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The effects of different immunocastration protocols on meat quality traits and boar taint compounds in male Bísaro pigs

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ABSTRACT

Immunocastration can be an alternative to surgical castration in Bísaro pigs when there is a need to keep animals on the farm until at least 8 months old. As an autochthonous breed, some particularities must be addressed when doing immunocastration, for which 3 different protocols were tested and two control groups were made with surgically castrated males (SC) and boars (Bo). Two protocols were made in prepubertal pigs, with two (E2) and three inoculations (L3) in the first cycle, and another in adults (A2) with only two inoculations. Physicochemical parameters and boar taint compounds quantification and sensory analysis of the meat from the studied pigs were assessed. Immunocastration provided intermediate values between surgically castrated pigs and entire males, with low levels of boar taint compounds. The L3 group provided closer results to SC, which was also corroborated by the sensory analysis. Although the other two protocols had no significant differences with Bo, there was a positive tendency towards them. As is, the L3 protocol was promising as a good alternative to surgical castration, maintaining the characteristic attributes of the Bísaro pig meat.

1. Introduction

The Bísaro pig is one of the most representative Portuguese autochthonous breeds [1] and is known for its robustness, highly valued meat quality [2] and for producing high-quality smoked-cured meat products holding multiple certifications [3]. To obtain such products, animals are usually reared to older ages, at least 8 months old [4]. In addition, male Bísaro pigs reach puberty earlier than other pigs from commercial crosses, 3–4 months old on average [5]. Consequently, they are routinely surgically castrated, usually at weaning, to avoid boar taint, hierarchical fighting, and unwanted pregnancies, as many are fattened in heterosexual drifts [6].

Alternatives are being considered to avoid this painful procedure. Raising entire males is being gradually implemented in many pig-breeding countries [7,8]. However, attempting to raise entire animals with enhanced genetics or altering their diet to reduce boar taint can pose significant challenges in terms of productivity and animal welfare when implemented in alternative systems, ultimately resulting in the need to slaughter animals at younger ages. A promising alternative is immunocastration, yet, like other pig breeds reared in alternative systems, the conventional two-dose protocol is insufficient as many farms include periods of extensive grazing with limited handling facilities and the risk of unintentional matting of females with male cohorts or wild boars. It's important for the immunocastration to begin as early as needed and be extended for longer periods, which is different from the short-term approach used in industrial systems. To address this need for adaptation, long-term immunocastration protocols specific to the Bísaro breed were proposed by Paixão et al. [9]. Immunocastration refers to the annulment of the GnRH activity, the primary hormone orchestrating the hypothalamic-pituitary-gonadal axis activity, thus suppressing the

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gonadal functionality. Improvac® (Zoetis) is the sole product licensed for swine immunocastration in Europe to reduce the boar taint in pork. Improvac® is a vaccine composed of a synthetic GnRH analog, conjugated with a carrier protein (diphtheria toxoid); the conjugate is combined with synthetic aqueous adjuvant (DEAE-Dextran) to promote the necessary immune response (in terms of level and duration of immunity) [10]. After a correct administration of the vaccine (two administrations four weeks apart) enough antibodies against GnRH were generated to block the GnRH secretion, and by consequence that of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [11]. The suppression of the hypothalamic-pituitary axis will drive a decrease in spermatogenesis and testicular steroids synthesis [12], as well as a decrease in androstenone and skatole content in the body [13].

The efficacy of Improvac® immunization may be variable with the farm and individuals. It has been reported that up to 3 % of animals do not respond or respond poorly to the two vaccinations [14]. A stressful environment and poor animal well-being, the stress at vaccination, a subclinical illness or an accidental failure during the vaccination are some of the factors that might impair the quality of the immune response [12]. Not noticing the reduction of the scrotal volume may help identify animals not responding to the vaccination, and a third administration of the vaccine may be pondered.

Immunocastration has been shown to impact carcass and meat quality traits [15,16]. Differences in carcass traits are more noticeable and widely agreed upon, while variations in meat parameters are generally minor and lack consistency [17]. In sum, carcasses and meat from immunocastrated animals will occupy an intermediate position between entire and surgically castrated animals [18–20], behaving more or less like surgically castrated animals depending on the onset and duration of the reproductive suppression until slaughter [21]. The same conclusions were achieved in heavier and older pigs from similar autochthonous breeds [22–26]. Conversely, boar taint can severely affect meat palatability and consumer acceptance. Boar taint is an unpleasant odor or taste that can be evident during the cooking or eating of pork or pork products derived from entire post-pubertal male pigs (boars) [27], and thus it is an essential sensory meat quality parameter. It is caused by the accumulation of androstenone and skatole in fat tissues. Androstenone is a testicular steroid acting as a pheromone, secreted into the boar saliva; it has a urine-like odor and is most likely found in older and heavier animals [28]. Skatole is produced by microbial degradation of the amino acid tryptophan and its metabolism is modulated by gonadal steroids in the liver and origins a more fecal-like odor [29].

