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Non-destructive evaluation of carcass and ham traits and meat quality assessment applied to early and late immunocastrated Iberian pigs

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ABSTRACT

Castration is a common practice in Iberian pigs due to their advanced age and high weight at slaughter. Immunocastration (**IC**) is an alternative to surgical castration that influences carcass and cut fatness. These traits need to be evaluated *in vivo* and *postmortem*. The aims of the present work were (a) to determine the relationship between ham composition measured with computed tomography (**CT**) and *in vivo* ultrasound (**US**) and carcass fat thickness measurements, (b) to apply these technologies to early (**EIP**) and late (**LIP**) immunocastrated Iberian pigs in order to evaluate carcass fatness and ham tissue composition and (c) to assess meat quality on these animals and to find the relationships between meat quality traits (namely, intramuscular fat (**IMF**)) and fat depot thicknesses. For this purpose, 20 purebred Iberian pigs were immunocastrated with three doses of Improvac ®, at either 4.5, 5.5 and 9 or 11, 12 and 14 months of age (EIP or LIP; respectively; $n = 10$ each) and slaughtered at 17 months of age. Fat depots were evaluated *in vivo* by US, in carcass with a ruler and in hams by CT. Carcass and cut yields, loin meat quality and loin acceptability by consumers were determined. Also, IMF was determined in the loin and three muscles of the ham. Carcass weight was 14.9 kg heavier in EIP vs LIP, and loin backfat thickness (US- and ruler-measured) was also greater in EIP. Similarly, CT-evaluated ham bone and fat contents were greater and smaller for EIP vs LIP, respectively. Loin and ham IMF were also greater in EIP, but the other meat quality parameters were similar. The acceptability of meat by consumers was high and it did not differ between IC protocols. Correlations between several fat depots measured with the different technologies were high. In conclusion, all these technologies allowed fat depot measurements, which were highly correlated despite being obtained at different anatomical locations.

Implications

The quantity (and quality) of fat in Iberian pig carcasses is an important characteristic. This work shows that measurements obtained by ultrasounds in live pigs, by a ruler in carcasses and by computed tomography in hams, are highly correlated despite their different nature and anatomical locations. Also, these technologies can be applied to immunocastrated Iberian pigs so that the information obtained could be used to optimize the vaccination protocol to reach the target carcass and meat quality traits.

Introduction

The high quality of Iberian pig products is mainly due to the meat's fat content and composition. Hams are their most valuable cuts and must reach high standards of sensory quality and fat content and composition. Imaging technologies such as computed tomography (**CT**) and ultrasound (**US**) can be used in livestock. US is used in live animals to determine fat thickness, muscle area and intramuscular fat content (**IMF;** Scholz et al., 2015). In the CT procedure, X-rays are more or less attenuated depending on tissue density. From these attenuation values, measured in Hounsfield units (HU), and by means of reconstruction algorithms, a 3D image can be obtained (Font i Furnols et al., 2009). This technology has been used to evaluate live pigs' body composition (Kolstad et al., 1996), carcass and cut composition (Font-i-Furnols et al., 2009), and IMF (Font-i-Furnols et al., 2019). The correlation between fat thicknesses obtained with US and CT in live pigs has been reported to be high, as well as the correlation between thicknesses

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determined by US in live pigs and by a reflectance-based device (Fat-O-Meat'er, Frontmatec, DK) in carcasses (Lucas et al., 2017). However, the relationships among loin and ham US measures and CT ham tissue composition are unknown.

An important sensory quality parameter in meat from entire male pigs is boar taint. This unpleasant odour and flavour perceived in fresh meat and cured meat products could affect acceptability by consumers (Font-i-Furnols, 2012) and is mainly due to androstenone and skatole. Androstenone has a urine-like odour, is synthesized in the testes and is most likely found in older and heavier animals, like the Iberian or the heavy Italian pigs (Bonneau et al., 2018). Skatole content has a faecal odour, is synthesized in the hind gut and is more related to the dietary composition. As well as androstenone, skatole level increases at puberty (Babol et al., 2004). Consequently, Iberian pigs are routinely castrated to avoid boar taint and hierarchical fighting, and also pregnancies in extensive systems. Surgical castration in piglets has been banned in some countries unless anaesthesia and analgesia are applied. An alternative method is immunocastration (**IC**), a vaccination against gonadotrophin-releasing hormone. In conventional pig breeds, two doses of the vaccine are recommended. The first dose primes the immune system, and the full effects take place after the second dose, for example, decreased testes size, sexual activity and boar taint, and increased daily gain, feed intake and fatness (Dunshea et al., 2001; Turkstra et al., 2002; Fàbrega et al., 2010). Three-dose IC is applied for pigs with long life cycles, like the Iberian pig, to ensure efficacy (Allison et al., 2009; Martinez-Macipe et al., 2016).In some Iberian pigs, two doses are given because they are intensively reared and slaughtered much earlier than extensively reared ones (Seiquer et al., 2019). The effect of IC on final carcass composition can be modified by adjusting the vaccination schedule, that is, applying an early or late vaccination or modifying the dosing interval, so the animal has more time for fattening after the second (effective) dose. Early vaccination resulted in fatter carcasses (Andersson et al., 2012) or did not (Aluwé et al., 2016). Moreover, early IC resulted in lower saturated fatty acid content than late IC (Zoels et al., 2020). Thus, modifications in the vaccination schedule might facilitate the obtainment of a desired quality in the final product.

