

[1] In Trial 1, 5 pigs were slaughtered after the growing and 8–10 pigs after fattening periods, with an average final BW of 98.1 and 154.2 kg, respectively; in Trial 2, 6–7 were slaughtered after the growing and 10 pigs after fattening periods, with an average final BW of 101.1 and 159.4 kg, respectively.

[2] pH_{45} and pH_{24} = pH measured at 45 min and 24 h; L^*_{24}, a^*_{24}, b^*_{24}, = Minolta colour parameters measured at 24 h; CFL = water losses by centrifuge.

[3] CD = Control diet (16.5 % CP); PRD_1 and PRD_2 = Protein-restricted diet, respectively, in Trial 1 (12.4 % CP) and Trial 2 (10.0 % CP); SE = Standard error.

[4] NS = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.[a-b]Means within a row with different superscripts differ (Diet x Period < 0.05).

was not sufficient to stimulate a response in the appetite of the PRD_1 animals, despite their slower growth.

Carcass and premium cuts yields remained unchanged when pigs were fed protein-restricted diets, which is consistent with findings from other studies (Fabro et al., 2013; Tous et al., 2014), including those in which ADG was previously affected (Teye et al., 2006; Suárez-Belloch et al., 2016). Similar findings were observed in Alentejano (Madeira et al., 2013), Cinta Senese (Sirtori et al., 2014) and Iberian (Barea et al., 2006) pigs. The lack of effects on growth and carcass traits may be attributed to the level of protein restriction. In fatty pigs, several studies indicate an excessive protein supply beyond actual requirements (Nieto et al., 2012; Rojas-Cano et al., 2014). In fact, diets are formulated to meet the demands of few specific essential amino acids, which often results in the supplementation of high levels of other amino acids that can be synthesized by the organism.

Protein restriction has been suggested as an effective nutritional approach to increase body fatness, particularly IMF, in commercial pig breeds (Alonso et al., 2010), Duroc crossbred (Teye et al., 2006) or heavy local pigs (Sirtori et al., 2014). In our study, IMF was high in *biceps femoris* followed by *semimembranosus* and *gluteus medius*, in agreement with Font-i-Furnols et al. (2019). Overall, our results partially support previous findings that reported greater fattening produced by this type of diet. The PRD_2 diet used in this study resulted in a higher IMF percentage in the three examined ham muscles and in LTL. The PRD_2 diet contained slightly less protein and lysine compared to PRD_1, and reduced lysine intake is related to increased intramuscular fat in pigs (Katsumata et al., 2005; Tous et al., 2014), even in the case of the Iberian breed (Palma-Granados et al., 2017b). However, the effectiveness of protein-restriction strategy to increase IMF is not entirely clear. In our study this effect was not observed in PRD_1. In the examination of the liver and LTL transcriptome of animals from Trial 1, there were no differentially expressed genes related to lipid metabolism between dietary groups, but some genes involved in immunological functions were identified in a previous study (Muñoz et al., 2021). Besides, Madeira et al. (2013) found no differences on IMF between diets in Alentejano pigs, suggesting that it could be because sufficient protein levels may have been supplied for the adipogenic characteristics of this local breed. Similarly, no differences in IMF were detected in Polish x Duroc pigs due to dietary protein content (Fiedorowicz et al., 2016).

In terms of other fatty depots, such as BFT or ham fat, no significant differences were detected. Similar findings were observed by Fabro et al. (2013), Tous et al. (2014), and Fiedorowicz et al. (2016). Suárez-Belloch et al. (2016) proposed that the lack of effect of protein restriction on ultrasonic BFT measurements may be attributed to the

Table 5
Effects of dietary protein content and productive phase on backfat fatty acids composition (g/100 g tissue) of Duroc x Iberian crossbred barrows[1].

