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Effect of male pig immunocastration on physical and chemical characteristics of Teruel dry-cured hams

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Abstract

Aim of study: To evaluate the effect of the type of male castration (surgical vs. immunological) on the characteristics of Teruel dry-cured hams.

Area of study: Teruel and Zaragoza (Spain).

Material and methods: A total of 14 dry-cured hams from Duroc × (Landrace × Large White) male pigs intended for the Spanish Protected Designation of Origin 'Teruel ham' were used. Half of them belonged to surgical castrated males (SCM) and the other half to immunocastrated males (IM). Ham weight losses during processing, physical and chemical characteristics were analyzed.

Main results: There were no differences ($p>0.05$) due to the type of castration in ham weight losses throughout processing, thickness of subcutaneous fat, marbling, maximum stress and intramuscular fatty acid profile. However, hams from IM presented lower ($p<0.05$) chroma than those from SCM. The type of castration had limited influence on chemical composition; only potassium nitrate and retinol contents were affected ($p<0.05$), being lower in IM than in SCM. For volatile compounds, IM showed lower ($p<0.05$) percentages of total alcohols and sulfur compounds and higher ($p=0.012$) proportion of total acids than SCM. All the hams had negligible androstenone content but IM presented higher ($p<0.05$) skatole and indole concentrations than SCM, being these levels low.

Research highlights: The type of castration in male pigs seems to have scarce influence on the quality of Teruel dry-cured hams, and therefore, immunocastration could be considered as a possible alternative to surgical castration.

Additional key words: barrow; immunological castration; processing losses; physical measurements; chemical composition; dry-cured ham.

Abbreviation used: a^* (redness); a_w (water activity); b^* (yellowness); C_{ab}^* (chroma); FA (fatty acid); GnRF (gonadotrophin releasing factor); h_{ab} (hue angle); IM (immunocastrated males); IMF (intramuscular fat); L^* (lightness); MUFA (monounsaturated fatty acids); PDO (Protected Designation of Origin); PUFA (polyunsaturated fatty acids); RH (relative humidity); SCM (surgically castrated males); SFA (saturated fatty acids).

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Introduction

Pigs destined for the Protected Designation of Origin (PDO) 'Teruel ham' have to be slaughtered at heavy weights (>130 kg of body weight) to meet the quality requirements established by the Consortium of this PDO (BOA, 2017). Consequently, male pigs have to be castrated to avoid boar taint, which is an unpleasant odor mainly originated by two compounds present in the adipose tissue: androstenone, a testicular steroid, and skatole, a product of bacterial degradation of tryptophan in the large intestine (Brunius et al., 2011; Škrlep et al., 2014). Besides, another compound, indole, could contribute to the formation of this odor (Brunius et al., 2011). Traditionally, it has been allowed to castrate male pigs by surgical methods without anesthesia or analgesia if it is practiced during the first days of animal life (BOE, 2002). However, it generates pain to piglets (Bonneau & Weiler, 2019), and therefore alternatives to this type of castration are being sought in the European Union (EC, 2010). Among them, producing entire males and immunocastration are the two most practical, short-term solutions, likely to thrive (Škrlep et al., 2014).

Rearing entire animals is being successfully practiced in several European countries, but it implies a reduction in the slaughter weight, which is not feasible for the PDO Teruel ham. However, immunocastration could be viable in this PDO. Immunocastration consists of the injection of several vaccines whose active substance is an inactive analogue of gonadotrophin releasing factor (GnRF) conjugated to an immunogenic carrier protein, triggering the formation of antibodies against endogenous GnRF, neutralizing it (Škrlep et al., 2014; Čandek-Potokar et al., 2017). It blocks the stimulation of the hypothalamic-pituitary-gonadal axis, preventing the formation of gonadal steroid hormones and causing regression of reproductive organs and some metabolic changes, which finally leads to the reduction of aggression, the increment of appetite and the elimination of androstenone formation (Škrlep et al., 2014).

Nevertheless, according to the meta-analysis of Poulsen-Nautrup et al. (2018), immunocastrated males (IM) seem to present lower fatness than surgically castrated males (SCM). It could penalize the end product quality, because an insufficient amount of subcutaneous fat covering the piece causes an increase of the seasoning losses and a decrease of the organoleptic characteristics of the dry-cured hams (Bosi & Russo, 2004). However, the effect of immunocastration could differ among production systems. To our knowledge, no scientific paper has been published about the influence of male pig immunocastration on the quality of Teruel dry-cured hams. To check the feasibility of this strategy to produce hams under this PDO, a trial was conducted to assess the effect of the type of castration (surgical vs. immunological) of male pigs on processing weight losses and on physical and chemical characteristics of Teruel dry-cured hams.

Material and methods

The raising and slaughter of the animals as well as the dry-curing process of the hams followed the regulations established by the *Consortium* of the PDO Teruel ham (BOA, 2017).

Experimental samples

A total of 14 fresh hams from Duroc × (Landrace × Large White) male pigs were utilized. The pieces came from the experimental animals used in the research of Pérez-Ciria et al. (2022), in which the impact of castration type and different feeds was evaluated on productive performances and carcass traits. All the experimental procedures used in that research followed the ethical committee requirements of the University of Zaragoza (ref. PI29/18). Concretely, the hams were chosen at random from pigs receiving the high-energy diet; seven belonged to SCM and seven belonged to IM. The surgical castration was practiced in the SCM group the first week of life and the immunization against GnRF in the IM group was carried out with Improvac® (Zoetis Belgium S.A., Louvain-la-Neuve, Belgium) using three doses, with approx. 25, 58 and 79 kg of body weight (56, 101 and 122 ± 3 d of age, respectively). The general management and feeding at the farm were the same for all of them. All pigs were slaughtered in the abattoir (Teruel, Spain) at the same day, with 142 ± 11.8 kg of body weight (199 ± 3 d of age). There, the left ham from each carcass was taken, trimmed and individually weighed (Table 1).