The objectives of the present work were (a) to determine the relationship between fat thickness measurements (evaluated *in vivo* by US and *post mortem* by a ruler) and ham composition evaluated by CT, (b) to apply these technologies to early (**EIP**) or late (**LIP**) immunocastrated Iberian pigs in order to evaluate carcass fatness and ham tissue composition, meat quality and the acceptability of pork loins by consumers and (c) to assess meat quality in EIP and LIP and to determine the relationship between meat quality traits (IMF) and the measurements of different fat depots.

Material and methods

Measurements in live pigs

Twenty purebred Iberian (Retinto strain, Valdesequera line) male pigs immunocastrated with three doses of the vaccine Improvac® (Zoetis, Madrid, Spain) were used in the present study. Three IC doses were applied at the age of 4.5, 5.5 and 9 months (pre-pubertal IC; early immunocastrated pigs; EIP; $n = 10$) and at 11, 12 and 14 months of age (pre-finishing IC; late-immunocastrated pigs; LIP; $n = 10$).These 10 LIP were fed *ad libitum* for 15 days (nutritional "flushing"; "flushed" LIP) right after the 3rd vaccination (approximately 3 months before slaughter) with the aim of improving IC efficacy. The 20 pigs of the present study were randomly selected *post-mortem* (from a larger study) from 25 of 39 EIP and 23 of 23 "flushed" LIP having fully atrophic testicles, that is, with a bilateral mean weight smaller than 150 g, thus excluding insufficiently responders in EIP group, according to previous studies(Hernández-García et al., 2018). All pigs were reared in extensive conditions. Piglets were placed in a big outdoor corral after weaning and later moved to large paddocks. Pigs were housed together until IC treatments were started. Thereafter, EIP and LIP were raised separately but in enclosures of the same type and space allowance. Pigs were in-group fed with commercial pig concentrates (see Supplementary Material S1) according to age, at a progressive rate from 1 to 1.8 kg/head/day from weaning to 115 kg of BW or until 13 months of age (whichever came first). Afterwards (except for the already mentioned 15-day flushing period in "flushed" LIP), the ration was progressively increased up to 4 kg/head/day. This ration was maintained for both groups during the last 2 months until approximately 17 months old with a BW of at least 160 kg.

The day before slaughter, echography was performed with a US scanner (ALOKA-500SSD, ALOKA Inc., Tokyo, Japan) and a 12-cm, 3.5 MHz probe. Total inner, middle and outer backfat thicknesses (excluding the skin) were measured at the middle of the transversal section of the *longissimus thoracis* (**LT**) muscle at the 10th rib level (intercostal space between 10th and 11th rib). On the ham region, fat thickness was measured over the *gluteus medius* (**GM**) muscle (above the *gluteus profundus*) at the level of the ischiatic spine (bone protuberance over the *acetabulum* of the pelvis). Gluteal muscle depth was recorded at the same level.

Slaughter and quality measurements

Slaughtering of all the experimental pigs was done in several slaughter days with the same pre-slaughter fasting and management protocol. When pigs reached the target age, pigs were weighed the day prior to slaughter, then given their last feed (in the morning) and then transported to a commercial abattoir in Castuera (155 km away; Badajoz, Spain), where they were slaughtered the following morning. Carcass weight was recorded and, together with the BW, used to calculate carcass yield (%). In addition, backfat thickness was measured with a ruler at the level of the 10th rib and at the last rib level. Also, the thickness of the three fat layers (inner, middle and outer) at these two levels was recorded. The ultimate pH was determined at the last rib level of the LT muscle at 24 h *post-mortem* with the pH-meter (Crison model MicropH 2201, Crison Instruments S.A., Alella, Spain). Also, on this muscle and at the same level, colour was measured at 24 h *post-mortem* after 30 min of blooming with the Chroma meter CR400 (Konica Minolta Holdings Inc., Chiyoda, Japan) and L*, a* and b* values were obtained.

The left carcass was cut in pieces and the weights of the prime cuts (ham, shoulder, loin (deboned and fat-trimmed), tenderloin, as well as "secreto" and "presa" (Martín, 2013)), from *latissimus dorsi* and *serratus ventralis*, respectively, were recorded. A piece of the loin region (including loin muscle and surrounding bone, fat and skin tissues) was cut from the left carcass between the 10th and the last rib, and the area of the LT muscle was determined in both sides, by tracing the perimeter in a transparent plastic sheet and subsequently using a planimeter (LI-3100C Area Meter, LI-COR Inc., Bad Homburg, DE). Then, a 2.5 cm-thick slice followed by two 5 cm-thick slices was cut, vacuum packed and frozen at −20 °C for further analyses (texture, IMF and consumers study). The last two pieces were transported to IRTA-Monells (Girona, Spain) together with the left hams, previously packed and frozen at −20 °C. After CT ham scanning, the *biceps femoris* (**BF**) and *semimembranosus* (**SM**) muscles were dissected and weighed. Samples of BF, SM and GM were vacuum-packed and frozen at −20 °C for further IMF analysis.