Trial 1

Traits[2]	Growing		Fattening		*P*-value[4]	
	CD[3] (SE)	PRD_2 (SE)	CD (SE)	PRD_2 (SE)	Diet	Period
Total Fat g/100 g	89.1 (0.73)	88.8 (0.73)	88.3 (0.58)	87.8 (0.52)	NS	NS
C14:0	1.34 (0.041)	1.28 (0.042)	1.28 (0.039)	1.31 (0.028)	NS	NS
C16:0	23.8 (0.33)	23.3 (0.33)	23.7 (0.26)	23.5 (0.23)	NS	NS
C16:1	2.53 (0.107)	2.58 (0.107)	2.19 (0.084)	2.25 (0.075)	NS	**
C18:0	12.4 (0.46)	11.9 (0.46)	13.1 (0.37)	12.6 (0.33)	NS	0.07
C18:1	43.1 (0.73)	45.1 (0.73)	44.4 (0.58)	45.3 (0.51)	*	NS
C18:2	13.0 (0.58)	12.2 (0.58)	11.5 (0.46)	11.3 (0.41)	NS	*
C18:3	0.86 (0.040)	0.76 (0.040)	0.73 (0.032)	0.70 (0.029)	0.09	*
C20:0	0.20 (0.008)[b]	0.17 (0.008)[a]	0.22 (0.007)[b]	0.21 (0.006)[b]	*	*
C20:1	0.91 (0.052)	0.86 (0.052)	1.03 (0.041)	1.09 (0.037)	NS	***
SFA	38.1 (0.68)	37.1 (0.68)	38.7 (0.54)	38.0 (0.48)	NS	NS
MUFA	47.0. (0.76)	49.0 (0.76)	48.0 (0.60)	49.0 (0.54)	*	NS
PUFA	14.9 (0.64)	13.9 (0.64)	13.3 (0.51)	13.0 (0.46)	NS	*

Trial 2

Traits[2]	Growing		Fattening		*P*-value[4]	
	CD[3] (SE)	PRD_2 (SE)	CD (SE)	PRD_2 (SE)	Diet	Period
Total Fat g/100 g	89.7 (0.59)	89.0 (0.64)	87.9 (0.50)	88.0 (0.50)	NS	*
C14:0	1.37 (0.041)	1.35 (0.040)	1.40 (0.029)	1.37 (0.030)	NS	NS
C16:0	23.7 (0.33)	23.3 (0.35)	23.3 (0.27)	23.2 (0.27)	NS	NS
C16:1	3.06 (0.135)	3.11 (0.146)	2.50 (0.113)	2.56 (0.113)	NS	***
C18:0	11.7 (0.36)	11.9 (0.39)	11.7 (0.30)	11.8 (0.30)	NS	NS
C18:1	45.8 (0.43)	47.6 (0.47)	47.0 (0.64)	48.1 (0.36)	**	*
C18:2	10.6 (0.38)	9.15 (0.41)	10.0 (0.31)	9.34 (0.312)	**	NS
C18:3	0.67 (0.026)	0.57 (0.029)	0.61 (0.022)	0.55 (0.022)	**	NS
C20:0	0.26 (0.009)	0.24 (0.010)	0.22 (0.008)	0.21 (0.008)	*	***
C20:1	1.05 (0.047)	1.04 (0.051)	1.37 (0.040)	1.34 (0.040)	NS	***
SFA	37.5 (0.12)	37.2 (0.57)	37.0 (0.44)	36.9 (0.44)	NS	NS
MUFA	50.3 (0.50)	52.2 (0.54)	51.3 (0.41)	52.3 (0.41)	**	NS
PUFA	12.2 (0.44)	10.6 (0.47)	11.7 (0.36)	10.8 (0.36)	**	NS

[1] In Trial 1, 5 pigs were slaughtered after the growing and 8–10 pigs after fattening periods, with an average final BW of 98.1 and 154.2 kg, respectively; in Trial 2, 6–7 were slaughtered after the growing and 10 pigs after fattening periods, with an average final BW of 101.1 and 159.4 kg, respectively.
[2] SFA, MUFA, PUFA = sums of saturated, monounsaturated and polyunsaturated fatty acids, respectively.
[3] CD = Control diet (16.5 % CP); PRD_1 and PRD_2 = Protein-restricted diet, respectively, in Trial 1 (12.4 % CP) and Trial 2 (10.0 % CP); SE = Standard error.
[4] NS = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.[a-b]Means within a row with different superscripts differ (Diet x Period < 0.05).

differences in the BW of the animals. The wide variability in BW in our study may also mask any potential effect of diet on fatness traits. However, other authors, such as Barea et al. (2006) observed a small but significant effect of protein restriction, with enhanced fat deposition in pigs as the protein content in the diet decreased (from 145 to 95 g CP /kg dry matter).