Dry-curing process and sampling

Upon arrival at the ham-curing facilities, hams were classified according to the weight. Then, the residual blood was removed by a bleeding-massaging machine that presses the femoral artery. The six phases of the dry-curing process were the following:

i) Salting; each ham was introduced in a salting tumbler and 2.5 g of nitrifying salt (a mixture of sodium chloride, maltodextrin, sodium ascorbate and potassium nitrate) per kg of meat mass were applied. Then hams were placed in stackable bins, coated with common salt and kept at 0-2°C and 75-90% of relative humidity (RH) for 0.8 days per kg of meat mass.
ii) Washing with water and molded.
iii) Resting; hams were hung in racks with hangers and stored from 3.5 to 5°C and from 80-82 to 72-77% of RH for 90 days.
iv) Drying; the temperature was gradually increased from 8 to 21°C and the RH reduced from 70-75 to 68-73% for 136 days. Finally, lard was applied manually to the muscular part of the hams to prevent the entry of microorganisms and to avoid over-drying.

Table 1. Impact of the type of castration of male pigs on weight losses (mean ± standard deviation) of dry-cured hams.

	Type of castration		p-value
	Surgical	Immunological	
Ham weight, kg			
Fresh	13.74 ± 1.30	13.79 ± 1.01	0.928
Dry-cured	9.36 ± 1.26	9.17 ± 0.94	0.328
Weight losses[1], %			
After salting	5.52 ± 1.11	6.30 ± 1.00	0.154
After resting	18.90 ± 2.72	20.04 ± 1.76	0.369
After drying	24.99 ± 3.47	26.41 ± 2.33	0.384
After maturing	29.71 ± 3.97	31.09 ± 2.56	0.457
After aging	32.03 ± 4.07	33.63 ± 3.15	0.427

[1] Relative to the fresh-ham weight.

v) Maturing; the temperature continued increasing from 25 to 28°C and the RH was maintained at 70-75% for 79 days.

vi) Aging; hams stayed in a natural dryer until reaching 32°C for 256 days.

The individual weight of all pieces was recorded after salting, resting, drying, maturing and aging.

Once the dry-curing process ended (19 months later), hams were manually boned, sectioned in three parts and individually vacuum packaged. The proximal part of each ham (the opposite part to the hoof) was chosen to carry out the laboratorial analyses and was stored at 4°C until then. One month later, one slice of the sectioned surface of each piece was removed with a slicer (Sammic S.L., Azkoitia, Gipuzkoa, Spain) to determine the color measurements in the piece and another slice was cut to carry out the image analyses. After muscle dissection, the *biceps femoris* muscle (170 ± 20 g) was destined to measure texture. This muscle was minced with a chopper (Moulinette chopper dpa1, Moulinex®, Groupe SEB Iberica S.A., Barcelona, Spain) to analyze the chemical composition, fatty acid (FA) profile of intramuscular fat (IMF) and volatile compounds. Finally, samples of subcutaneous fat (approximately 100 ± 20 g) were taken to determinate boar taint compounds.

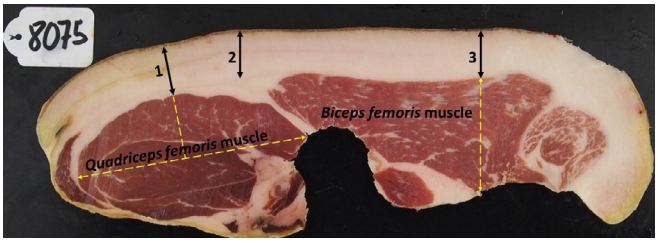

Figure 1. Subcutaneous fat thickness measured at three points; at the midpoint of the *quadriceps femoris* muscle (1), between the *quadriceps femoris* muscle and the *biceps femoris* muscle (2), and at the right side of the *biceps femoris* muscle (3).

Color traits

Color was evaluated on subcutaneous fat and on the muscles *quadriceps femoris* and *biceps femoris* using a spectrophotometer (CM-2600d, Konica Minolta Holdings, Inc., Osaka, Japan), previously calibrated, with illuminant D65 and observer angle of 10º, in CIELAB color space (CIE, 1986). The mean of three random readings of each section was used to obtain lightness (L^*), redness (a^*), yellowness (b^*), chroma and hue angle (h_{ab}).

Subcutaneous fat thickness and marbling by image analysis

One photograph of each slice was taken following Ripoll et al. (2019a). All images were transferred to a computer and no image editing was applied other than the cropping of the image. Subcutaneous fat thickness was measured at three points (Fig. 1): at the midpoint of the *quadriceps femoris* muscle, between the *quadriceps femoris* muscle and the *biceps femoris* muscle, and at the right side of the *biceps femoris* muscle. Marbling was estimated in the *biceps femoris* muscle following the methodology described by Mendizabal et al. (2005). The program ImageJ v1.48 (National Institutes of Health, USA) was used to determine subcutaneous fat thickness and marbling.