While the effectiveness of the immunocastration for the Bísaro pig has already been demonstrated [9], we need to rule out the impact of this technique on meat and carcass quality, especially when it could potentially affect the production of cured meat products. The necessity to validate these protocols implies guaranteeing that most of the organoleptic parameters of the meat are preserved. Therefore, the objective of the present study was to evaluate meat quality traits from long and short-term immunocastrated male Bísaro pigs, while comparing them to entire and surgically castrated pairs.

2. Materials and methods

This study was conducted on a Bísaro pig farm in Bragança (Portugal) from August 2019 to September 2020. All the animals were handled according to the Portuguese law on animal welfare in experimental research (Decree-Law No. 1/2019, January 10th), the Directive 2010/63/EU of the European Parliament, and the European Council of September 22, 2010. The study protocol was approved by the animal welfare body (ORBEA) of the University Trás-os-Montes and Alto Douro (reference 1767-e-DCV-2019).

2.1. Study design

Fifty-one male Bísaro pigs were used in the present study. All animals were housed indoors until they were 29 weeks old and thereafter in outdoor pens. They were fed *ad libitum* and added beet supplementation once outdoors. The animals were then slaughtered at 57 weeks old.

Three immunocastration protocols were performed. Vaccination schemes are depicted in Fig. 1. Two of them, E2 and L3 were performed in prepubertal pigs, already described in Paixão et al. [9]. In that study [9], comprehensive *in vivo* data were collected, including testicular measurements, which were employed as indicators to assess the efficacy of immunocastration. Priming started at 9 weeks old in the Early group (E2, n = 6), at 13 weeks old in the Late group (L3, n = 11) and in the third group, post-pubescent Adults, priming was done at 49 weeks old (A2, n = 10). Groups E2 and L3 had two inoculation cycles while in the A2 group, only one inoculation cycle was performed, with the booster injection done 4 weeks after priming at 49 weeks old. No deaths were recorded in this study, except in group E2. This group had 9 animals, of which one died at 10 weeks old and two in the slaughterhouse's lairage; neither death was related to the immunocastration procedure.

Two control groups were used. A group of entire males, Boars (Bo, n = 11) was used to compare the size of the testis and assess the effect of immunocastration, as described in Paixão et al. [9]. The other group, of surgically castrated males (SC, n = 14), was used to compare meat quality traits, as Bísaro pigs are mostly castrated at weaning. Both control groups were also slaughtered at 57 weeks old.

2.2. Meat sampling

The animals were fasted for 15 h, and their live weight was recorded the day before slaughter. The pigs were transported for a period not exceeding 2 h and sacrificed in an official slaughterhouse, complying with the current welfare hygienic legislation for food of animal origin (Council Regulation No 1/2005/EC; Council Directive 93/119/EC; Regulation No 853/2004/EC).

The animals were followed along the slaughter line until evisceration. A portion of fat from the neck area was collected and identified into individual containers to guarantee traceability, then stored at 4 °C and transported to the laboratory for further analysis. Carcasses were split lengthwise and hot carcass weights were individually recorded to calculate carcass yield. The Longissimus thoracis et lumborum (LTL) muscle from the left side of the carcasses of all the animals in this experiment was used for meat quality assessment.

2.3. Physicochemical analysis

At 45 min postmortem, still in the slaughterhouse line, the pH_{45min} was recorded using pH meter WTW 330i (Weilheim, Germany) after calibration with pH 4.01 and 7.00 buffers. Measurements were made in duplicate, between the 13th-14th thoracic vertebrae. LTL muscle, between the 7th thoracic vertebrae and 3rd lumbar vertebrae, was excised from the carcass at 24 h after slaughter, when carcass left halves were dissected into commercial cuts. LTL samples were taken refrigerated at 4 °C to Laboratório de Tecnologia, Qualidade e Segurança Alimentar (TeQSA) at the University of Trás-os-Montes and Alto Douro (UTAD), Vila Real (Portugal), where they were trimmed of fat and connective tissue and sliced for further analysis. Meat quality traits in fresh cuts included pH_{24h}, color coordinates (L^*, luminosity; a^*, red-green; b^*, yellow-blue; C*, chroma; and h°, hue angle), drip loss, cooking loss, and shear force. A sample of approximately 100 g was vacuum packed and stored at −20 °C for subsequent chemical analysis and another sample of approximately 400 g was vacuum packed and stored at −20 °C for sensory analysis. Fat samples from the neck area were also preserved at −20 °C for further boar taint compound quantification.