The fatty acid composition plays a crucial role in various attributes of meat quality, including tissue firmness, product shelf life and eating quality (Wood et al., 2008). Previous studies have reported alterations in the FA profile due to lower protein content (Tous et al., 2014; Suárez-Belloch et al., 2016), characterized by an increase in MUFA percentage and a reduction in PUFA. In our study, the main finding in the analysis of FA composition in subcutaneous fat was a higher proportion of oleic acid and MUFA under protein restriction. These differences in oleic acid were already established at the end of the growing period in both trials and remained consistent at the end of the fattening period. The sum of PUFA also showed significant changes between diets, particularly at the end of the feeding treatment (growing period), with significantly lower percentages in the restricted diets in both trials. These changes were somewhat less pronounced at the final commercial weight. However, contradictory results have been reported by other authors. Madeira et al. (2013) found a minimal effect of restricted diets on subcutaneous adipose tissue of Alentejano pigs, with no statistical difference in oleic acid but lower PUFA percentage. Tejeda et al. (2020), in an evaluation of the effect of dietary protein level (12 % vs. 6.5 %) in Iberian pigs at 174 kg BW, did not observe differences in the proportions of oleic acid and MUFA of subcutaneous fat.

Modifications in feeding can also impact meat quality attributes beyond IMF content and FA profile. The results of this study indicate a low impact of protein restriction on quality traits such as texture, colour or water losses. Similarly, Madeira et al. (2013) did not observe any effect of the protein and lysine-restricted diet on quality traits in Alentejano pig, while Sirtori et al. (2014) found very clear undesirable effects, including higher b^* and L^* colour values and water losses, when applying high protein restriction to Cinta Sinese pigs. Teye et al. (2006) and Suárez-Belloch et al. (2016) also observed an increase in all colour parameters and tenderer meat in pigs fed low protein diets. In our study, meat from pigs on PRD_1 and PRD_2 diets showed higher thawing water loss, with similar trends for the other water losses traits, as well as increased redness and yellowness. These results suggest that animals fed protein restriction diets may enhance some characteristic appreciate by consumers, such as redder meat, but not improve overall quality of the meat.

5. Conclusions

The final slaughter weights achieved in these experiments, at 8–9 months of age, reveal the difficulty in finding a strategy to meet the requirements of the Standard Quality law for this type of intensive management, where the animals are typically Iberian pigs crossed with Duroc boars from modern selected lines. In addition, the effectiveness of this nutritional strategy in improving intramuscular fat is unclear, and further studies with a larger number of animals are needed. Furthermore, the production system or o farm management should be considered as factors before implementing such strategy. Nevertheless, this study indicates that the amount of protein in the diets intended for Duroc x Iberian crossbred pigs could be further reduced without negative effects on performance and carcass quality traits.

Ethics approval and consent to participate

No ethics approval was required for these experiments.

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CRediT authorship contribution statement

Patricia Palma-Granados: Visualization, Writing – original draft, Writing – review & editing. **Juan M. García-Casco:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Maria Font-i-Furnols:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **María Muñoz:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Miguel A. Fernández-Barroso:** Investigation. **Carmen Caraballo:** Investigation. **Adrián López-García:** Formal analysis, Investigation. **Albert Brun:** Investigation, Writing – review & editing. **Marina Gispert:** Investigation, Writing – review & editing. **Elena González-Sánchez:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

References

Alberti, P., Panea, B., Ripoll, G., Sañudo, C., Olleta, J.L., Hegueruela, I., Serra, X., 2005. Serie Ganadería Nº 3, Medición del Color. Estandarización de las Metodologías Para Evaluar la Calidad Del Producto (Animal Vivo, Canal, Carne y Grasa) en los Rumiantes. Monografías INIA, pp. 216–225.

Alonso, V., Campo, M.D., Provincial, L., Roncales, P., Beltran, J.A., 2010. Effect of protein level in commercial diets on pork meat quality. Meat Sci. 85, 7–14. https://doi.org/10.1016/j.meatsci.2009.11.015.

Bardera, A., Martínez, R., Boada, I., Font-i-Furnols, M., Gispert, M., 2012. VisualPork. Towards the simulation of a virtual butcher. In: Presentation at Farm Animal Imaging (FAIM) conference. Dublin, Ireland.

Barea, R., Nieto, R., Lara, L., García, M.A., Vílchez, M.A., Aguilera, J.F., 2006. Effects of dietary protein content and feeding level on carcass characteristics and organ weights of Iberian pigs growing between 50 and 100 kg body weight. Anim. Sci. 82, 405–413. https://doi.org/10.1079/ASC200645.

Barea, R., Nieto, R., Vitari, F., Domeneghini, C., Aguilera, J.F., 2011. Effects of pig genotype (Iberian v. Landrace× Large White) on nutrient digestibility, relative organ weight and small intestine structure at two stages of growth. Animal 5, 547–557. https://doi.org/10.1017/S1751731110002181.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.