Texture

The measure of maximum stress was performed following Honikel (1998)held in Helsinki in 1992, a group of scientists with many years of experience in the field of

meat quality assessment convened in February 1993 for the first time, and subsequently in 1994 and 1995, in Kulmbach at the German Federal Centre for Meat Research under the auspices of the OECD research project Management of Biological Resources. Three specific areas were discussed in order to develop internationally accepted reference methods: water-holding capacity tenderness colour of meat. In the autumn of 1997 the methods were brought into their final form at the Meat Industry Research Institute of New Zealand (MIRINZ. Each sample was cut in prism-shaped pieces with a 100 mm^2 (10 × 10 mm) cross-section with the fiber direction parallel to a long dimension of at least 30 mm. A total of 8-10 prisms per sample were sheared perpendicular to the fiber orientation using a Warner-Bratzler device, with a cross-head speed of 2.5 mm/s, attached to an Instron universal testing machine (Model 5543, Instron Ltd, Buckinghamshire, UK) attached to a computer. Maximum stress was the load at maximum peak shear force per unit of cross-section (Ripoll et al., 2019b).

Chemical composition

Moisture, ash, protein and IMF were analyzed following the procedures described in BOE (1979). Moisture was determined using an oven (Memmert UFE500, Schwabach, Germany) at 102°C during 48 h and ash by a muffle (Model 10-PR/400, Forns Hobersal S.L., Caldes de Montbui, Barcelona) at 550°C during 7 h. Protein was analyzed utilizing a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden) and IMF by an ANKOM^{XT15} Extraction System (ANKOM Techonology, Macedon, NY) after hydrolysis (ANKOM^{HCL} Hydrolysis System).

Sodium chloride was determined following Matissek et al. (1998). A total of 3 g of each sample and 50 mL of milli-Q water were agitated in a shaker-incubator (Rotabit, J.P. Selecta S.A., Abrera, Barcelona) at 190 rpm during 30 min using a magnet. Finally, after the addition of 2 mL of nitric acid, samples were analyzed in a titrator (SM Titrino 702, Metrohm Hispania, Madrid, Spain).

Potassium nitrate and sodium nitrite were also analyzed following the official methods of analysis of meat products described in BOE (1979, 1981, 1982). In the case of potassium nitrate, 4 g of each sample were weighted in an Erlenmeyer flask of 250 mL and 150 mL of ethyl alcohol were added. Samples were agitated in a thermostatic bath (Bunsen BTG, Bunsen, Humanes de Madrid, Madrid) during 1 h. Once cooled, 5 mL of each of the Carrez reagents I and II, prepared with zinc acetate dihydrate and potassium hexacyanoferrate (II) trihydrate, respectively, were added, and milli-Q water were also added to level the flask at 250 mL. The content of this flask was filtered in a flask of 100 mL until its level. The filtrated was discarded and the remaining part was put in another flask of 250 mL, which was placed in a heating plate (Combiplac, J.P. Selecta S.A., Abrera, Barcelona) to evaporate ethyl alcohol, until achieving a volume of 50 mL. Then, this volume was transferred to the flask of 100 mL and milli-Q water was added to level it and it was flipped. Later, a total of 10 mL was transferred to a 50 mL flask and 1 mL of brucine-sulfanilic acid and 10 mL of sulphuric acid were added (color reaction) and it was left to rest 10 min in the dark. This flask was made up to 40 mL with milli-Q water and left to rest 15 min in the dark. Later, it was cooled and levelled. Lastly, a spectrophotometer (Shimadzu UV-1700 Pharmaspec, Kyoto, Japan) was used to determine potassium nitrate content at 410 nm. The procedure to determine sodium nitrite was similar, except for the reagent used for the color reaction, which was prepared mixing equal parts of two solutions. The first solution contained 1.50 g of sulfanilic acid, 50 mL of acetic acid and approx. 200 mL of milli-Q water to make up to 250 mL. The second solution contained 0.075 g of 1-naphthylamine, 50 mL of acetic acid and approximately 200 mL of milli-Q water to make up to 250 mL.

Collagen and water activity (a_w) were determined by near-infrared spectroscopy (measuring range: 850-1100 nm). Each sample was put in a circular small cup of 8.8 mm of depth and 134 mm of diameter that was introduced in the FoodScan™2 equipment (FOSS Iberia S.A., Barcelona).

The contents of α-tocopherol, γ-tocopherol, δ-tocopherol, retinol and cholesterol were determined following the methods described by Bertolín et al. (2018) using ultra-high performance liquid chromatography (ACQUITY UPLC H-Class liquid chromatograph (Waters, Milford, MA, USA) equipped with a silica-based bonded phase column (Acquity UPLC HSS T3, 1.8 µm × 2.1 mm × 150 mm column; Waters, USA), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ Detector; Waters, USA) and a fluorescence detector (2475 Multi λ Fluorescence Detector; Waters, USA)). To determine lipid oxidation, the content of malondialdehyde was analyzed, following Bertolín et al. (2019) using ultra-high performance liquid chromatography coupled to a fluorescence detector.

