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Impact of gilt immunocastration on weight losses and instrumental and chemical characteristics of Teruel dry-cured ham

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ABSTRACT

A total of 32 fresh hams intended for the Spanish Protected Designation of Origin "Teruel ham" were used to evaluate the impact of gilt immunocastration (*vs.* entire gilts) on weight losses during the dry-curing process. After processing, 20 dry-cured hams (10 of each group) were chosen at random to assess instrumental and chemical characteristics. Hams from immunocastrated gilts tended ($P = 0.057$) to present lower weight losses, they were fattier ($P < 0.05$) at both subcutaneous and intramuscular levels and had lower ($P < 0.05$) water activity and volatile compounds that provide unpleasant odors than those from entire gilts. However, immunocastration increased ($P < 0.05$) slightly sodium chloride and sodium nitrite contents, being normal levels. Fatty acid profile was not significantly affected ($P > 0.05$). It can be concluded that, in general, immunocastration could be a good strategy in gilts to improve the quality of Teruel dry-cured ham.

1. Introduction

In recent years, strategies have been sought to reduce the number of unsuitable carcasses for the Spanish Protected Designation of Origin (PDO) "Teruel ham" due to limited fat covering in the ham (Latorre, García-Belenguer, & Ariño, 2008). Fat thickness > 16 mm is required for a correct dry-curing process because it prevents an excessive drying of the pieces and improves organoleptic characteristics (Bosi & Russo, 2004). In addition, hams destined for this PDO have moderate marbling (Rodríguez-Sánchez, Calvo, Suárez-Belloch, & Latorre, 2014), parameter positively related to juiciness and tenderness (Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000). This problem of lack of fat (at subcutaneous and intramuscular levels) has been found principally in females (Latorre et al., 2008), because male pigs are surgically castrated to avoid boar taint, and castration increases the deposition of fat tissue (Weatherup, Beattie, Moss, Kilpatrick, & Walker, 1998). Surgical castration of females reared indoors is banned in the European Union (Official Journal of the European Union, 2009) but they could be immunocastrated. In industrial gilts, immunocastration consists of the application of one vaccine in two doses, whose active substance is a gonadotrophin releasing factor (GnRF) analogue-protein conjugate. Antibodies against GnRF are generated, inhibiting follicle-stimulating and luteinizing hormones, and thus, suppressing temporarily the ovarian function. The effects of gilt immunocastration have been studied on fresh pork characteristics (Daza, Latorre, Olivares, & López-Bote, 2014; Pérez-Ciria et al., 2021) but not on the quality of the dry-cured ham. Therefore, the goal of the current study was to evaluate the effect of gilt immunocastration on weight losses and on instrumental and chemical characteristics of Teruel dry-cured hams.

2. 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 (Boletín Oficial de Aragón, 2017).

2.1. Experimental samples

A total of 32 fresh hams from Duroc × (Landrace × Large White) gilts were used in the trial. These pieces came from the experimental animals

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utilized in the work of Pérez-Ciria, Miana-Mena, Falceto, Mitjana, and Latorre (2021) in which the influence of gilt immunocastration and different diets was evaluated on productive performances and carcass quality. All the experimental procedures used in that trial followed the ethical committee requirements of the University of Zaragoza (ref. PI29/18). Concretely, the hams were chosen at random from gilts that received a high-energy diet; 16 belonged to entire gilts (EG) (intact throughout the study) and 16 belonged to immunocastrated gilts (IG). The immunization of that group against GnRF was carried out with Vacsincel® (Zoetis Spain S.L., Alcobendas, Madrid, Spain). The priming dose and the booster were applied at 102 and 122 ± 3 days of age, respectively (with 58 and 77 kg of average body weight). 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 133 kg of average body weight (199 days of age). There, the left ham from each carcass was taken, trimmed and individually weighed.

2.2. Dry-curing process and sampling

All hams were processed as one group. 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 85% sodium chloride, 7% maltodextrin, 2% sodium ascorbate and 6% 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. 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), a total of 20 hams (10 from EG and 10 from IG), chosen at random, were manually boned, sectioned in three parts and individually vacuum packaged (Fig. 1). The proximal part of each ham (the opposite part to the hoof; part 1 in Fig. 1) was chosen to carry out the laboratorial analyses and was stored at 4 °C until then. One month later, one slice of approximately 5 mm of the sectioned surface of each piece was removed with a slicer (Sammic S.L., Azkoitia, Gipuzkoa, Spain), crosswise to the bone, to determine the color parameters 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. Finally, 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.