The ultimate pH (pH_{24h}), 24 h postmortem, was measured at the laboratory in duplicate with the same equipment as the one used for

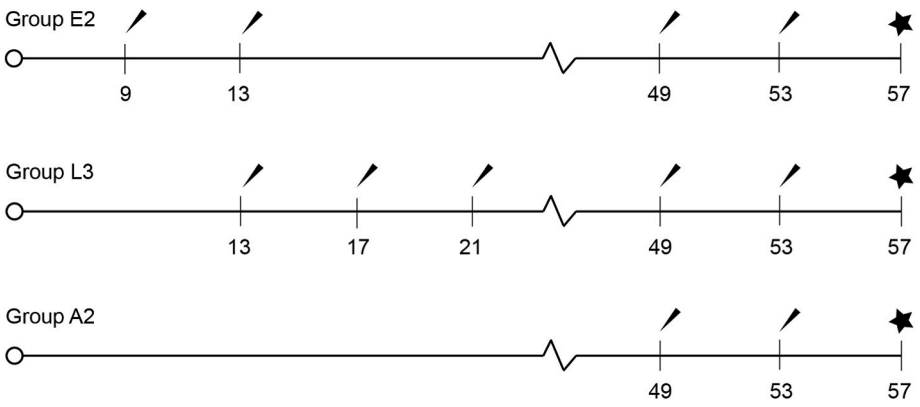

Fig. 1. Scheme of the vaccination protocols (weeks). Arrow: injection; Star: slaughter.

pH_{45min}.

The color was measured in a slice of about 2 cm thickness with a Minolta Chroma Meter CR-310 colorimeter (Osaka, Japan) and assessed using the color coordinates L^*, a^*, and b^*, C* and h° [30]. The color was measured on the meat surface after 60 min of blooming, by placing the samples in trays covered with polyethylene film and stored at 4 °C. The colorimeter was calibrated with a standard white ceramic plate and D65 illuminant.

Heme pigments were obtained by stirring for 30 sec. 10.0 g of minced meat in 40 mL acetone and 2 mL of water and 1 mL HCl 12 M. The suspension in a sealed flask was kept for 1 h in the dark, filtered (Whatman n°1) and the absorbance was read at 640 nm (Jasco V-530 UV/VIS Spectrophotometer, Tokyo, Japan) in a cell of 1 cm path length. Absorbance values are multiplied by 680 [31] and expressed in total heme pigments (mg/g) multiplied by 0.026 factor [32].

Drip loss was determined using the suspension method of Honikel [33]. Loin samples of about 65 g were put into a plastic net and suspended in a closed plastic bag for 3 days at 4 °C. Drip loss was expressed as a percentage of mass loss relative to the initial mass of the sample.

Cooking loss was evaluated in slices of similar geometry with about 100 g. The samples were then placed individually inside polyethylene bags and heated in a water bath at 80 °C until an internal temperature of 75 °C was reached (monitored with thermocouples introduced in the core). Heated samples were cooled in an ice bath until they were at 4 °C. Cooled samples were removed from the bag, dried with filter paper, and weighed. Cooking loss was expressed as the percentage of mass loss relative to the initial sample mass [34]. After determination, samples were packed in plastic bags and stored overnight at 4 °C for shear force measurements.

Meat samples used to determine the cooking losses were cut into cuboid shape sub-samples (4–6) of 1 cm^2 cross-section and 3–4 cm in length with muscle fibers parallel to the length of the cuboid. After room temperature equilibrium, the sub-samples were placed with muscle fibers perpendicular to the direction of a Warner–Bratzler rectangular hole probe coupled to a TA.XT.plus texturometer (Stable Micro Systems, Godalming, UK), with a 30 kg load cell., blade velocity 200 mm/min and trigger force of 5.00 g. Maximum shear force values were recorded and the values were expressed in N/cm^2.

The approximate chemical composition comprised the determination of moisture, fat, protein, and ashes. The moisture content was performed by drying 5.00 g of homogenized sample in an oven at 103 °C to constant mass according to ISO 1442:1997. Results were expressed in percentage by mass. The lipid content was determined following the protocol established by the American Oil Chemists' Society (AOCS) Official Procedure Am 5-04 using the fat extractor Ankom XT10 (ANKOM Technology Corp., Macedon, NY, USA). The determination of the total nitrogen content was carried out by the Kjeldhal method (AOAC, 1990). The digestion of 1.00 g of homogenized sample was carried out in an Inkjel 1255P digester (beher Labor-Technik, Germany) with the addition of 1.5 catalyst pellets (7.5 g), composed of potassium sulfate and copper sulfate (Merck 1.15348) and 17 mL of concentrated sulfuric acid. The total digestion time was 150 min with a gradual increase in temperature. For distillation, 100 mL of 35 % sodium hydroxide, 50 mL of distilled water and 30 mL of 4 % boric acid with indicator (bromocresol green and methyl red) were used and subsequently titrated with 0.1 N HCl. Distillation and titration were performed on a VELP UDK 159 apparatus (Velp Scientifica Srl, Usmate, Italy). Total nitrogen content was converted to protein content using factor 6.25 and expressed as a percentage. The ash content was determined after the incineration of 1500 g of the homogenized sample in a muffle furnace at 550 °C, according to ISO 936:1998. Results were expressed as a percentage by mass.