Fatty acid profile of IMF

Firstly, all samples were lyophilized. Then the FA extraction and methylation was carried out following Lee et al. (2012). A Bruker Scion 436-GC gas chromatograph (Bruker, Billerica, MA, USA) equipped with SP-2560 capillary column (100 m × 0.25 mm ID × 0.20 µm film thickness; Supelco, Saint Louis, MO, USA) was used for FA determination. The identification of the FAs was done using certified reference materials (GLC-401, GLC-463, GLC-532, GLC-538, GLC-642 and GLC-643, Nu-Chek Prep Inc., Elysian, MN, USA). The FAs were quantified based on the guidelines described in ISO 12966-4 (2015) as mg of FA/100 mg of total FAs (% of total FAs). The percentages of total saturated FAs (SFA), monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA), n-3 and n-6 and the ratios PUFA/SFA and n-6/n-3 were calculated from individual FA percentages.

Volatile compounds

Static headspace technique by using a Turbomatrix HS16 sampler (PerkinElmer, Massachusetts, USA) was used to analyze the volatile profile. A total of 4 g of each homogenized sample were placed in vials of 20 mL that were hermetically closed. The samples were thermostatized at 130ºC for 20 min and 1 min of pressurization time. The injection was carried out over 12 s at 25 psi and an inlet temperature of 220ºC. A Clarus 500 gas chromatograph coupled with a mass spectrometer (Perkin Elmer, MA, USA) equipped with a DB-Wax capillary column (60 m × 0.25 mm ID × 0.25 µm film thickness; Agilent Technologies, California, USA) was used to separate and identify the extracted compounds. A flow of 1 mL/min of helium was used as carrier gas. The oven temperature was 45ºC held for 2 min, 45-200ºC at a rate 4ºC/min, and finally to 225ºC at 10ºC/min, and held for 5 min. The mass spectrometer used the electron impact mode with an ionization potential of 70 eV and an ion source temperature of 200ºC. The interface temperature was 220ºC. The mass spectrometer scanned in full scan mode (35-300 m/z). A TurboMass vers. 5.4.2 Workstation was used for the gas chromatograph-mass spectrometer system. Tentative identification of the volatile components was achieved by comparison of the mass spectra with mass spectral data from the Nist MS Search Program 2.0 library and by comparison of previously reported Retention Index with those calculated using a n-alkane (C7-C25) series under the same analysis conditions according to the Van Den Dool & Kratz´s (1963) equation. The relative percentage was expressed as a mass fraction of the total peaks area and fluorobenzene was used as internal standard. The percentages of total aldehydes, ketones, hydrocarbons, alcohols, sulfur compounds, furans and acids were calculated from individual volatile compound percentages.

Boar taint compounds

Androstenone, skatole and indole concentrations in the subcutaneous fat samples were measured by high-performance liquid chromatography as described Pérez-Ciria et al. (2021), using the same laboratory equipment. The concentrations were expressed as µg/g of liquid fat.

Statistical analysis

All statistical analyses were performed using SAS vers. 9.4 (SAS Institute Inc., Cary, NC, USA). Data were analyzed using the GLM procedure. The model included the type of castration (surgical vs. immunological) as fixed effect. Fresh ham weight and final dry-cured ham weight were included as covariates, when significant ($p<0.05$), to analyze ham weight losses and the rest of the variables studied, respectively. Androstenone concentration was not statistically analyzed since all values in both types of male pigs were below the detection level of the equipment used (0.20 µg/g of fat); consequently, a descriptive analysis was carried out with this variable.

Normality of the residuals was checked with Shapiro-Wilk's test using the UNIVARIATE procedure. In cases in which normality was not achieved, variables were transformed with or or Napierian logarithm or or before statistical analysis if it was possible. When normality could not be found with data transformation, Mann-Whitney U-test was carried out to analyze these variables. Homogeneity of variances was checked with Levene's test. When homoscedasticity was not achieved, Welch's test was applied.

The pig was the experimental unit. Data are presented in tables as original mean ± standard deviation. A p-value <0.05 was considered as a significant difference.

Results

Ham weight losses during processing

As shown in Table 1, throughout dry-curing process (composed by the phases of salting, resting, drying, maturing and aging), the type of castration did not influence ($p>0.05$) on ham weight losses. Thus, the final weight of the end product (dry-cured ham) from IM was similar ($p=0.328$) to that from SCM.

Physical characteristics

There were no differences ($p>0.05$) on color traits of subcutaneous fat between SCM and IM (Table 2). However, hams from IM presented lower ($p<0.05$) value in both muscles studied than those from SCM. Besides, IM also showed lower *a** values in the *quadriceps femoris* ($p=0.044$) and *b** in the *biceps femoris* ($p=0.045$) than SCM.

Hams from IM had similar ($p>0.05$) subcutaneous fat thickness and marbling than those from SCM (Table 3). Likewise, the type of castration had no effect ($p=0.283$) on maximum stress (67.57 ± 12.62 vs. 60.07 ± 12.38 in SCM and IM, respectively; data not shown in Table 3).

Chemical composition

The moisture, ash, protein and IMF of hams were not affected ($p>0.05$) by the type of castration (Table 4). Regarding salt contents, sodium chloride and sodium nitrite concentrations were also similar ($p>0.05$), but IM presented lower ($p=0.042$) potassium nitrate content than SCM. No influence ($p>0.05$) of the type of castration was observed on collagen content, a_w, and the contents of tocopherols, cholesterol and malondialdehyde. However, hams

Table 2. Effect of the type of castration of male pigs on color traits (mean ± standard deviation) of dry-cured hams.