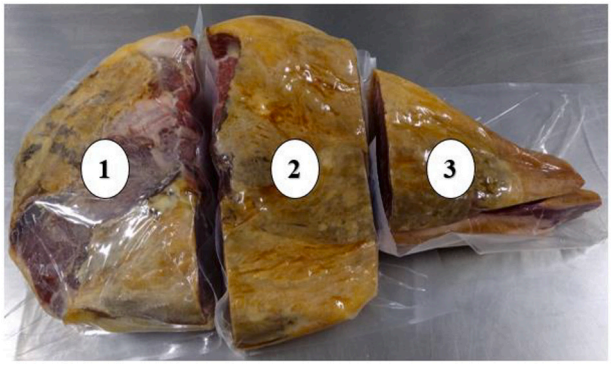

Fig. 1. Dry-cured hams sectioned in three parts.

2.3. Color parameters

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 10°, in CIELAB color space (CIE, 1986). The average of three random readings of each section was used to obtain lightness (L^*), redness (a^*), yellowness (b^*), chroma (C_{ab}^*) and hue angle (h_{ab}).

2.4. Subcutaneous fat thickness and marbling by image analysis

One photography of each slice was taken following the procedure carried out by Ripoll, Alcalde, Argüello, and Panea (2019). 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. 2): 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 in the work of Mendizabal, Purroy, Indurain, and Insausti (2005). The program ImageJ v1.48 (National Institutes of Health, USA) was used to determine subcutaneous fat thickness and marbling.

2.5. Texture

The measure of maximum stress was performed following the procedure described by Honikel (1998). 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 (vee-shaped, 76 mm × 140 mm, 1.016 mm thickness), with a cross-head speed of 2.5 mm/s, attached to an Instron universal testing machine (Model 5543, Instron Ltd., Buckinghamshire, United Kingdom) attached to a computer. Maximum stress was the load at maximum peak shear force per unit of cross-section (Ripoll et al., 2019).

2.6. Chemical composition

Moisture, ash, protein and IMF were analyzed following the procedures of Boletín Oficial del Estado (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, Spain) at 550 °C during 7 h. Protein was

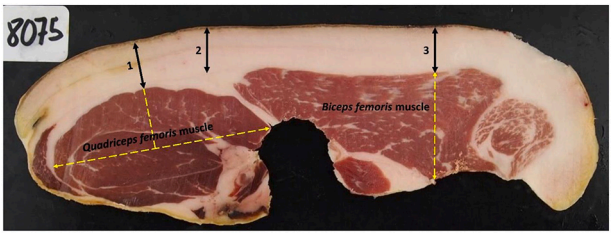

Fig. 2. Subcutaneous fat thickness measured at three points: at the midpoint of the *quadriceps femoris* muscle (1), between the *quadriceps femoris* muscle and the *biceps femoris* muscle (2), and at the right side of the *biceps femoris* muscle (3).

analyzed utilizing a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden) and IMF by an ANKOM^{XT15} Extraction System (ANKOM Techonology, Macedon, New York, USA) after being hydrolyzed the samples (ANKOM^{HCL} Hydrolysis System).

Sodium chloride was determined following the procedure described by Matissek, Schnepel, and Steiner (1998). For that, a total of 3 g of each sample were weighted and 50 mL of milli-Q water were added. Samples were agitated in a shaker-incubator (Rotabit, J.P. Selecta S.A., Abrera, Barcelona, Spain) at 190 rpm during 30 min using a magnet and 2 mL of nitric acid were added. Finally, 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 Boletín Oficial del Estado (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, Spain) 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, Spain) 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 were 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 approximately 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, Spain). The accuracy for the determination of collagen was 0.01 and for a_w 0.001.

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

2.7. Fatty acid profile of IMF

Firstly, all samples were lyophilized. Then the FA extraction and methylation was carried out following the methodology of Lee, Tweed, Kim, and Scollan (2012). A Bruker Scion 436-GC gas chromatograph (Bruker, Billerica, Massachusetts, USA) equipped with SP-2560 capillary column (100 m × 0,25 mm ID × 0,20 μm film thickness; Supelco, Saint Louis, Missouri, 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, Minnesota, USA). The FAs were quantified based on the guidelines described in ISO 12966-4 (2015) as mg/100 g of muscle (wet matter). The contents of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 and n-6 and the ratios PUFA/SFA and n-6/n-3 were calculated from individual FA concentrations.