Androstenone (AND) and skatole (SKA) contents from fat samples were measured using an HPLC analysis based on the method described by Hansen-Moller [35]. Liquid fat was extracted from the adipose tissue after microwave heating (800 W, 2 min). Methanol was added, and samples were sonicated and centrifuged before passing through a 0.2 μm filter. Manual derivatization was performed at room temperature for 5 min adding 40 μL of BF3, 50 μL of deionized water, and 75 μL of dansylhidrazine 0.1 %. An HPLC system (Thermo Scientific UltiMate 3000, Waltham, MA, USA) equipped with AkzoNobel® Kromasil 100-5C18 250 × 4.6 mm 5 μm column (Bohus, Sweden) operating at 40 °C. The composition of the mobile phase buffers was as follows: (A) acetic acid 0.1 %, (B) acetonitrile, (C) tetrahydrofuran, and (D) methanol 95 %. The used gradient profile, with a 2 mL/min flow, follows: 0.0–5.0 min: 45 % A, 55 % B; 5.0–6.0 min: 40 % A, 55 % B, 5 % C; 6.0–6.1 min: 20 % A, 30 % B, 30 % C, 20 % D; 6.1-12-0 min: 40 % B, 40 % C, 20 % D; 12.0–12.1 min: 45 % A, 55 % B; 12.1–13.0 min: 45 % A, 55 % B. Analytes were detected with a fluorescence detector with excitation at 285 nm and emission at 340 nm (0–6.0 min) for skatole, and excitation at 346 nm and emission at 521 nm (6.1–13 min) for androstenone. Twenty microliters of sample were injected. An external calibration method was used for quantification, with a calibration curve linearity coefficient of 0.999 for both compounds. Limits of detection (LoD) were 16.02 and 1.53 ng/mL for androstenone and skatole, respectively. Recovery values were 102,84 % for androstenone and 99,72 % for skatole. The method validation was performed, with repeatability <2.46 % RSD for SKA, <6.85 % RSD for AND, and intermediate precision was <2.87 % RSD for SKA, and <6.98 % RSD for AND [36].

2.4. Sensory analysis

In the first phase, twenty-two adults (thirteen females) were tested based on their sensory performance based on the ISO 8586:2012 [37] methods (5 sessions for each person). Additionally, boar taint was evaluated, and people with anosmia to AND or SKA were excluded. Ten

panelists were excluded due to their low performance. The training of the remaining 12 panelists was done according to a procedure adapted from Garrido et al. [38]. Standard solutions were prepared for AND (5α-androst-16-en-3-one, M 272.43 g mol−1, Sigma-Aldrich A8008, Saint Louis, USA) and SKA (3-methylindole, M 131.17 g mol−1, Sigma-Aldrich M51458, Saint Louis, USA) using Vaseline oil. Working solutions and the solutions made from them were stored in amber glass vials with cotton inside. Several tests were made using the vials to train the panelists, such as descriptive, ranking, classification, and triangular tests with the concentrations described in Garrido et al. [38], over the course of 16 sessions. The training was also carried out with loins from pigs with known levels (low and high) of boar taint compounds over 5 sessions.

Meat and neck fat samples were thawed at 4 °C for 24 h before assessment. Eight pigs per treatment were randomly selected, except for group E2 which only had 6 animals. Neck fat samples were sliced into 1.5 × 1.5 cm pieces with 0.5 cm thickness and put in a commercial microwave at 700W for 60 s or until the fat slightly melted [39–41]. No additional seasoning was included to ensure the boar taint remained unaltered [38,42]. Loin fillets (1.5 cm) were cooked and served as described in Silva [43]. Pieces measuring approximately 2 × 2 cm were extracted from the cooked fillets. Two pieces of the fat samples and two of the meat samples were then carefully wrapped in aluminum foil individually and maintained at a temperature of 60 °C until they were evaluated, which took place within 30 min after the cooking process. Each sample was presented with a unique three-digit identifier, and the order in which the samples were presented was randomized to mitigate any potential sequencing bias. All tests were performed in a sensory analysis laboratory, with individual booths, consistent illumination conditions and room temperature between 18 and 25 °C. Spring water at room temperature and bread were used as palate cleansers between sample evaluations. Assessors were asked to smell and taste the samples and evaluate - global intensity, boar taint, urine, manure; and texture (juiciness, tenderness, and chewiness), on a scale from 1 to 9 without intermediate values. Fat samples were only evaluated for aroma attributes.

2.5. Statistical analysis

Meat quality traits and sensory analysis comparison were conducted with IBM SPSS Statistics (v.29) software. Variables were tested to assess their distribution and the normality of the data using Shapiro-Wilk test. The data for live and carcass weight, pH_{45min}, color coordinates a^* and b^*, heme, protein, and ashes, seeing as they belong to the normal distribution, were analyzed by ANOVA with group set as fixed factor. In case of significant differences ($p < 0.05$) Tukey's HSD test was used to further differentiate treatments. Nonetheless, the variables dressing, pH_{24h}, L^*, C*, h°, drip loss, cooking loss, shear force, moisture, intramuscular fat, androstenone, skatole, and the sensory analysis parameters did not fit the normal distribution. In such cases, non-parametric tests were performed and differences between treatments were identified with the Kruskal-Wallis test, with a Bonferroni correction for pairwise comparisons. The values expressed in the results are means to improve understanding).

3. Results and discussion

3.1. Physicochemical traits

Carcass and physicochemical traits are shown in Table 1.