The IMF content of the LT, BF, GM and SM muscles was determined by means of the near-IR FoodScan™ equipment (Foss Analytics, Hillerød, DK) at an 850–1050 nm wavelength (RMSE = 0.17%, Font-i-Furnols et al., 2012a). Loin marbling was evaluated by two trained operators using the National Pork Producers Council scale (NPPC 1999, National Pork Board, 2010). Cooking losses were calculated by weighing after placing a 5 cm-thick slice of loin in a double boiler at 75 °C for 45 min. For texture analysis, the cooked loin was cut in a minimum of eight pieces of $1 \times 1 \times 2$ cm^3, sectioned perpendicularly to the muscle

fibres, and the average value was calculated. Texture profile analysis was performed with the Texturometer TA-XT2i (Stable Micro Systems Ltd., Surrey, UK) applying compression of 80% strain. From this test, the hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience were calculated. Also, a Warner–Bratzler test was performed with the same texturometer and the shear force was determined.

Computed tomography scanning and image analysis

The hams were thawed at 4 °C 1 week before the scanning. Hams were scanned with the GE HiSpeedZx/I CT equipment (GE HealthCare, Madrid, ES). Scanning protocols were similar to those applied for carcasses by Font-i-Furnols et al. (2009; settings:140 kV, 145 mA, 10 mm thick, 512 × 512 matrix), but adapting the displayed field of view to 350 mm. Image analysis was performed with the software VisualPork (Bardera et al., 2012) and the distribution of volume by HU was determined. Fat was considered as the volume between HU-149 and HU-1, lean as the volume between HU 0 and HU + 140 and bone as the volume between HU + 141 and HU + 1400 (Font-i-Furnols et al., 2015).Water losses due to freezing and thawing could have affected the volumes, mainly those of lean. However, the procedure was the same for all the animals, and carcass water losses were not significantly different between treatments (EIP: 3.7 ± 1.3%; LIP: 3.2 ± 0.5%; *P*-value = 0.31). The proportion of each tissue was also calculated. Furthermore, lean and fat volume were converted into lean and fat weight by means of the density value 1.04 g/cm^3 (Daumas and Monziols, 2018) and 0.9196 g/cm^3 (Farvid et al., 2005), respectively.

In the exploratory image, the length and width of the hams were calculated. From the axial image at the level of the joint between the femur and pelvic bones (Fig.1a), the fat thickness (excluding the skin) was obtained perpendicularly to the skin at the junction between the BF and *tensor fasciae latae* muscles. Furthermore, subcutaneous fat area (excluding skin) and the area of the whole section of the image were measured. Fat thickness was also measured in the axial image obtained just beside the patella in the caudal direction, at the junction between BF and SM muscles (Fig.1b). Fat and whole area of the section were also calculated in this image.

Consumer study

Sample preparation

The frozen loins were cut into 1.5 cm thick slices. Loins were thawed at 4 °C for 24 h before consumer evaluation and cooked in an oven (FAGOR Innovation Class A; Fagor Electrodomésticos, S.Coop., Mondragón, Spain) pre-heated at 200 °C without turning the slices until reaching 76 °C internally, measured using thermocouple K probes (Beamex Oy Ab, Pietarssari, Finland) inserted into the steak centre. After cooking, subcutaneous fat was trimmed keeping only 2 mm and steaks were cut perpendicular to the fat into 1.5×4 cm pieces. Each piece was wrapped in aluminium foil, coded with a 3-digit number and kept warm in a heater to avoid cooling before being served to consumers.

Consumer selection and experimental design

One hundred consumers were recruited in Barcelona. Consumers were selected to mimic the Spanish distribution by gender and sex (men = 47, women = 53; <25 years old = 10, 21–40 years old = 27, 41–60 years old = 41, >60 years old = 22). Five sessions (n = 20 consumers/session) were conducted. Two fresh loin samples (one of each treatment) were presented monadically to each consumer. Each loin was evaluated by 10 consumers. The order of presentation of samples was changed between consumers of the same session. Each consumer rated overall acceptability, tenderness, odour and flavour using a 9-point category scale from 1 ('dislike extremely') to 9 ('like extremely') without the intermediate level 5 ('neither like nor dislike'). Consumers were asked to eat unsalted and toasted bread and rinse their mouths out with water before tasting each sample.

Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute Inc., Cary, NC, USA). Correlations between the different measurements obtained with US, a ruler and CT, and between fatness and IMF were obtained by the CORR procedure. To study differences between IC protocols (treatment effect) on the carcass and ham composition and on meat quality characteristics, a GLM procedure was used. The statistical model included treatment as a fixed effect. In the consumer study, the average consumer scores for each animal were obtained and the model included treatment as fixed effect and the pair of samples evaluated by the same consumers as random effect (Supplementary Material S2). Differences between least square means were obtained with the *t*-test, and $P < 0.05$ was considered significant.

Results

Relationship between different non-destructive measures of carcass quality

Correlations between the measurements obtained in the animals *in vivo*, in the carcass and ham, using different technologies were all significant ($P < 0.01$) (Table 1). Correlations were high, despite the fact that some measurements were lengths, other were areas and proportions, and some measurements were taken in the loin and ham region. It is worth noting that correlations between ham fat area (CT) and loin

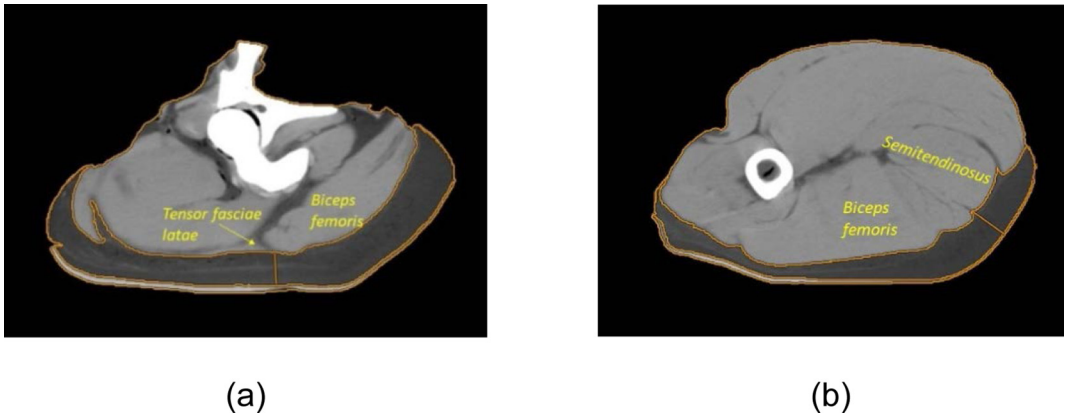

Fig. 1. Area and fat thickness measurements in the computed tomography image of a pig ham at the level of the joint between the femur and pelvic bones (a) and in the computed tomography image obtained just beside the patella in the caudal direction (b).

Table 1
Correlations between ultrasound measurements taken in live pigs in the loin and ham regions, loin measurements taken in the carcass with a ruler and ham measurements taken in the ham by computed tomography ($n = 20$).[1]

	Ultrasound		Ruler	
	Loin fat thickness	Ham fat thickness	Fat thickness 10th rib	Fat thickness last rib
Computed tomography (ham)				
Fat thickness	0.74	0.80	0.75	0.84
Fat area	0.83	0.71	0.81	0.88
Fat weight	0.79	0.76	0.83	0.84
Lean weight	−0.65	−0.61*	−0.58	−0.71
Ham composition				
Fat, %	0.85	0.81	0.83	0.89
Lean, %	−0.83	−0.79	−0.82	−0.88
Bone, %	−0.76	−0.80	−0.77	−0.80
Ultrasounds				
Loin fat thickness		0.78	0.87	0.91
Ham fat thickness	0.78		0.77	0.74

[1] All the correlations were significant ($P < 0.001$) except those with * which had a $P < 0.01$.

Table 3
Influence of early (pre-pubertal, EIP) and late (pre-finishing, LIP) immunocastration of pigs on the carcass and loin characteristics measured with a ruler ($n = 10$ each).

	Immunocastration		RMSE	*P*-value
	Pre-pubertal	Pre-finishing		
BW (kg)	161.95	161.35	5.94	0.824
Age (months)	16.68	16.88	0.45	0.337
Carcass weight (kg)	135.83	120.98	5.76	<0.001
Carcass yield (%)	83.90	74.96	2.22	<0.001
Carcass length (cm)	81.00	81.00	2.24	1.000
10th rib level				
Loin area (cm^2)	28.54	28.97	4.52	0.841
Fat thickness (cm)				
Total	8.36	5.58	1.47	<0.001
Inner	1.47	1.22	0.47	0.250
Middle	5.23	3.01	1.13	<0.001
Outer	1.66	1.35	0.23	0.007
Last rib level				
Loin area (cm^2)	31.18	34.58	3.83	0.070
Fat thickness (cm)				
Total	5.97	4.46	0.97	0.003
Inner	1.76	1.35	0.51	0.091
Middle	2.94	1.99	0.58	0.002
Outer	1.27	1.12	0.35	0.352

fat thickness (US and ruler) were higher ($r = 0.80$) than the correlation between fat area (CT) and fat thickness (US) when both were taken in the ham ($r = 0.71$). The lowest correlations were those between US- and ruler-measured fat thicknesses and the CT-estimated lean weight ($r \leq -0.71$). Correlations between fat measurements were positive, thus suggesting that fat increases proportionally in most body regions. Correlations between fat and lean, or between fat and bone proportion, were negative, indicating that fat deposition increases as lean and osseous accretion rates decrease.