2.8. 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 130 °C 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 220 °C. A Clarus 500 gas chromatograph coupled with a mass spectrometer (PerkinElmer, Massachusetts, 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 45 °C held for 2 min, 45–200 °C at a rate 4 °C/min, and finally to 225 °C at 10 °C/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 200 °C. The interface temperature was 220 °C. The mass spectrometer scanned in full scan mode (35–300 m/z). A TurboMass version 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 equation of Van den Dool and Kratz (1963). 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, acids, hydrocarbons, alcohols, furans and sulfur compounds were calculated from individual volatile compound percentages.

2.9. Statistical analysis

All statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Data were analyzed using the GLM procedure. The model included the type of gilt (EG or IG) 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. Least square means were compared using the PDIFF option.

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 $\sqrt{x}$ or $\sqrt[4]{x}$ or Napierian logarithm or x^2 or $1/(x+1)$ 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 gilt was the experimental unit ($n = 16$ for weight losses and $n = 10$ for the rest of the variables). A P-value <0.05 was considered as a significant difference and a P-value between 0.05 and 0.10 as a tendency.

3. Results and discussion

To the best of our knowledge, this is the first paper evaluating the effect of immunocastration on dry-cured hams from gilts. Therefore, in some parameters, studies on immunocastration in male pigs (entire *vs.* immunocastrated males) have been used for the discussion.

3.1. Ham weight losses during processing

As shown in Table 1, even though fresh ham weight was similar ($P = 0.720$) in EG and in IG, hams from IG tended ($P = 0.057$) to be heavier after the dry-curing process than those from EG. The reason is that pieces from IG tended ($P < 0.10$) to present lower weight losses after resting, drying, maturing and aging phases, giving 2.2%-point difference at the end. Therefore, the production of dry-cured hams from IG could be economically positive for ham cellars. Škrlep et al. (2016) did find significant that hams from immunocastrated males had lower processing losses than those from entire males. These results would be expected since immunocastrated pigs, both females and males, usually showed greater fat thickness covering the ham than entire pigs (Daza et al., 2014; Škrlep et al., 2020), and adipose tissue contains less water than muscular tissue and hinders exchanges between muscular part and external environment (Bosi & Russo, 2004).

3.2. Instrumental characteristics

Gilt immunocastration had no effect ($P > 0.10$) on color parameters of ham subcutaneous fat (Table 2). However, hams from IG tended ($P = 0.057$) to show lower C_{ab}* value in the *quadriceps femoris* muscle and this effect was significant ($P = 0.017$) in the *biceps femoris* muscle. Besides, IG tended ($P = 0.055$) to have lower b* value than EG only in the *biceps femoris* muscle. It has to be noted that, according to Zanardi, Novelli, Ghiretti, Dorigoni, and Chizzolini (1999), L* and h_{ab} parameters are those strictly linked with human perception of pork color, and none of them were affected ($P > 0.10$) by gilt immunocastration. Therefore, as for color of fat and muscle, hams from IG would result considerably similar to those from EG for consumers.

On the other hand, hams from IG had thicker ($P < 0.05$) subcutaneous fat depth than those from EG at *quadriceps femoris* and *biceps femoris* muscles (Table 3). It was expected because castration increases fat deposition (Peinado, Medel, Fuentetaja, & Mateos, 2008), and it would corroborate the differences observed in the weight loss of the pieces. However, this effect was not significant ($P = 0.685$) detected in the marbling, although numerically IG also presented a higher value than EG (5.72 *vs* 5.29%). A similar effect was observed by Gamero-Negrón, García, Reina, and Sánchez del Pulgar (2018) in dry-cured loins from female pigs and by Čandek-Potokar et al. (2020) in dry-cured hams from male pigs when pig immunocastration was researched.

Table 1
Impact of gilt immunocastration on weight losses (least square means) of dry-cured hams.

	Type of gilt		SEM[a] ($n = 16$)	*P*-value
	Entire	Immunocastrated		
Ham weight, kg				
Fresh	13.1	13.3	0.355	0.720
Dry-cured	8.63	8.91	0.100	0.057
Weight losses[b], %				
After salting	5.88	5.32	0.290	0.180
After resting	20.4	19.1	0.478	0.070
After drying	27.0	25.4	0.599	0.071
After maturing	32.1	30.1	0.702	0.057
After aging	34.8	32.6	0.771	0.057

[a] SEM: standard error of the mean.
[b] Relative to the fresh-ham weight.

Table 2
Effect of gilt immunocastration on color parameters (least square means) of dry-cured hams.