Live weight and carcass weight did not differ between castrated groups (SC, E2, L3 and A2). These findings are in accordance with several studies, in which the castration method did not influence these traits [44–47]. The Bo group also did not differ ($p < 0.05$) from SC and E2 groups, a fact also reported by several studies that showed no differences existed between castrated males and entire males [11,47–51]. However, some authors reported higher carcass weights in castrated animals compared to entire males [15,18,52], which was not verified in our study. The carcass weight was higher in Bo than in immunocastration protocols L3 and A2. Diverging results can be observed depending on the immunocastration protocol studied. Turkstra et al. [53] reported heavier carcasses from entire males as the present study did, but immunocastrated males could not be differentiated from either the entire or the surgically castrated males. This agrees with our finding for the E2 group, with intermediate values between entire males and surgically castrated males [19,54].

The dressing percentage was significantly lower ($p < 0.05$) in the L3 group compared to the other groups, which had no differences recorded among them. This parameter is often lower in entire males than in surgically castrated males due to the genital tract size [55]. The regression of the genital tract in immunocastrated males, making it smaller than in entire males, could also explain the lower dressing percentage compared to surgically castrated males [56]. The L3 group also presented better results regarding the suppression of reproductive activity, as their testis size remained within the prepubertal range for the longest duration compared to the other studied protocol [9].

Table 1
Carcass and physicochemical traits of the LTL muscle in entire males, surgically castrated males and males subjected to different immunocastration protocols.

Parameter	Bo (n = 11)	SC (n = 14)	E2 (n = 6)	L3 (n = 11)	A2 (n = 9)	SEM	p-value
Live Weight (kg)	171.35^{a}	160.03 ^{ab}	155.53 ^{ab}	149.87 ^{b}	146.18 ^{b}	2.29	0.002
Carcass Weight (kg)	136.04^{a}	127.75 ^{ab}	123.08 ^{ab}	117.23 ^{b}	115.50 ^{b}	1.98	0.003
Dressing (%)	79.33 ^{ab}	79.85^{a}	79.07 ^{ab}	78.24 ^{b}	79.03 ^{ab}	0.35	0.027
pH_{45min}	6.15 ^{ab}	6.23 ^{ab}	6.15 ^{ab}	6.10 ^{b}	6.45^{a}	0.04	0.045
pH_{24h}	5.41 ^{b}	5.61^{a}	5.52 ^{ab}	5.56^{a}	5.55^{a}	0.02	<0.001
L^*	51.41	52.74	50.27	51.36	48.42	0.61	0.147
a^*	21.94^{a}	19.22 ^{b}	22.85^{a}	22.29^{a}	21.65^{a}	0.25	<0.001
b^*	6.25	5.73	7.36	7.03	6.27	0.20	0.073
C*	22.54^{a}	22.83^{a}	24.04^{a}	23.39^{a}	20.10 ^{b}	0.28	0.000
h°	16.12	15.89	17.73	17.41	16.40	0.40	0.600
Heme (mg/g)	1.60 ^{ab}	1.44 ^{b}	1.97^{a}	1.65 ^{ab}	1.87^{a}	0.05	0.006
Drip loss (%)	4.15	3.87	4.65	4.89	3.17	0.22	0.105
Cooking loss (%)	27.75 ^{ab}	24.44 ^{b}	32.28^{a}	33.64^{a}	24.36 ^{b}	0.62	<0.001
Shear force (N/cm^2)	54.95 ^{ab}	53.05 ^{ab}	46.94 ^{b}	51.64 ^{ab}	77.21^{a}	2.69	0.015
Moisture (%)	73.08^{a}	71.39 ^{ab}	72.79 ^{ab}	72.26 ^{b}	72.67 ^{ab}	0.18	0.006
Protein (%)	22.79	23.53	23.39	23.42	23.51	0.11	0.124
Intramuscular Fat (%)	1.50^{a}	3.24 ^{b}	1.56 ^{ab}	1.84 ^{ab}	1.61 ^{ab}	0.20	0.048
Ashes (%)	1.14	1.10	1.15	1.12	1.12	0.01	0.166

All values are means.
[1] Bo: entire males, boars; SC: surgically castrated; E2: early immunocastration protocol; L3: late immunocastration protocol; A2: adult immunocastration protocol.
[a-b] Means within a row with superscript letters are significantly different ($p < 0.05$).