Quality measurement in live pigs and carcasses

Table 2 depicts loin and ham US measurements for each IC protocol, with no treatment differences for loin area and gluteal depth. However, total loin backfat was 1.7 mm thicker in EIP vs LIP, mainly due to the middle fat layer, which was 1.4 mm thicker in EIP. Similarly, ham fat cover was significantly greater (0.9 mm thicker) in EIP. By experimental design, age at slaughter was not significantly different between treatments (16.7 vs 16.9 months in EIP and LIP, respectively; Table 3). Moreover, BW was not different between treatments, but, since carcass was 14.9 kg heavier in EIP, carcass yield was 8.9% greater in this group. Loin area at the 10th rib level did not differ between treatments, but, at the last rib level, it tended ($P = 0.07$) to be greater in EIP. Backfat at the 10th rib level was 2.8 cm thicker in EIP, which also had thicker middle and outer layers. Similarly, at the last rib level, total backfat was 1.5 mm thicker in EIP, but there were no treatment differences for the outer layer, and only a trend ($P = 0.09$) for the inner layer.

Regarding prime cut weights, only "secreto" and tenderloin were significantly heavier in EIP (Table 4). In contrast, ham yield was 1.75% heavier for LIP. The proportions of SM and BF ham muscles were also greater for LIP. Since EIP carcasses were heavier but cut weights were not, this made cut yield increase for LIP, which had a 1.5, 0.6, 0.2 and 0.1 kg heavier shoulder, loin, "presa" and tenderloin, respectively. Table 5 depicts CT ham composition. In the two positions applied, fat amount was significantly greater in EIP. Similarly, fat volume and proportion were significantly higher and lean volume and proportion lower in EIP. Ham linear dimensions did not differ between treatments. In contrast to ham weight, total ham volume was not greater for LIP; therefore, LIP had more muscular and leaner hams. There were no treatment differences in ultimate pH, colour, texture parameters or acceptability by consumers (Table 6). Marbling and IMF were 1.3 points and 1.2% higher, respectively, in EIP loins. Similarly, IMF contents from BF and GM ham muscles were higher in EIP.

Table 2
Influence of early (pre-pubertal, EIP) and late (pre-finishing, LIP) immunocastration protocol on the body measurements obtained in live pigs with ultrasounds at 17 months of age ($n = 10$ each).

Ultrasound measurements	Immunocastration		RMSE	*P*-value
	Pre-pubertal	Pre-finishing		
10th rib level				
Loin area (cm^2)	23.86	22.35	2.15	0.168
Fat thickness (cm)				
Total	8.48	6.79	1.09	0.006
Inner	2.16	1.83	0.39	0.107
Middle	4.67	3.25	0.76	0.002
Outer	1.65	1.71	0.31	0.723
Ham region				
Muscle depth GM[1] (cm)	6.75	6.79	0.59	0.884
Fat thickness GM[1] (cm)	4.38	3.47	0.70	0.012

[1] Gluteus medius muscle (GM) depth and subcutaneous fat thickness measured at the level of the ischiatic spine (bone protuberance over the acetabulum of the pelvis).

Table 4
Influence of early (pre-pubertal, EIP) and late (pre-finishing, LIP) immunocastration of pigs on the carcass and cut characteristics ($n = 10$ each).

	Immunocastration		RMSE	*P*-value
	Pre-pubertal	Pre-finishing		
Cut weights (kg)				
Shoulder	11.00	10.70	0.48	0.180
Loin	1.94	2.12	0.23	0.086
Latissimus dorsi (secreto)	0.32	0.27	0.05	0.019
Serratus ventralis (presa)	0.72	0.70	0.05	0.267
Tenderloin	0.41	0.37	0.04	0.030
Ham	14.51	13.97	0.88	0.193
Semimembranosus	0.74	0.77	62.01	0.264
Biceps femoris	1.25	1.34	111.51	0.096
Cut yield (%)				
Shoulder	16.21	17.70	0.95	0.003
Loin	2.86	3.50	0.37	0.001
Latissimus dorsi (secreto)	0.47	0.44	0.07	0.360
Serratus ventralis (presa)	1.02	1.20	0.08	<0.001
Tenderloin	0.54	0.68	0.07	<0.001
Ham	21.35	23.10	0.99	0.001
Semimembranosus	5.11	5.52	0.40	0.037
Biceps femoris	8.64	9.57	0.64	0.004

Table 5
Influence of early (pre-pubertal, EIP) and late (pre-finishing, LIP) immunocastration of pigs on the morphological characteristics and tissue ham composition evaluated by computed tomography ($n = 10$ each).