	Type of castration		p-value
	Surgical	Immunological	
Subcutaneous fat			
*L**	73.24 ± 1.77	73.45 ± 2.03	0.839
*a**	2.94 ± 0.94	3.49 ± 0.88	0.216
*b**	9.95 ± 1.39	10.11 ± 1.48	0.844
C_{ab}^*	10.41 ± 1.43	10.72 ± 1.51	0.700
h_{ab}	73.53 ± 4.68	70.93 ± 4.29	0.300
Quadriceps femoris muscle			
*L**	37.49 ± 1.47	37.59 ± 1.55	0.910
*a**	12.10 ± 1.35	10.73 ± 0.88	0.044
*b**	7.65 ± 2.79	5.29 ± 2.00	0.094
C_{ab}^*	14.54 ± 1.43	12.07 ± 1.28	0.005
h_{ab}	31.79 ± 10.84	25.74 ± 8.47	0.267
Biceps femoris muscle			
*L**	44.78 ± 2.25	43.73 ± 3.43	0.536
*a**	13.17 ± 1.22	11.90 ± 1.43	0.116
*b**	8.52 ± 1.01	7.08 ± 1.25	0.045
C_{ab}^*	15.72 ± 1.06	13.92 ± 1.19	0.015
h_{ab}	32.93 ± 4.34	30.87 ± 6.00	0.499

L:* lightness. *a*:* redness. *b*:* yellowness. C_{ab}^*: chroma. h_{ab}: hue angle.

from IM presented lower (p=0.039) retinol content than those from SCM.

Fatty acid profile of IMF

Table 5 shows the impact of the type of castration (surgical vs. immunological) of male pigs on the FA profile of the *biceps femoris* muscle of dry-cured hams. No significant differences (p>0.05) were observed between SCM and IM in total SFA, MUFA, PUFA, n-3 and n-6 proportions and neither in PUFA/SFA and n-6/n-3 ratios.

Volatile compounds

A total of 39 volatile compounds were identified in the *biceps femoris* muscle of dry-cured hams (Table 6), including the following groups: aldehydes (9), ketones (7), hydrocarbons (6), alcohols (11), sulfur compounds (1), furans (2) and acids (3).

The type of castration had no influence (p=0.618) on total aldehydes percentage. However, within this group, hams from IM presented higher (p=0.006) proportion of heptanal, nonanal and (E)-2-octenal than those from SCM. No impact (p=0.708) of the type of castration was observed

Table 3. Impact of the type of castration of male pigs on subcutaneous fat thickness and marbling (mean ± standard deviation) of dry-cured hams.

	Type of castration		p-value
	Surgical	Immunological	
Subcutaneous fat thickness, mm			
At the *quadriceps femoris*	13.59 ± 5.73	14.52 ± 4.93	0.473
Between *quadriceps* and *biceps femoris*	14.14 ± 4.47	15.29 ± 3.38	0.321
At the *biceps femoris*	15.34 ± 3.80	14.17 ± 3.43	0.628
Marbling of the *biceps femoris*, %	6.24 ± 2.96	5.70 ± 3.20	0.750

Table 4. Effect of the type of castration of male pigs on chemical composition (mean ± standard deviation) of the *biceps femoris* muscle of dry-cured hams (results expressed on wet matter basis).

	Type of castration		p-value
	Surgical	Immunological	
Moisture, %	55.66 ± 1.18	55.80 ± 2.12	0.881
Ash, %	6.77 ± 0.66	6.85 ± 0.62	0.995
Protein, %	31.06 ± 2.08	31.71 ± 1.32	0.524
Intramuscular fat, %	6.06 ± 2.76	5.25 ± 2.74	0.502
Sodium chloride, g/100g	5.17 ± 0.58	5.14 ± 0.63	0.719
Potassium nitrate, mg/kg	120.29 ± 27.34	93.59 ± 14.85	0.042
Sodium nitrite, mg/kg	0.81 ± 0.21	0.62 ± 0.21	0.123
Collagen, %	1.40 ± 0.43	1.36 ± 0.43	0.856
Water activity	0.91 ± 0.01	0.90 ± 0.01	0.857
α-Tocopherol, µg/g	4.29 ± 0.89	3.37 ± 0.95	0.086
γ -Tocopherol, µg/g	0.25 ± 0.07	0.26 ± 0.05	0.752
δ -Tocopherol, µg/g	0.02 ± 0.00	0.02 ± 0.00	0.763
Retinol, ng/g	21.16 ± 6.78	15.50 ± 2.77	0.039
Cholesterol, mg/g	0.88 ± 0.03	0.88 ± 0.03	0.769
Malondialdehyde, mg/kg	0.54 ± 0.23	0.47 ± 0.20	0.554

on the percentage of total ketones. It is worth noting that inside this group, hams from IM showed lower (p=0.009) proportion of 2,3-butanedione and higher (p=0.006) of 2-octanone. The percentage of total hydrocarbons was also similar (p=0.183) in both experimental groups regardless of the type of castration. Within this group, hams from IM had lower percentage of α-pinene (p=0.027) and limonene (p=0.012) and greater of methylbenzene (p=0.024) than those from SCM. Immunocastration of male pigs reduced (p=0.019) the percentage of total alcohols in comparison to surgical castration, mainly due to the lower proportion of 1-octen-3-ol (p=0.009) and 1-octanol (p=0.008), although hams from IM presented higher (p=0.006) 2-methyl-1-propanol percentage than those from SCM. Likewise, hams from IM showed lower (p=0.039) percentage of total sulfur compounds than those from SCM owing to the lower (p=0.039) proportion of dimethyl disulfide. The type of castration had no effect (p=0.183) on the percentage of total furans, although IM presented lower (p=0.015) proportion of 2-ethylfuran than SCM. Finally, hams from IM did show greater (p=0.012) percentage of total acids than those from SCM, because of the higher proportions of 2-methylpropanoic (p=0.006), butanoic (p=0.009) and hexanoic (p=0.024) acids.