The mean values of pH_{45min} observed were higher in A2 (6.45) and lower in L3 (6.10) ($p < 0.05$), but not different from the other studied groups. The pH at 45 min postmortem is a valuable parameter to assess the extent of glycolysis in the early postmortem period and is directly related to meat quality [57,58]. Two of the major issues in pork meat are related to PSE (pale, soft, exudative) and DFD (dark, firm, dry) meats. PSE meats are characterized by values of pH at 45 min postmortem of less than 5.8 [57,58], which was not observed in the present study, i.e., no PSE meats were found. PSE meat's prevalence is associated with antemortem acute stress situations and mishandling of carcasses after slaughter [57,58]. Acute stress can lead to the fast rate of acidification of the muscles immediately postmortem, so low pH values (<5.8) are reached early postmortem when the temperature of the carcass is still high, originating a high denaturation of muscle proteins [57,58]. This reduces the water-holding capacity, increasing parameters such as drip loss and L^* parameter [57–59]. Temperature is also an important factor to consider in pig slaughter. Pigs are highly sensitive to thermal stress, especially high temperatures, and after slaughter, if the carcass is not properly cooled the glycolysis rate will rise and promote the decrease of the pH in the muscle [57–59]. The A2 group was slaughtered in the middle of winter, while the other studied groups were slaughtered from spring to summer, which might explain the slightly lower values for the groups slaughtered with higher temperatures. Čobanović [59] also reported that pigs slaughtered in the summer had lower pH_{45min} values with a tendency to PSE meat (5.93). The denaturation of muscle proteins reduces the water-holding capacity, increasing parameters such as drip loss and the L* parameter [57–59].

The ultimate pH, which indicates the completion of the glycolysis, was significantly lower in Bo compared to the other groups, although not different from E2, indicating higher acidification in the muscle. Considering the value of pH at 24h post-mortem higher than 6 as an indicator of DFD meats [57,58], the present study did not find any meat with these characteristics. Lower pH values are associated with lighter-colored pork meat due to increased water-holding capacity and improved stability of pigments, in opposition to higher pH levels (>6), which can result in a darker meat color due to the conversion of myoglobin to metmyoglobin [57,58]. Several authors observed that castration does not affect the ultimate pH of the meat [60,61], and the method of castration also has no impact on pH [18,62,63].

Regarding color parameters, there were no significant differences ($p > 0.05$) between groups for L^* (lightness), b^* (yellowness) and h°. The L*, which refers to the brightness of the meat surface, also aids in the determination of PSE or DFD meats. As mentioned, the meat color is influenced by the pH at 45 min and 24 h post mortem, in which lower levels of pH_{45min} are related to higher values of L^*, and higher ultimate pH are with lower L^* values [57–59]. Despite not having significant differences, the lower mean value of L^* (48.42) of A2 might be explained by the highest mean value of pH_{45min} (6.45) compared to the other groups. The myoglobin concentration is another factor that influences meat color, directly associated with the a^* and b^* coordinates [57,58]. The a^* (redness) values varied among the groups, with the SC group exhibiting the lowest a^* value (19.22; $p < 0.05$). Heme content was also significantly lower ($p < 0.05$) in the SC group compared to the E2 and A2 groups, indicating that the differences in a^* value are related to myoglobin concentration. Despite studying different muscles (pork bellies and LTL muscle), some authors also reported higher a^* values in meat from immunocastrated males than in the meat from surgically castrated males [51,64] and discrepancies in the b^* score: Jeong et al. [64] found higher values for entire males and Aluwé et al. [51] had entire males with less yellow. In the present study, there was a tendency for lower yellow scores in entire males compared to the pre-pubescent immunocastration protocols, but similar to the adult immunocastration protocol. The C* (chroma) value was significantly higher in the E2 group compared to the other groups, indicating a more saturated color. Batorek et al. [18], in a meta-analysis, found no differences in the color coordinates when comparing meat from immunocastrated males to meat from surgically castrated males and entire males.

Drip loss, a measure of water loss during storage, did not differ significantly among the groups. The lower mean value for the A2 group is consistent with the higher pH_{45min} values and lower L^* values [57–59]. Drip loss is an important meat quality trait because the consumer values meat and meat products that do not exude a lot of water. Batorek et al. [18] found no significant differences between immuno- and surgically castrated males, whereas an increased drip loss existed in immunocastrated males compared with entire males. Also Aluwé et al. [51] found enhanced drip loss in immunocastrated males compared to surgically castrated males, contrasting to the findings reported herein: despite the absence of significance, the drip loss was higher in early castrated males and boars compared to surgically castrated males or adult males castrated at culling.

The weight loss experienced during the cooking process is called cooking loss. This loss is mostly water, but also soluble proteins, vitamins, and minerals [65,66], thus impacting meat quality. Several authors report that neither castration nor the castration method has an impact on cooking loss [46,48,62,64,67], which is not in accordance with our findings. Cooking loss was significantly higher ($p < 0.05$) in the pre-pubescent immunocastration protocols (E2 and L3) compared to SC and A2, the Bo presenting intermediated values. Two key factors that strongly influence cooking loss are intramuscular fat content and pH levels (both 45 min and 24 h post-mortem). Higher intramuscular fat content is associated with lower cooking losses [68], as is the case with the SC group. Conversely, lower pH_{45min} levels are linked to higher cooking losses [68], which aligns with our findings as the E2 and L3 groups exhibited lower pH_{45min}. In addition to low pH_{45min}, these two groups also presented higher drip loss values, which also indicates reduced water-holding capacity [68]. These observations agree with Boler et al. [49] found that immunocastrated males had higher cooking loss compared to surgically castrated males, and Aluwé et al. [51] who reported that immunocastrated males exhibited increased cooking loss compared to entire males.