	Immunocastration		RMSE	*P*-value
	Pre-pubertal	Pre-finishing		
Length (mm)	899.70	891.18	20.83	0.386
Width (mm)	289.88	282.75	14.88	0.299
Fat weight (kg)[1]	5.13	3.79	0.62	<0.001
Lean weight (kg)[2]	6.57	7.47	0.5	0.001
Position 1[3]				
Fat thickness (mm)	40.04	30.40	3.95	<0.001
Fat area (cm^2)	85.62	64.18	10.03	<0.001
Total area (cm^2)	320.70	316.02	16.02	0.523
Position 2[4]				
Fat thickness (mm)	35.03	25.21	3.87	<0.001
Fat area (cm^2)	119.74	91.02	17.14	0.002
Total area (cm^2)	315.47	299.17	20.47	0.092
Tissue volume (dm^3)				
Fat	5.58	4.12	0.68	<0.001
Lean	6.32	7.18	0.48	<0.001
Bones	0.87	0.92	0.06	0.105
Total ham	12.77	12.22	0.81	0.147
Ham composition (%)				
Fat	43.55	33.74	3.73	<0.001
Lean	49.62	58.74	3.33	<0.001
Bones	6.83	7.53	0.57	0.013

[1] Fat weight obtained by fat volume and applying a density of 0.9196 g/cm^3.
[2] Lean weight obtained by lean volume and applying a density of 1.04 g/cm^3.
[3] Position 1: Joint between the femur and pelvic bones (Fig.1a).
[4] Position 2: First image beside the patella in caudal direction (Fig. 1b).

Table 6
Influence of early (pre-pubertal, EIP) and late (pre-finishing, LIP) immunocastration of pigs on the meat quality characteristics and consumer acceptability of loin ($n = 10$ each).

	Immunocastration		RMSE	*P*-value
	Pre-pubertal	Pre-finishing		
Ultimate pH	6.11	6.11	0.31	1.000
Lightness-L*	31.48	32.74	2.34	0.243
Redness-a*	6.73	6.92	1.07	0.701
Yellowness-b*	1.09	1.12	0.72	0.917
Cooking loss (%)	21.25	22.42	3.84	0.504
Marbling[1]	3.31	2.06	0.96	0.016
Intramuscular fat (%)				
Longissimus	4.30	3.08	1.05	0.022
Semimembranosus	2.77	2.49	0.62	0.327
Biceps femoris	4.14	3.03	0.84	0.009
Gluteus medius	3.92	2.75	0.89	0.009
Texture analysis of loin				
Warner–Bratzler shear force (N)	5.54	5.08	1.61	0.533
Hardness (kN)	11.14	11.02	1.88	0.888
Adhesiveness (N·s)	−1.50	−1.60	0.47	0.633
Springiness	0.49	0.51	0.03	0.082
Cohesiveness	0.51	0.53	0.03	0.084
Chewiness (kN)	2.85	3.14	0.69	0.366
Resilience (N·s)	0.19	0.20	0.02	0.081
Acceptability by consumers[2]				
Overall	6.35	6.30	0.79	0.877
Tenderness	6.07	5.95	1.05	0.813
Odour	6.47	6.56	0.58	0.729
Taste	6.52	6.35	0.78	0.644

[1] Measured with the National Pork Producers Council pattern (NPPC), scale 1 (very low) to 10 (very high).
[2] Overall, tenderness, odour and taste acceptability evaluated with a scale from 1 (extremely dislike) to 9 (extremely like) without the intermediate level.

Relationship between intramuscular fat content and fat depot measurements

Table 7 shows low correlations between US fat thicknesses and IMF contents. The highest correlations were found between CT fat measurements and IMF content in BF, GM and LT muscles ($0.45 \le r \le 0.60$).Loin marbling was highly correlated with loin IMF content and also with BF and GM ham muscles. The lowest correlations were found between SM and BF ($r = 0.66$) or GM ($r = 0.69$), and the highest between BF and GM ($r = 0.91$) or LT ($r = 0.90$). Percent IMF was very high for all muscles evaluated.

Table 7
Correlations between fat deposition – evaluated in live pigs with ultrasounds (US), in carcasses with a ruler, and in the ham with computed tomography (CT) and intramuscular fat measurements of the loin and different muscles of the ham.

Fat deposition	Intramuscular fat content			
	Semimembranosus	*Biceps femoris*	*Gluteus medius*	*Longissimus*
Ultrasounds				
Loin fat thickness	0.15	0.25	0.27	0.31
Ham fat thickness	0.10	0.21	0.23	0.31
Ruler				
Fat thickness 10th rib	0.18	0.43+	0.45*	0.44+
Fat thickness last rib	0.30	0.45*	0.33	0.43+
Computed tomography				
Fat thickness, P1[1]	0.43+	0.57**	0.53*	0.60**
Fat area, P1[1]	0.26	0.52*	0.49*	0.58**
Fat thickness, P2[2]	0.28	0.56*	0.51*	0.58**
Fat area, P2[2]	0.24	0.48*	0.45*	0.52*
Ham fat volume	0.27	0.50*	0.52*	0.54*
Ham fat, %	0.32	0.56**	0.55*	0.55*
Fat weight	0.27	0.50*	0.52*	0.54*
Visual evaluation				
Loin marbling[3]	0.44+	0.61**	0.63**	0.69**
Intramuscular fat content				
Biceps femoris	0.66**			
Gluteus medius	0.69***	0.91***		
Longissimus thoracis	0.80***	0.90***	0.87***	