Boar taint compounds

Table 7 shows the impact of the type of castration of male pigs on the boar taint compounds. All hams, both from SCM and IM groups, presented low androstenone concentrations, below the detection level of the equipment (0.20 µg/g) (data not shown). However, IM hams had higher skatole (p=0.004) and indole (p=0.029) concentrations than those from SCM.

Discussion

Ham subcutaneous fat thickness is the main factor affecting dehydration losses during the dry-curing process, presenting lower losses those hams with thicker subcutaneous fat (Čandek-Potokar & Škrlep, 2011). In a meta-analysis, Poulsen-Nautrup et al. (2018) reported that IM had less backfat thickness in the carcass than SCM, and thus, it was expected to find this effect in dry-cured hams too. However, in the present study, the type of castration had no significant effect on this parameter and neither on ham weight losses throughout the dry-curing process, which agrees with Pinna et al. (2015). Čandek-Potokar et al. (2020) did find that IM presented greater ham weight losses, since the fresh hams from these pigs had lower fat thickness than those from SCM. This disagreement among authors could be due, at least in part, to the different period elapsed between the application of the second dose of immunocastration and the slaughter, because this dose is the one that really stimulates the protective immune response (Font-i-Furnols et al., 2012). In the current trial and in the study of Pinna et al. (2015), the second dose was injected much earlier than in the work of Čandek-Potokar et al. (2020) (approx. 14 vs. 4-5

Table 5. Impact of the type of castration of male pigs on fatty acid profile (% of total fatty acids) of the *biceps femoris* muscle of dry-cured hams (mean ± standard deviation).

	Type of castration		p-value
	Surgical	Immunological	
C8:0	0.045 ± 0.015	0.040 ± 0.015	0.491
C9:0	0.022 ± 0.009	0.018 ± 0.009	0.480
C10:0	0.129 ± 0.026	0.130 ± 0.026	0.944
C11:0	0.044 ± 0.012	0.048 ± 0.014	0.525
C12:0	0.085 ± 0.015	0.090 ± 0.012	0.293
C12:1-9c	0.057 ± 0.041	0.032 ± 0.021	0.201
C13:0	0.007 ± 0.004	0.006 ± 0.005	0.723
C14:0	1.081 ± 0.220	1.038 ± 0.105	0.746
C14:1	0.016 ± 0.010	0.022 ± 0.010	0.250
C15:0	0.043 ± 0.010	0.050 ± 0.013	0.267
C16:0	21.729 ± 0.582	21.686 ± 0.157	0.854
C16:1-7c	0.322 ± 0.025	0.350 ± 0.039	0.134
C16:1-9c	2.203 ± 0.552	2.013 ± 0.469	0.501
C16:1-11c	0.177 ± 0.035	0.201 ± 0.043	0.271
C17:0	0.157 ± 0.034	0.188 ± 0.027	0.065
C17:1-9c	0.109 ± 0.035	0.118 ± 0.021	0.578
C18:0	11.400 ± 0.837	11.886 ± 0.649	0.214
C18:1-9c	30.800 ± 3.910	31.186 ± 2.711	0.834
C18:1-11c	3.314 ± 0.352	2.953 ± 0.364	0.083
C18:2-n6	18.529 ± 2.694	18.529 ± 1.874	1.00
C18:3-n3	0.479 ± 0.061	0.513 ± 0.067	0.342
C18:3-n6	0.443 ± 0.154	0.411 ± 0.143	0.698
C19:0	0.029 ± 0.022	0.031 ± 0.015	0.969
C19:2-n6	0.012 ± 0.012	0.022 ± 0.009	0.094
C20:0	0.093 ± 0.019	0.095 ± 0.015	0.868
C20:1	0.537 ± 0.128	0.539 ± 0.065	0.971
C20:2-n6	0.462 ± 0.055	0.465 ± 0.047	0.927
C20:3-n6	0.677 ± 0.153	0.608 ± 0.073	0.314
C20:4-n6	4.933 ± 1.233	4.674 ± 0.793	0.465
C20:5-n3	0.139 ± 0.021	0.130 ± 0.012	0.347
C21:0	0.007 ± 0.004	0.006 ± 0.005	0.870
C22:0	0.024 ± 0.010	0.026 ± 0.011	0.675
C22:3-n3	0.285 ± 0.080	0.286 ± 0.016	0.325
C22:4-n6	0.771 ± 0.147	0.757 ± 0.114	0.659
C22:5-n3	0.399 ± 0.130	0.376 ± 0.071	0.689
C22:5-n6	0.328 ± 0.058	0.343 ± 0.046	0.598
C22:6-n3	0.118 ± 0.063	0.101 ± 0.034	0.544
C24:0	0.020 ± 0.012	0.019 ± 0.014	0.903
Total SFA[1]	34.886 ± 0.609	35.343 ± 0.516	0.156
Total MUFA[2]	37.529 ± 4.818	37.429 ± 3.294	0.965
Total PUFA[3]	27.571 ± 4.593	27.214 ± 2.955	0.866
PUFA/SFA	0.790 ± 0.127	0.769 ± 0.076	0.723
Total n-3	1.419 ± 0.204	1.407 ± 0.138	0.723
Total n-6	26.143 ± 4.360	25.829 ± 2.805	0.875
n-6/n-3	18.400 ± 1.245	18.386 ± 1.040	0.982

[1] SFA: saturated fatty acids. [2]MUFA: monounsaturated fatty acids. [3]PUFA: polyunsaturated fatty acids.

weeks before slaughter), and therefore the metabolic and hormonal status of IM was similar to that of SCM before, which produces similar results between IM and SCM.