Shear force was significantly higher ($p < 0.05$) in the A2 group compared to the E2 group, suggesting reduced tenderness in adult immunocastrated pigs along with increased tenderness when early immunocastration protocols are used. As an indicator of meat tenderness, the shear force is influenced by several factors, such as pH ultimate and intramuscular fat content. When the ultimate pH has higher values, there is an increased water-holding capacity, and myofibrillar proteolysis by calpains [57] which can explain the reduced shear force in high pH meats [69]. However, the higher shear force in A2 cannot be explained by ultimate pH or intramuscular fat, which does not differ between these groups. Some authors report a lower shear force in immunocastrated males compared to entire males [41,42] or the surgically castrated males [41], which agrees with our study but only for the early immunocastration protocols; the shear force was the highest in the A2 group (immunocastration protocols implemented before culling). These findings disagree with some studies [40,42–45,49] that reported no differences between the pre-pubescent immunocastration protocols, entire males, and surgically castrated males, in tenderness and other meat quality parameters.

Moisture content was significantly higher ($p < 0.05$) in the Bo group compared to the L3 group. Protein and ash content did not show significant differences. Intramuscular fat content was significantly higher ($p < 0.05$) in SC compared to Bo, which is supported by some studies [60,61]. Our study found no significant differences between the immunocastration protocols and the control groups (Bo and SC), indicating intermediate levels of intramuscular fat content, as reported by Jeong et al. [64] and Batorek et al. [56]. Batorek et al. [18] found no statistical difference between immunocastrated males and surgically castrated males, but the intramuscular fat was more abundant in immunocastrated males than in entire males, which was not observed in the current study. Also, Aluwé et al. [63] reported higher intramuscular fat content in surgically castrated males, compared to immunocastrated

males and entire males. The lower content of fat found in the Bo group, previously reported, may explain the higher moisture content of meat in this group [70].

Both androstenone and skatole are compounds associated with boar taint, and the results are presented in Fig. 2.

Castration effectively reduces boar taint regardless of the method [19,48,50,51,71–73], although there seems to be a lower effect on skatole than androstenone in both castration methods [18]. Androstenone levels in Bo were significantly higher than in the other studied groups. As expected, testicles ablation nullified the androstenone content in surgically castrated animals. Immunocastration had a similar effect on this boar taint compound: three males out of six in E2, nine males out of eleven in L3, and four males out of nine in A2, had no detectable androstenone in their neck fat (data not shown). This attests to the protocol's efficacy regarding androstenone depletion from the body fat reserves. A meta-analysis by Batorek et al. [18] detected a small but significant difference between immunocastrated males and surgically castrated males regarding the androstenone and skatole levels. While in the present study, there was not a significant difference in androstenone levels in the castrated groups, we also observed a tendency in their absolute values, in which immunocastrated males had higher values than surgically castrated males. It should also be considered that a non-responder in the E2 group showed levels of 113.57 ng/g liquid fat of androstenone (data not shown), possibly from wrong handling or poor health at the moment of the inoculation [12,14,50,74–76]. Weiler [73] reported similar results in androstenone and low skatole levels in all the studied groups (immuno- or surgically castrated, and entire males). As expected, the skatole levels in the present study exhibited the highest concentration in the Bo group and the lowest in A2 ($p < 0.05$). Once again, this difference might be related to the slaughter season, where higher skatole concentration values are observed in the summer months [59]. During hot summer temperatures, backfat of pigs can experience increased levels of indole and skatole when they are housed in dirty pens with feces and urine, especially in cases of high stocking rates [77], since skatole can be reabsorbed through pig's skin when in contact with fecal contamination [78]. Boars raised during the autumn/winter season may have an accelerated onset of puberty due to reduced daylight exposure. Consequently, they may exhibit higher levels of plasma testosterone and estradiol, increased skatole levels in fat, and heavier testis weights [79]. However, no distinct seasonal effect was observed on fat androstenone [80]. Several studies locate the concentration levels for skatole in entire males between 100 ng/g and 200 ng/g of liquid fat [8,41,81–87], whereas the present study observed much lower values: only one pig had levels higher than 100 ng/g, and eight animals had lower than 30 ng/g. This could be due to the beet supplementation implemented on the farm, which has already been proven to reduce skatole levels [87,88].

Table 2
Sensory analysis of the neck fat and LTL muscle in entire males, surgically castrated males and males subjected to different immunocastration protocols.