	Type of gilt		SEM[a] ($n = 10$)	*P*-value
	Entire	Immunocastrated		
Subcutaneous fat				
Lightness, L*	71.8	74.0	1.054	0.171
Redness, a*	3.36	3.28	0.277	0.850
Yellowness, b*	9.90	9.69	0.336	0.670
Chroma, C_{ab}*	10.5	10.3	0.330	0.632
Hue angle, h_{ab}	71.4	71.1	1.558	0.894
Quadriceps femoris muscle				
Lightness, L*	37.8	38.1	0.515	0.730
Redness, a*	11.6	10.9	0.457	0.302
Yellowness, b*	6.58	5.09	0.623	0.108
Chroma, C_{ab}*	13.4	12.1	0.464	0.057
Hue angle, h_{ab}	29.2	24.7	2.722	0.259
Biceps femoris muscle				
Lightness, L*	43.7	43.8	0.762	0.961
Redness, a*	12.6	11.8	0.313	0.120
Yellowness, b*	8.80	7.45	0.468	0.055
Chroma, C_{ab}*	15.4	14.0	0.377	0.017
Hue angle, h_{ab}	34.9	32.0	1.591	0.210

[a] SEM: standard error of the mean.

Table 3
Impact of gilt immunocastration on subcutaneous fat thickness and marbling (least square means) of dry-cured hams.

	Type of gilt		SEM[a] ($n = 10$)	*P*-value
	Entire	Immunocastrated		
Subcutaneous fat thickness, mm				
At the *quadriceps femoris*	12.6	16.3	1.201	0.040
Between *quadriceps* and *biceps femoris*[b]	14.0	16.5	1.434	0.130
At the *biceps femoris*	12.7	17.0	1.144	0.017
Marbling of the *biceps femoris*, %	5.29	5.72	0.728	0.685

[a] SEM: standard error of the mean.
[b] Least square means and SEM of the original data and *P*-value obtained with the transformed data.

Likewise, there was no difference ($P = 0.433$) on maximum stress between EG and IG (60.5 *vs.* 56.2 N/cm^2, respectively) (data not shown in tables). Škrlep et al. (2016) and Čandek-Potokar et al. (2020) did not find differences on texture profile of the *biceps femoris* muscle between entire males and immunocastrated males.

3.3. Chemical composition

As shown in Table 4, hams from IG showed lower ($P = 0.001$) moisture percentage and higher ($P = 0.049$) IMF content and tended ($P = 0.091$) to present greater ash proportion than those from EG. Gamero-Negrón, Sánchez del Pulgar, and García (2015) also found higher IMF content in dry-cured loins from IG than in those from EG. Likewise, Škrlep et al. (2016) observed that immunocastrated males had greater IMF proportion in the *biceps femoris* muscle than entire males. Our results about IMF supports the differences detected in subcutaneous fat thickness and the numerical effect found on marbling, corroborating that gilt immunocastration increases body fat deposition. Besides, the higher IMF content in hams from IG could improve the development of typical aromatic and textural properties (Kaltnekar et al., 2016).

The pieces from IG presented higher ($P < 0.05$) sodium chloride and sodium nitrite contents than those from EG, which exerts a greater preservative effect. The values of both compounds were within the

Table 4
Effect of gilt immunocastration on chemical composition (least square means) of the *biceps femoris* muscle of dry-cured hams.[a]

	Type of gilt		SEM[b] (n = 10)	P-value
	Entire	Immunocastrated		
Moisture, %	57.1	55.3	0.320	0.001
Ash, %	6.58	7.02	0.175	0.091
Protein, %	31.3	31.8	0.262	0.243
Intramuscular fat, %	4.39	5.54	0.387	0.049
Sodium chloride, g/100 g	4.68	5.24	0.176	0.037
Potassium nitrate, mg/kg	98.8	117.1	8.217	0.132
Sodium nitrite, mg/kg	0.42	0.66	0.061	0.014
Collagen[c], %	1.23	1.36	0.097	0.346
Water activity, a_w	0.91	0.90	0.002	0.015
α-Tocopherol, μg/g	4.02	3.97	0.189	0.869
γ-Tocopherol, μg/g	0.28	0.26	0.021	0.639
δ-Tocopherol, μg/g	0.02	0.02	0.001	0.252
Retinol, ng/g	17.4	20.6	1.406	0.126
Cholesterol, mg/g	0.85	0.89	0.017	0.074
Malondialdehyde, mg/kg	0.50	0.49	0.044	0.905