BOE, 2014. BOE-A-2014-318. Royal decree 4/2014, of 10 January, by Which the Quality Standard for Meat, Ham, Shoulder and Cane Of Iberian loin is Approved. Boletín oficial del Estado (Spain).

Combes, S., Lepetit, J., Darche, B., Lebas, F., 2004. Effect of cooking temperature and cooking time on Warner-Bratzler tenderness measurement and collagen content in rabbit meat. Meat Sci. 66, 91–96. https://doi.org/10.1016/S0309-1740(03)00019-6.

De Blas, C., Mateos, G.G., García Rebollar, P., 2013. Tablas FEDNA de Composición y Valor Nutritivo de Alimentos Para la Fabricación de Piensos Compuestos, 2rd ed. (In Spanish.) Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain.

De Pedro, E., Casillas, M., Miranda, C.M., 1997. Microwave oven application in the extraction of fat from the subcutaneous tissue of Iberian pig ham. Meat Sci. 45, 45–51. https://doi.org/10.1016/S0309-1740(96)00097-6.

Fabro, C., Sgorlon, S., Guiatti, D., Stefanon, B., Susmel, P., 2013. Productive response of Duroc x Large white and commercial Hybrid x Large white crosses fed high and low protein diets. Ital. J. Anim. Sci. 12, e82. https://doi.org/10.4081/ijas.2013.e82.

Fiedorowicz, E., Sobotka, W., Stanek, M., Drazbo, A., 2016. The effect of dietary protein restriction in finishing pigs on the fat content, fatty acid profile, and atherogenic and thrombogenic indices of pork. J. Elem. 21, 3.

Font-i-Furnols, M., Tous, N., Esteve-Garcia, E., Gispert, M., 2012. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. Meat Sci. 91, 448–453. https://doi.org/10.1016/j.meatsci.2012.02.030.

Font-i-Furnols, M., Carabús, A., Pomar, C., Gispert, M., 2015. Estimation of carcass and cuts composition from computed tomography images of growing live pigs of different genotypes. Animal 9, 166–178. https://doi.org/10.1017/S1751731114002237.

Font-i-Furnols, M., Brun, A., Gispert, M., 2019. Intramuscular fat content in different muscles, locations, weights and genotype-sexes and its prediction in live pigs with computed tomography. Animal 13, 666–674. https://doi.org/10.1017/S1751731118002021.

Font-i-Furnols, M., García-Gudiño, J., Izquierdo, M., Brun, A., Gispert, M., Blanco-Penedo, I., Hernández-García, F.I., 2021. Non-destructive evaluation of carcass and ham traits and meat quality assessment applied to early and late immunocastrated Iberian pigs. Animal 15, 100189. https://doi.org/10.1016/j.animal.2021.100189.

García-Casco, J.M., Muñoz, M., Silió, L., Rodríguez, C., 2014. Genotype by environment interaction for carcass traits and intramuscular fat content in heavy Iberian pigs fattened in two different free-range systems. SJAR 12, 388–395. https://doi.org/10.5424/sjar/2014122-4840.

Honikel, K.O., 1997. Reference methods supported by OECD and their use in Mediterranean meat products. Food Chem. 59, 573–582. https://doi.org/10.1016/S0308-8146(97)00002-2.

Horrillo, A., Gaspar, P., Muñoz, Á., Escribano, M., González, E., 2023. Fattening Iberian pigs indoors vs. outdoors: production performance and market value. Animals 13, 506. https://doi.org/10.3390/ani13030506.

Katsumata, M., Kobayashi, S.I., Matsumoto, M., Tsuneishi, E., Kaji, Y., 2005. Reduced intake of dietary lysine promotes accumulation of intramuscular fat in the Longissimus dorsi muscles of finishing gilts. Anim. Sci. 76, 237–244. https://doi.org/10.1111/j.1740-0929.2005.00261.x.

Lebret, B., 2008. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. Animal 2, 1548–1558. https://doi.org/10.1017/S1751731108002796.

Madeira, M.S., Costa, P., Alfaia, C.M., Lopes, P.A., Bessa, R.J., Lemos, J.P., Prates, J.A., 2013. The increased intramuscular fat promoted by dietary lysine restriction in lean but not in fatty pig genotypes improves pork sensory attributes. J. Anim. Sci. 91, 3177–3187. https://doi.org/10.2527/jas.2012-5424.

Muñoz, M., Bozzi, R., Garcia-Casco, J.M., Núñez, Y., Ribani, A., Franci, O., García, F., Ovilo, C., et al., 2019. Genomic diversity, linkage disequilibrium and selection signatures in European local pig breeds assessed with a high density SNP chip. Sci. Rep. 9, 1–14. https://doi.org/10.1038/s41598-019-49830-6.