+: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$ M; ***: $P < 0.001$.
[1] P1 (Position 1): Joint between the femur and pelvic bones (Fig. 1a).
[2] P2 (Position 2): First image beside the patella in caudal direction (Fig.1b).
[3] National Pork Producers Council pattern (NPPC), scale 1: very low to 10: very high.

Discussion

Quality compositional measurement relationships

Lucas et al. (2017) found good correlations between fat thickness measured in the same locations in live pigs with US and in carcass with Fat-O-Meat'er and CT. In the present work, fat measurements were also highly correlated, even though there were different type of measurements (thickness, area, proportion) taken with different technologies (US in live pigs, ruler in the carcass and CT in the ham) and at different locations (loin or ham). Therefore, although US is less accurate than CT (Scholz et al., 2015), US fat cover thickness over the loin and over the ham region is useful to classify live pigs by fat deposition which may be of interest for productive and genetic purposes, even allowing fatness prediction for the ham, a highly valuable cut in Iberian pigs. These correlations between measurements might indicate that fat deposition is proportional among different locations. It has to be considered that correlations might be overestimated due to wide differences in body composition between the two treatment groups.

Body, carcass and ham composition by immunocastration protocol

All the technologies used (US, ruler and CT) showed that carcasses and hams from EIP were fatter than those from LIP. Several works report an increase in fat deposition after the second vaccine (Fàbrega et al., 2010; Carabús et al., 2017), probably due to an increase in the feed intake and growth of immunocastrated pigs (Millet et al., 2011). EIP received the second vaccine 6.5 months before LIP. The second vaccine (after which the IC effects could start) took place 11.5 and 5 months

before slaughter for EIP and LIP, respectively. Therefore, EIP were allowed more months to deposit fat, thus physiologically functioning as castrated pigs earlier. In both cases, a third vaccine was given because it is recommended for pigs slaughtered at an older age to ensure boar taint control (Allison et al., 2009; Martinez-Macipe et al., 2016). Likewise, Turkstra et al. (2002) reported greater backfat thickness and smaller lean meat content for early vs late IC. In the present work, fat thickness differences can be related to the fact that EIP carcasses were heavier than LIP carcasses. Indeed, if carcass weight was included as a covariate, significant differences in fat thickness measured with US and ruler between LIP and EIP disappeared (results not shown), although CT measurements of fat thickness and fat proportion were still significantly higher in EIP than in LIP, although differences in fat weight disappeared.

In the present study, the loin muscle area measured in live pigs and in carcass was not significantly ($P > 0.05$) different between treatments. Lower subcutaneous fat thickness might be associated with greater loin area in conventional pig breeds (Gispert et al., 2007) and Iberian pigs (Martinez-Macipe et al., 2016). When 2-dose early and late IC protocols were applied, no differences were reported in loin thickness or carcass lean content (Andersson et al., 2012; Aluwé et al., 2016), or the late IC resulted in leaner carcasses (Turkstra et al., 2002). In the present work, ham's lean content was higher in LIP, which suggests an effect of an IC schedule on lean content.

Due to the physiological implications of IC, the design of this study included a uniform slaughter age (17 months), but EIP and LIP also reached similar BW. In other works with different breeds, the second (last) vaccine dose increased daily feed intake and average daily gain (Turkstra et al., 2002), and, consequently, BW increased in the interval from the second vaccine to slaughter (Fàbrega et al., 2010) in comparison with entire males. In the present study, the overall average daily gain during the last 3 months (right after the 3rd vaccination of LIP) and the BW at the time of this 3rd vaccination were significantly greater in LIP than in EIP (0.597 ± 0.024 vs 0.524 ± 0.017 kg/d and 118.4 ± 2.3 vs 102.9 ± 4.4 kg, respectively; mean ± SE). The 15-day flushing in LIP may have contributed to this greater ADG, whereas the greater BW at last vaccination could be due to the well-known anabolic effect of testosterone. However, growth performance results should be interpreted with caution due to several concurrent influences, like the nutritional flushing period and the greater intestinal capacity of LIP (as detailed below), and also because of the small number of animals used and their unknown feed intake, as these 20 pigs were two subsets within two greater groups with known in-group feed intake. Even though BW was similar between protocols, carcass weight was on average 15 kg heavier in EIP, which, consequently, had a 8.94% greater carcass yield. In the same direction but to a much less extent, Turkstra et al. (2002) and Andersson et al. (2012) reported that the early IC resulted in 1.0 and 0.6% greater carcass yield than late or standard IC, respectively. Similarly, Aluwé et al. (2016) showed that pigs receiving the 2nd vaccine 6 weeks before slaughter presented 0.6% higher carcass yield than those who received the 2nd dose of the vaccine 4 weeks before slaughter. The lower carcass yield of LIP could be explained by a greater weight of the digestive tract (and consequently its content) and of other organs removed during carcass preparation, as it was hypothesized for entire males, immunocastrated males or later-immunocastrated males (Gispert et al., 2010; Millet et al., 2011; Andersson et al., 2012). It was later showed by Boler et al. (2014), who found that empty gastrointestinal tract, liver and kidneys (among other organs) were heavier in IC males (slaughtered 33–47 days after the second vaccination) than in barrows that had a 1.43% greater carcass yield but a 1.0% smaller lean cutting yield for the same slaughter BW (130 kg). However, differences in intestinal capacity were plausibly greater for the older animals of the present study. In EIP and LIP carcasses, loin subcutaneous fat thickness was greater at the 10th rib level than at the last rib level. Similarly, in females from three genotypes of conventional pig breeds and in immunocastrated Duroc, Carabús et al. (2014) and Font-i-Furnols et al. (2012b) reported smaller fat thickness at the last rib level than between the 11th and 12th.