[a] Results expressed on wet matter basis.
[b] SEM: standard error of the mean.
[c] Least square means and SEM of the original data and P-value obtained with the transformed data.

normal range for this kind of product (Rodríguez-Sánchez et al., 2014). It has to be noted that experts from the World Health Organization and from the Food and Agriculture Organization of the United Nations recommend avoiding daily consumption above 5 g of sodium chloride to prevent diet-related chronic diseases (WHO/FAO, 2003). Potassium nitrate followed the same line, but the effect was not significant (P = 0.132). These differences could be because salt and nitrites are more concentrated in the muscle of those animals with lower moisture content (IG). Also, some factors during salting could have differed between hams, which might have influenced the formation of the surface brine and the diffusion of sodium, chloride and nitrite ions. Arnau (2007) indicated that these factors are: the way to place hams and the ham situation in the stackable bins during salting, the lean surface shape, the size and pH of the hams, the water holding capacity of the muscles and the content of water in the surface of the hams. Therefore, further studies are needed to understand better the effect of female immunocastration on salt contents of dry-cured ham.

Hams from IG had similar (P = 0.346) collagen proportion than those from EG, confirming the lack of effect found on texture analysis. The lower moisture content and the higher sodium chloride concentration detected in hams from IG led to lower (P = 0.015) a_w than that observed in EG, which would suggest lower microbial growth and thus a greater shelf life (Blesa et al., 2008). In male pigs, results are contradictory; Čandek-Potokar et al. (2020) did not observe differences in a_w of the *biceps femoris* muscle between entire and immunocastrated males and Škrlep et al. (2016) found that immunocastrated males tended to present higher a_w than entire males.

No significant differences (P > 0.05) were found in α-tocopherol, γ-tocopherol, δ-tocopherol and retinol concentrations. It is worth noting that hams from IG tended (P = 0.074) to show greater cholesterol content than those from EG, being this compound a major factor in the pathogenesis of atherosclerosis (Connor & Connor, 2002). However, this cholesterol content (0.89 mg/g of ham) is not worrisome, because the maximum daily consumption recommended by healthy reasons is 300 mg of cholesterol (WHO/FAO, 2003). Both types of hams presented similar (P = 0.905) content of MDA, confirming the results of Gamero-Negrón et al. (2015) with dry-cured loins and shoulders from EG and IG, and those of Čandek-Potokar et al. (2020) by comparing dry-cured hams from entire and immunocastrated males. This result is in accordance with the lack of effect found on tocopherol concentrations, because these compounds have antioxidant function (Di Mascio, Murphy, & Sies, 1991).

3.4. Fatty acid profile of IMF

Table 5 shows the impact of gilt immunocastration on FA profile of the *biceps femoris* muscle of dry-cured hams. This immunization against GnRF had no effect (P > 0.10) on total SFA and MUFA concentrations and either on PUFA/SFA and n-6/n-3 ratios. However, hams from IG tended to present lower total PUFA (P = 0.057), n-3 (P = 0.098) and n-6 (P = 0.062) contents than those from EG, although, as seen previously, lipid oxidation (the content of MDA) was not affected. The reason could be that in IG the higher IMF content counterbalances the effect that fat tends to be less polyunsaturated. These last findings were due to the fact that IG presented or tended to show lower values of C18:2n-6 (P =

Table 5
Impact of gilt immunocastration on fatty acid profile (mg/100 g of muscle, on wet matter basis) (least square means) of the *biceps femoris* muscle of dry-cured hams.