Muñoz, M., Fernández-Barroso, M.A., López-García, A., Caraballo, C., Nuñez, Y., Óvilo, C., González, E., García-Casco, J.M., 2021. Consequences of a low protein diet on the liver and longissimus dorsi transcriptome of Duroc× Iberian crossbred pigs. Animal 15, 100408. https://doi.org/10.1016/j.animal.2021.100408.

Nieto, R., Lara, L., Barea, R., García-Valverde, R., Aguinaga, M.A., Conde-Aguilera, J.A., Aguilera, J.F., 2012. Response analysis of the Iberian pig growing from birth to 150 kg body weight to changes in protein and energy supply. J. Anim. Sci. 90, 3809–3820. https://doi.org/10.2527/jas.2011-5027.

Palma-Granados, P., Haro, A., Lara, L., Aguilera, J.F., Nieto, R., Seiquer, I., 2017a. Differences on meat colour and composition between Landrace×Large White and Iberian pigs under identical nutritional and management conditions. Anim. Prod. Sci. 58, 2132–2142. https://doi.org/10.1071/AN16375.

Palma-Granados, P., Haro, A., Seiquer, I., Lara, L., Aguilera, J.F., Nieto, R., 2017b. Similar effects of lysine deficiency in muscle biochemical characteristics of fatty and lean piglets. J. Anim. Sci. 95, 3025–3036. https://doi.org/10.2527/jas.2017.1364.

Rojas-Cano, M.L., Ruiz-Guerrero, V., Lara, L., Nieto, R., Aguilera, J.F., 2014. An estimation of the protein requirements of Iberian× Duroc 50: 50 crossbred growing pigs. J. Anim. Sci. 92, 1595–1603. https://doi.org/10.2527/jas.2013-6947.

Rosenvold, K., Andersen, H.J., 2003. Factors of significance for pork quality - a review. Meat Sci. 64, 219–237. https://doi.org/10.1016/S0309-1740(02)00186-9.

Sirtori, F., Crovetti, A., Acciaioli, A., Pugliese, C., 2014. Effect of dietary protein level on carcass traits and meat properties of Cinta Senese pigs. Animal 8, 1987–1995. https://doi.org/10.1017/S1751731114002006.

Suárez-Belloch, J., Latorre, M.A., Guada, J.A., 2016. The effect of protein restriction during the growing period on carcass, meat and fat quality of heavy barrows and gilts. Meat Sci. 112, 16–23. https://doi.org/10.1016/j.meatsci.2015.10.006.

Tejeda, J.F., Hernández-Matamoros, A., Paniagua, M., González, E., 2020. Effect of free-range and low-protein concentrated diets on growth performance, carcass traits, and meat composition of Iberian pig. Animals 10, 273. https://doi.org/10.3390/ani10020273.

Tejerina, D., García-Torres, S., Cava, R., 2012. Water-Holding capacity and instrumental texture properties of M. Longissimus Dorsi and M. Serratus Ventralis from Iberian pigs as affected by the production system. Livest. Sci. 148, 46–51. https://doi.org/10.1016/j.livsci.2012.05.005.

Teye, G.A., Sheard, P.R., Whittington, F.M., Nute, G.R., Stewart, A., Wood, J.D., 2006. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Sci. 73, 157–165. https://doi.org/10.1016/j.meatsci.2005.11.010.

Tous, N., Lizardo, R., Vila, B., Gispert, M., Font-i-Furnols, M., Esteve-Garcia, E., 2014. Effect of reducing dietary protein and lysine on growth performance, carcass characteristics, intramuscular fat, and fatty acid profile of finishing barrows. J. Anim. Sci. 92, 129–140. https://doi.org/10.2527/jas.2012-6222.

Ventanas, S., Ventanas, J., Jurado, Á., Estévez, M., 2006. Quality traits in muscle biceps femoris and back-fat from purebred Iberian and reciprocal Iberian× Duroc crossbred pigs. Meat Sci. 73, 651–659. https://doi.org/10.1016/j.meatsci.2006.03.009.

Wang, Y.M., Yu, H.T., Zhou, J.Y., Zeng, X.F., Wang, G., Cai, S., Huang, S., Zhu, Z.P., Tan, J.J., Johnston, L.J., Levesque, C.L., Qiao, S., 2019. Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. Anim. Feed Sci. Technol. 256, 114256 https://doi.org/10.1016/j.anifeedsci.2019.114256.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S. I., Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78, 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019.