Regarding color traits, in contrast to the current trial, Pinna et al. (2015) did not find that the muscle tissue of dry-cured hams from IM showed lower *a**, *b** and values

Table 6. Effect of the type of castration of male pigs on volatile compounds (chemical compound area/ internal standard-fluorobenzene-area expressed as %) of the *biceps femoris* muscle of dry-cured hams (mean ± standard deviation).

	Type of castration		p-value
	Surgical	Immunological	
Aldehydes			
3-Methylbutanal	68.900 ± 6.340	68.643 ± 3.285	0.851
Hexanal	0.455 ± 0.956	1.406 ± 0.824	0.121
Heptanal	-	0.110 ± 0.051	0.006
Octanal	0.015 ± 0.016	0.027 ± 0.011	0.120
(E)-Hept-2-enal	0.005 ± 0.008	0.046 ± 0.054	0.096
Nonanal	-	0.085 ± 0.024	0.006
(E)-2-Octenal	-	0.028 ± 0.019	0.006
Benzaldehyde	0.006 ± 0.005	0.262 ± 0.332	0.572
(E)-2-Nonenal	0.006 ± 0.007	0.008 ± 0.008	0.949
Total aldehydes	69.386 ± 6.581	70.600 ± 3.247	0.618
Ketones			
2-Propanone	14.100 ± 5.907	17.351 ± 1.540	0.534
2-Butanone	2.830 ± 0.745	3.307 ± 0.612	0.325
2,3-Butanedione	4.205 ± 1.624	1.358 ± 0.330	0.009
2-Pentanone	0.050 ± 0.054	0.006 ± 0.002	0.221
2-Heptanone	0.130 ± 0.135	0.177 ± 0.098	0.149
3-Octanone	0.036 ± 0.051	0.009 ± 0.004	0.202
2-Octanone	-	0.009 ± 0.004	0.006
Total ketones	21.350 ± 7.760	22.221 ± 1.549	0.708
Hydrocarbons			
Hexane	0.170 ± 0.150	0.716 ± 0.768	0.097
Heptane	0.413 ± 0.423	0.858 ± 1.646	1.00
Octane	0.900 ± 0.663	1.697 ± 1.295	0.271
α-Pinene	0.247 ± 0.178	0.009 ± 0.008	0.027
Methylbenzene	0.006 ± 0.006	0.297 ± 0.199	0.024
Limonene	0.081 ± 0.109	-	0.012
Total hydrocarbons	1.818 ± 1.204	3.576 ± 3.207	0.183
Alcohols			
Ethanol	0.151 ± 0.120	0.163 ± 0.136	0.754
2-Propanol	2.281 ± 4.297	0.033 ± 0.052	0.053
2-Butanol	0.008 ± 0.011	0.003 ± 0.003	0.789
2-Methyl-1-propanol	-	0.116 ± 0.134	0.006
Butanol	0.003 ± 0.007	0.003 ± 0.004	0.715
3-Methyl-1-butanol	0.503 ± 0.893	0.527 ± 0.526	0.174
1-Pentanol	0.071 ± 0.053	0.108 ± 0.040	0.108
2-Heptanol	0.006 ± 0.004	0.004 ± 0.003	0.179
Hexanol	0.087 ± 0.101	0.096 ± 0.065	0.707
1-Octen-3-ol	1.079 ± 0.756	0.053 ± 0.020	0.009
1-Octanol	0.030 ± 0.029	0.002 ± 0.003	0.008
Total alcohols	4.218 ± 3.860	1.108 ± 0.777	0.019
Sulfur compounds			
Dimethyl disulfide	1.902 ± 1.560	0.242 ± 0.115	0.039
Total sulfur compounds	1.902 ± 1.560	0.242 ± 0.115	0.039
Furans			
2-Ethylfuran	1.160 ± 0.389	0.402 ± 0.235	0.015
2-Pentylfuran	0.016 ± 0.017	0.383 ± 0.370	0.706
Total furans	1.176 ± 0.394	0.785 ± 0.431	0.183
Acids			
2-Methylpropanoic acid	-	0.031 ± 0.018	0.006
Butanoic acid	0.004 ± 0.003	0.503 ± 0.222	0.009
Hexanoic acid	0.151 ± 0.246	0.927 ± 0.580	0.024
Total acids	0.154 ± 0.249	1.461 ± 0.580	0.012

-: Undetected value

Table 7. Impact of the type of castration of male pigs on skatole and indole concentrations (μg/g of liquid fat) of subcutaneous fat of dry-cured hams (mean ± standard deviation).

	Type of castration		p-value
	Surgical	Immunological	
Skatole	0.053 ± 0.005	0.066 ± 0.008	0.004
Indole	0.052 ± 0.003	0.059 ± 0.006	0.029

than that from SCM. According to Zanardi et al. (1999), human perception of pork color is strongly influenced by L^* and h_{ab} values, which were not affected by the type of castration in the subcutaneous fat or in the muscle tissue. Consequently, in terms of color, consumers would find hams from IM to be similar to those from SCM.