	Type of gilt		SEM[a]	P-value
	Entire	Immunocastrated	(n = 10)	
C8:0	0.66	0.59	0.081	0.569
C9:0	0.30	0.34	0.037	0.428
C10:0	1.91	1.89	0.162	0.926
C11:0	0.60	0.63	0.070	0.807
C12:0	1.18	1.21	0.107	0.829
C12:1-9c[b]	1.34	0.81	0.489	0.676
C13:0	0.06	0.05	0.016	0.629
C14:0	15.2	15.5	1.259	0.837
C14:1[b]	0.26	0.30	0.050	0.498
C15:0	0.66	0.63	0.054	0.730
C16:0	325	320	18.75	0.831
C16:1-7c	5.26	4.74	0.194	0.079
C16:1-9c	31.1	29.3	2.688	0.654
C16:1-11c	2.73	2.49	0.149	0.278
C17:0	2.72	2.73	0.201	0.974
C17:1-9c	1.62	1.42	0.128	0.271
C18:0	177	174	9.006	0.840
C18:1-9c	456	464	32.53	0.863
C18:1-11c	46.8	45.0	3.032	0.688
C18:2n-6[b]	287	264	9.033	0.078
C18:3n-3	7.60	7.20	0.417	0.504
C18:3n-6	5.14	5.38	0.414	0.688
C19:0	0.39	0.34	0.056	0.493
C19:2n-6[b]	0.13	0.22	0.043	0.149
C20:0	1.50	1.41	0.101	0.560
C20:1	7.28	7.71	0.646	0.644
C20:2n-6	6.72	6.65	0.275	0.869
C20:3n-6[b]	9.72	8.86	0.511	0.221
C20:4n-6[b]	79.5	70.9	3.614	0.087
C20:5n-3	2.24	1.81	0.134	0.037
C21:0[b]	0.08	0.09	0.025	0.949
C22:0	0.31	0.32	0.040	0.794
C22:3n-3[b]	3.60	4.14	0.412	0.445
C22:4n-6	11.6	10.4	0.387	0.049
C22:5n-3	6.15	5.23	0.247	0.018
C22:5n-6	5.96	5.08	0.417	0.150
C22:6n-3	2.09	1.97	0.142	0.566
C24:0	0.29	0.34	0.063	0.576
Total SFA[c]	528	520	30.44	0.860
Total MUFA[d]	552	555	39.03	0.949
Total PUFA[b,e]	428	392	13.53	0.057
PUFA/SFA	0.83	0.76	0.038	0.245
Total n-3	21.7	20.4	0.541	0.098
Total n-6[b]	406	372	13.33	0.062
n-6/n-3	18.9	18.3	0.638	0.534

[a] SEM: standard error of the mean.
[b] Least square means and SEM of the original data and P-value obtained with the transformed data.
[c] SFA: saturated fatty acids.
[d] MUFA: monounsaturated fatty acids.
[e] PUFA: polyunsaturated fatty acids.

0.078), C20:4n-6 ($P = 0.087$), C20:5n-3 ($P = 0.037$), C22:4n-6 ($P = 0.049$) and C22:5n-3 ($P = 0.018$). Gamero-Negrón et al. (2015), in dry-cured shoulders of Iberian × Duroc females, did not find differences on total SFA, MUFA and PUFA contents between EG and IG, but in dry-cured loins, those authors did observe that IG had greater total SFA percentage and lower total PUFA content. Therefore, further studies are needed to understand better the effect of gilt immunocastration on the fatty acid profile of cured products.

3.5. Volatile compounds

A total of 40 volatile compounds, generated mainly by proteolysis and lipolysis (Toldrá, 1998), were identified in the *biceps femoris* muscle of the experimental dry-cured hams (Table 6), including the following groups: aldehydes (9), ketones (7), acids (3), hydrocarbons (7), alcohols (11), furans (2) and sulfur compounds (1).

Despite the lower percentage of octanal ($P = 0.006$), (*E*)-hept-2-enal ($P = 0.023$), nonanal ($P = 0.024$), (E)-2-octenal (a trend, $P = 0.059$) and (E)-2-nonenal ($P = 0.040$) found in hams from IG, immunocastration had no significant influence ($P = 0.603$) on the proportion of total aldehydes, being this group the most abundant (around 70% of the total). Škrlep et al. (2016) did not find differences on total aldehydes with male pig immunocastration. These compounds play an important role in ham aroma due to their low perception thresholds and their distinctive characteristic odors (Pugliese, Sirtori, Calamai, & Franci, 2010). According to Flores, Grimm, Toldrá, and Spanier (1997), these compounds generate green, oily, fatty and tallowy flavors.

Hams from IG presented similar percentage ($P = 0.738$) of total ketones than EG, in agreement with the results of Škrlep et al. (2016) comparing immunocastrated and entire male pigs. Within this group of compounds, IG tended ($P = 0.080$) to present a higher proportion of 2,3-butanedione, which is responsible for buttery notes (Flores et al., 1997), and lower proportions of 2-heptanone ($P = 0.011$), 3-octanone ($P = 0.002$) and 2-octanone ($P = 0.002$) than EG. The 2-heptanone and 2-octanone are methyl ketones having a very strong odor and being neat contributors to ham aroma (García et al., 2013; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998). The sensory attributes of 2-heptanone are blue cheese and spicy and a great intensity of these perceptions has been described as a symptom of bad quality, because this compound can be formed by microorganisms (Berdagué, Denoyer, Le Quéré, & Semon, 1991; Creuly, Larroche, & Gros, 1992; Luna, Aparicio, & García-González, 2006; Sabio et al., 1998). In contrast, 2-octanone has been associated to the olfactory characteristics green and herbaceous (Berdagué et al., 1991). Also, 3-octanone is a remarkable ketone since its very low odor threshold makes it to contribute to ham aroma with spicy, mushroom and dirty notes (García-González, Aparicio, & Aparicio-Ruiz, 2013).