Meat quality by immunocastration protocol

Meat quality, in terms of pH, colour, cooking losses and texture did not differ between IC protocols, indicating the feasibility of both strategies to produce the same quality in meat characteristics. The differences found between protocols are related to the IMF content of several muscles. Except for the SM, the other muscles studied had more IMF in EIP vs LIP. This can be partly due to the higher weight and fatness of EIP carcasses. Accordingly, marbling was also higher in the loin of EIP. A recent work (Zoels et al., 2020) showed no differences in IMF from early vs late IC (immunized at 8 and 12 vs 12 and 16 or 12 and 18 weeks of age). However, that work was carried out with lean pigs, with low IMF content (Pietrain × Large White/Landrace; 1.35–1.53%) in which differences in fat deposition are more difficult to appreciate. In contrast, in the present work, fatty and older pigs were used, with an important amount of IMF content.

Acceptability of the meat by consumers was good and similar for meat from both IC protocols, probably because no important differences in meat quality have been detected between different treatments. Differences in loin IMF seem to be not enough, or IMF levels too high, to produce differences in the meat acceptability according to consumers, in agreement with previous works (Channon et al., 2004), but in contradiction with others (Fortin et al., 2005; Font-i-Furnols et al., 2012a). Thus, from the consumers' point of view, both protocols are suitable to produce good enough meat.

Intramuscular fat content and relationship with fat depots

A positive relationship between fat depots and IMF content has been reported, although this relation depends on the muscle and the type of fat measurement (Font-i-Furnols et al., 2019). The highest correlations obtained between fat measures and IMF content were between CT fat measures and IMF of LT, BF and GM. Surprisingly, the IMF of the SM ham muscle was less correlated with ham fatness than with loin IMF content. These correlations were much lower when the ham or loin fat depot measures were obtained by US in live pigs. Correlations between the fat measures obtained in the carcass with a ruler and the IMF content were intermediate. Nevertheless, the three technologies are different, were applied to different items (pig, carcass and ham) and have different precision, and all of these circumstances could have an influence on these results.

Correlation of loin marbling with loin IMF ($r = 0.69$) was significant but lower than those found by Font-i-Furnols et al. (2012a; $r = 0.89$) and higher than those reported by Font-i-Furnols et al. (2019), which varied between 0.50 and 0.66, depending on the loin location .Correlations between loin marbling and IMF of ham muscles were moderate (> 0.60) except for the SM muscle. Font-i-Furnols et al. (2019) also found variation in these correlations depending on the compared muscles. In fact, this correlation can be highly variable and it might depend on the methodology for IMF determination, the marbling scale used, the operator that performs the evaluation, the muscle or the location within the muscle evaluated and the range of IMF content.

Conclusion

In conclusion, different types of fat measurements, taken at different locations on the live animal, carcass or ham prime cuts and using different non-destructive technologies (those *in vivo* being also non-invasive) are consistent. Because of that, ultrasonography of live pigs might be a suitable technology to evaluate carcass and ham characteristics in terms of fat tissue deposition, because results are consistent with those obtained *post-mortem* with a ruler directly on the carcass and with those obtained by using CT on the ham. Although the effects of IC

protocol should be confirmed with a larger number of animals and with a defined feed intake for the two groups used in this study, in the conditions of the present work, EIP had greater carcass weight, fatter carcasses and a more marbled meat than LIP at the same slaughter age, but these differences did not affect meat quality and acceptability by consumers. Finally, this study shows a great potential for the application of US and CT to immunocastrated Iberian pigs so that the information obtained could be used to optimize the vaccination protocol to reach the target carcass and meat quality traits.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100189.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. Confidential data.

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Declaration of interest

The authors have no conflict of interest to report.

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