In the current trial, the absence of effect of the type of castration on moisture, protein, IMF and sodium chloride contents agrees with the study of Pinna et al. (2015). Nevertheless, Čandek-Potokar et al. (2020) found that hams from IM presented lower moisture and greater protein and sodium chloride contents than those from SCM, which was also related to the lower subcutaneous fat thickness found in IM that increased the dehydration of their hams. In this last study, IM was in an intermediate position between entire males and SCM according to IMF content and showed lower visually marbling than SCM. In the current study, the high variability found in IMF content and marbling could have contributed partially to the lack of significant differences in these variables. On the other hand, the lower potassium nitrate concentration in hams from IM was not a relevant finding, since this compound has to be converted into nitrite to exert preservative effects (Toldrá et al., 2009), and the concentration of sodium nitrite was not affected by the type of castration. The similar collagen content detected in SCM and IM, together with the similar moisture and IMF percentages and FA profile of IMF, led to the type of castration had not influence on maximum stress.

Moreover, in the present study, a_w did not differ between treatments probably because of similarities in moisture and sodium chloride contents, in agreement with the findings of Pinna et al. (2015). However, Čandek-Potokar et al. (2020) found that IM had lower a_w than SCM, which could indicate a better shelf life of the hams. On the other hand, despite the fact that hams from IM had lower content of retinol, an antioxidant substance (Boulet et al., 2020), it did not translate into differences in malondialdehyde concentration, in agreement with the results of Čandek-Potokar et al. (2020). It is worth noting that numerical differences in this variable could suggest a certain tendency towards less oxidation in hams from IM, which would imply that these hams would last longer without going rancid. Additionally, the similar FA profile found between treatments could have contributed to the lack of effect on lipid oxidation. Font-i-Furnols et al. (2012), studying subcutaneous fat of dry-cured hams, did not find either difference between SCM and IM in total SFA, PUFA, n-3 and n-6 percentages and in PUFA/SFA and n-6/n-3 ratios. From the results of the current trial, it could be deduced that immunocastration does not harm the FA profile of dry-cured hams.

Regarding volatile compounds, generated mainly by proteolysis and lipolysis (Toldrá, 1998), aldehydes play a relevant role in the overall flavor of dry-cured hams due to their low odor thresholds and high concentrations (García-González et al., 2013). Hams from IM by having a greater percentage of heptanal, nonanal and (E)-2-octenal could present more fatty, greasy, ham-like, rancid, leaves, pungent and fruity notes (García-González et al., 2008) than those from SCM. Additionally, the higher percentage of the methyl-ketone 2-octanone in hams from IM could contribute to a greater fruity, floral and green herbaceous scents (Luna et al., 2006; García-González et al., 2008). The hydrocarbons α-pinene, methylbenzene and limonene had relatively low odor thresholds, especially α-pinene (García-González et al., 2008). Therefore, ham consumers could appreciate that hams from IM present lower sharp, pine, citric, fresh and wood olfactory notes and higher strong, plastic and glue notes (Luna et al., 2006; García-González et al., 2008). On the other hand, in general, the flavor of alcohols is considered irrelevant because of their higher odor threshold compared to other carbonyl compounds (Drumm & Spanier, 1991). Therefore, the lower percentage of total alcohols in hams from IM than in those from SCM would not be relevant. However, the alcohols 1-octen3-ol and 1-octanol had lower odor thresholds (García-González et al., 2008), and consequently, these compounds could play an important role in the aroma of dry-cured hams. Thus, hams from IM could show a lower mushroom-like, earthy, dust, fatty and sharp scents than those from SCM (García-González et al., 2008). Sulfur compounds have been associated to undesirable aromas (García-González et al., 2013), and therefore, hams from IM could have a more pleasant aroma than those from SCM, due to the lower percentage of the unique sulfur compound detected, dimethyl disulfide. The olfactory notes of this compound have been defined as dirty socks or cauliflowers (Flores et al., 1997; García-González et al., 2008). In spite of the fact that male immunocastration reduced 2-ethylfuran percentage and increased organic acids proportion, the contributions of these compounds to dry-cured ham aroma appear to be low (García-González et al., 2013; Pugliese et al., 2015). Therefore, it could be interesting to carry out a sensory evaluation to try to see if really consumers appreciated different olfactory notes between hams from SCM and those from IM intended for the PDO Teruel ham.

In respect of boar taint compounds, immunocastration was an efficient strategy for decreasing androstenone concentration. In fact, hams from both groups (SCM and IM) showed values under the detection level of the analy-

sis equipment. However, in the case of skatole and indole concentrations, IM failed to reduce their concentrations as much as SCM. Nevertheless, it is worth noting that in both SCM and IM, androstenone, skatole and indole concentrations were under the threshold values for sensory acceptance (0.5 to 1.0 µg/g, 0.20 to 0.25 µg/g and 0.10 µg/g, respectively) (Walstra et al., 1999; Font-i-Furnols et al., 2012). Therefore, in general, immunocastration would also avoid boar taint in Teruel dry-cured hams.

In conclusion, the type of castration (surgical vs. immunological) of male pigs intended for the PDO Teruel ham has limited effect on weight losses throughout the processing, physical characteristics, chemical composition and FA profile of IMF of the end product. On the other hand, immunocastration affects some volatile compounds, which could have positive influence on ham flavor, and provides hams with low levels of boar taint contributors.

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