Gilt immunocastration had no significant influence ($P > 0.05$) on any individual acid or hydrocarbon, nor on the total percentage of acids or hydrocarbons. However, hams from IG presented lower ($P = 0.044$) proportion of total alcohols than those from EG, owing to the lower percentages of 1-pentanol ($P = 0.005$), hexanol ($P = 0.009$), 1-octen-3-ol ($P = 0.002$) and 1-octanol ($P = 0.043$). Conversely, Škrlep et al. (2016) found in hams from male pigs that immunocastration tended to increase the relative abundance of total acids and alcohols. We have no explanation for it but we will study it in more detail in the future. Alcohols are compounds with multiple origins (Škrlep et al., 2016) and their influence in the overall aroma seems to be low, since they have higher flavor thresholds compared to carbonyl compounds (Drumm & Spanier, 1991). Nevertheless, in general, the straight chain primary alcohols, such as 1-pentanol, hexanol and 1-octanol, did affect the overall flavor (Garcia et al., 1991). According to García-González, Tena, Aparicio-Ruiz, and Morales (2008), the aroma notes of 1-pentanol are pungent, strong and balsamic, of hexanol fruity and green and of 1-octanol fatty and sharp. In addition, unsaturated alcohols, such as 1-octen-3-ol, may play an important role in the odor because they have a lower threshold value (Sabio et al., 1998). This compound has a mushroom-like, earthy and dust aroma notes (García-González et al., 2008). Therefore, the lower presence of 1-octen-3-ol in hams from IG could indicate a positive effect on their sensory characteristics.

Table 6
Effect of gilt immunocastration on volatile compounds (chemical compound area/internal standard-fluorobenzene- area expressed as %) (mean ± standard error) of the *biceps femoris* muscle of dry-cured hams.

	Type of gilt		*P*-value
	Entire	Immunocastrated	
Aldehydes			
3-Methylbutanal	67.70 ± 1.095	69.30 ± 2.622	0.395
Hexanal	1.126 ± 0.326	1.322 ± 0.349	0.794
Heptanal	0.172 ± 0.042	0.082 ± 0.020	0.120
Octanal	0.062 ± 0.014	0.016 ± 0.004	0.006
(*E*)-Hept-2-enal	0.080 ± 0.025	0.010 ± 0.006	0.023
Nonanal	0.144 ± 0.029	0.049 ± 0.009	0.024
(E)-2-Octenal	0.039 ± 0.009	0.015 ± 0.004	0.059
Benzaldehyde	0.306 ± 0.127	0.252 ± 0.137	0.940
(E)-2-Nonenal	0.019 ± 0.006	0.006 ± 0.001	0.040
Total aldehydes	69.65 ± 0.896	71.07 ± 2.483	0.603
Ketones			
2-Propanone	17.44 ± 0.757	17.50 ± 2.395	0.738
2-Butanone	2.718 ± 0.274	4.519 ± 0.954	0.105
2,3-Butanedione	1.031 ± 0.183	1.428 ± 0.165	0.080
2-Pentanone	0.006 ± 0.001	0.017 ± 0.009	1.000
2-Heptanone	0.635 ± 0.176	0.195 ± 0.073	0.011
3-Octanone	0.053 ± 0.015	0.007 ± 0.002	0.002
2-Octanone	0.054 ± 0.014	0.009 ± 0.002	0.002
Total ketones	21.94 ± 0.902	23.68 ± 2.497	0.738
Acids			
2-Methylpropanoic acid	0.188 ± 0.129	0.021 ± 0.004	0.079
Butanoic acid	0.920 ± 0.194	0.596 ± 0.066	0.320
Hexanoic acid	1.415 ± 0.262	1.368 ± 0.223	0.970
Total acids	2.523 ± 0.342	1.985 ± 0.182	0.256
Hydrocarbons			
Hexane	0.126 ± 0.033	0.119 ± 0.070	0.208
Heptane	0.189 ± 0.063	0.232 ± 0.050	0.527
Octane	1.814 ± 0.369	1.259 ± 0.288	0.157
α-Pinene	0.010 ± 0.004	0.012 ± 0.004	0.519
Methylbenzene	0.290 ± 0.071	0.444 ± 0.074	0.120
Ethylbenzene	0.005 ± 0.003	0.002 ± 0.002	0.732
Limonene[a]	0.007 ± 0.006	–	0.094
Total hydrocarbons	2.440 ± 0.356	2.067 ± 0.297	0.396
Alcohols			
Ethanol	0.474 ± 0.250	0.137 ± 0.032	0.970
2-Propanol	0.094 ± 0.026	0.051 ± 0.022	0.265
2-Butanol	0.004 ± 0.001	0.004 ± 0.001	1.000
2-Methyl-1-propanol	0.067 ± 0.013	0.128 ± 0.041	0.320
Butanol	0.060 ± 0.036	0.001 ± 0.001	0.100
3-Methyl-1-butanol	0.552 ± 0.324	0.293 ± 0.102	0.852
1-Pentanol	0.225 ± 0.030	0.102 ± 0.016	0.005
2-Heptanol	0.023 ± 0.007	0.009 ± 0.003	0.119
Hexanol	0.397 ± 0.082	0.103 ± 0.024	0.009
1-Octen-3-ol	0.132 ± 0.011	0.050 ± 0.009	0.002
1-Octanol	0.011 ± 0.002	0.005 ± 0.002	0.043
Total alcohols	2.038 ± 0.428	0.884 ± 0.170	0.044
Furans			
2-Ethylfuran	0.420 ± 0.113	0.171 ± 0.109	0.031
2-Pentylfuran	0.526 ± 0.289	0.001 ± 0.001	0.002
Total furans	0.946 ± 0.353	0.171 ± 0.109	0.017
Sulfur compounds			
Dimethyl disulfide	0.468 ± 0.125	0.159 ± 0.050	0.069
Total sulfur compounds	0.468 ± 0.125	0.159 ± 0.050	0.069

[a] Undetected value.

Hams from IG showed lower ($P = 0.017$) percentage of total furans, owing to the lower 2-ethylfuran ($P = 0.031$) and 2-pentylfuran ($P = 0.002$) proportions, which seem to derive from Maillard reaction and from autoxidation of linoleic acid, respectively (Drumm & Spanier, 1991). Škrlep et al. (2016) also observed lower normalized areas of 2-ethylfuran and 2-pentylfuran in hams from immunocastrated males than in those from entire males. According to Elmore, Mottram, Enser, and Wood (1999), it is unlikely that furans contribute significantly to the flavor characteristics because their odor threshold values are relatively high. Nevertheless, 2-pentylfuran has a quite low odor threshold and green fruity notes, and thus, it could play an important role in the overall aroma (García-González et al., 2008).

Finally, hams from IG tended to present lower ($P = 0.069$) percentage of the only sulfur compound detected (dimethyl disulfide) than those from EG. This compound arises from the oxidation of methane thiol (Flores et al., 1997) and has a low odor threshold (García-González et al., 2008), and thus, it could be an important contributor to ham flavor. In the literature (Flores et al., 1997; García-González et al., 2008) it has been reported that dimethyl disulfide presents an unpleasant aroma defined as dirty socks or cauliflowers. Therefore, hams from IG could have a better flavor than those from EG. Lastly, it is worth noting that the higher amount of fat found in hams from IG than in those from EG could be an important reason of the differences in the proportions of volatile compounds, although further research has to be carried out in this field.

4. Conclusions

Under our experimental conditions, it can be concluded that gilt immunocastration increases the fat deposition of dry-cured hams, both subcutaneous and intramuscular, tending to reduce weight losses during the dry-curing process, which is positive for Teruel ham elaboration. Immunization against GnRF of gilts does not affect (or very slightly) some quality variables of hams as color, texture, oxidation or fatty acid profile, but it seems to increase the contents of sodium chloride and sodium nitrite, although the concentrations detected were normal ranged for this type of product. Additionally, immunocastration could prolong the shelf life of dry-cured hams by reducing their water activity and favor their aroma by reducing some unpleasant odors.

CRediT authorship contribution statement

Leticia Pérez-Ciria: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Guillermo Ripoll:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing. **María Á. Sanz:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing. **Mireia Blanco:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Francisco J. Miana-Mena:** Conceptualization, Methodology, Investigation. **Maria A. Latorre:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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