Parameter	Bo (n = 8)	SC (n = 8)	E2 (n = 6)	L3 (n = 8)	A2 (n = 8)	SEM	p-value
Neck Fat							
Aroma							
Pork Intensity	6.0	6.4	5.9	6.0	5.9	0.1	0.200
Boar taint	3.5^{a}	2.3 ^{ab}	2.5 ^{ab}	2.1 ^{b}	2.7 ^{ab}	0.1	0.030
Urine	2.3	1.9	2.3	1.9	2.1	0.1	0.397
Manure	1.9	1.5	1.8	1.6	1.7	0.1	0.419
LTL							
Aroma							
Pork Intensity	5.4	6.0	5.6	5.8	5.9	0.1	0.059
Boar taint	4.1^{a}	1.8 ^{b}	3.2 ^{ab}	2.4 ^{b}	2.8 ^{ab}	0.2	0.001
Urine	3.0^{a}	1.6 ^{b}	2.8 ^{ab}	1.9 ^{b}	2.2 ^{ab}	0.1	0.001
Manure	2.5^{a}	1.3 ^{b}	1.9 ^{ab}	1.7 ^{ab}	1.6 ^{ab}	0.1	0.002
Flavor							
Pork Intensity	5.3 ^{b}	5.9^{a}	5.5 ^{ab}	5.8 ^{ab}	5.7 ^{ab}	0.1	0.028
Boar taint	3.2^{a}	1.6 ^{b}	2.5 ^{ab}	1.8 ^{b}	2.3 ^{ab}	0.1	0.001
Urine	2.2^{a}	1.5 ^{b}	2.0 ^{ab}	1.5 ^{b}	1.8 ^{ab}	0.1	0.018
Manure	1.5	1.1	1.2	1.1	1.1	0.0	0.052
Juiciness	5.0 ^{ab}	5.9 ^{ab}	6.0^{a}	5.6 ^{ab}	4.8 ^{b}	0.1	0.012
Tenderness	5.3 ^{ab}	6.2 ^{ab}	6.4^{a}	5.9 ^{ab}	4.7 ^{b}	0.2	0.011
Chewiness	5.3 ^{ab}	6.2 ^{ab}	6.4^{a}	5.9 ^{ab}	4.7 ^{b}	0.2	0.007

All values are means.
[1] Bo: entire males, boars; SC: surgically castrated; E2: early immunocastration protocol; L3: late immunocastration protocol; A2: adult immunocastration protocol.
[a-b] Means within a row with superscript letters are significantly different ($p < 0.05$).

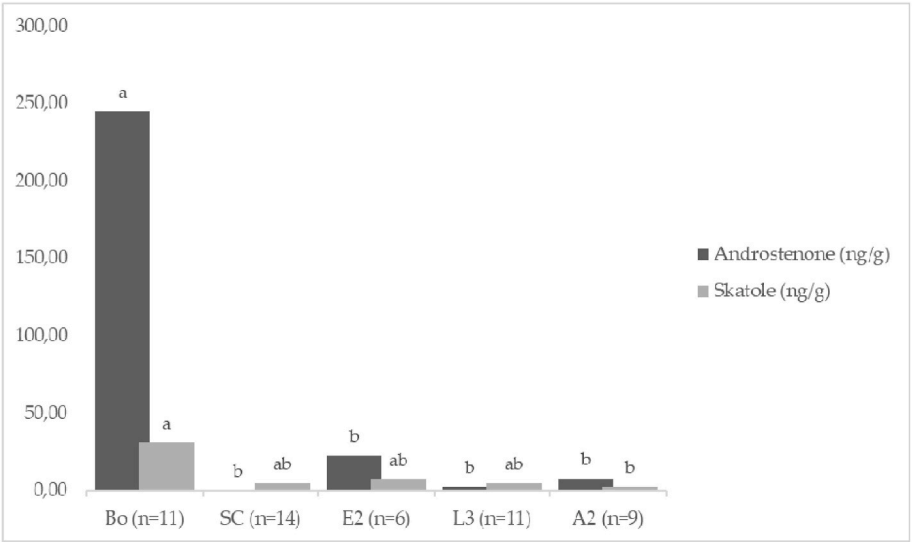

Fig. 2. Androstenone and Skatole content (ng/g) in the neck fat of entire males (Bo), surgically castrated males (SC) and males subjected to different immunocastration protocols (E2: early immunocastration protocol; L3: late immunocastration protocol; A2: adult immunocastration protocol). Androstenone (SEM 18.06; $p < 0.001$); Skatole (SEM 3.28 $p = 0.026$).

3.2. Sensory analysis

Sensory analysis results are presented in Table 2. Neck fat samples were only subjected to aroma testing whereas LTL boneless loin fillets were scored according to the aroma, flavor and texture.

Considering the intensity of the pork smell in meat samples, the panelists were not able to detect differences between the studied groups. Regardless of the use of a trained panel or consumers, most studies found that meat from surgically castrated males was similar to that from immunocastrated males, either in its smell or flavor [19,51,62–64,71,72,89]. Boar taint was significantly lower in SC and L3 groups compared to Bo, whereas E2 and A2 did not differ from all the studied groups. This supports the hypothesis that the immunocastration protocol L3 has the best results among the studied protocols, as presented by Paixão et al. [9]. The Urine attribute presented the same results as the boar taint, which is in accordance with the association of AND to the urine descriptor [90,91]. On the other hand, SKA is mainly described as manure [92], and only differences in the aroma were found between Bo and SC, whereas in terms of flavor, panelists could not detect differences in this attribute. Bonneau et al. [93] also found that consumer evaluation does not differ from meat with low levels of AND, even if AND concentrations are high. This suggests that SKA plays a key role in boar taint perception: the unpleasant odor associated with androstenone can be strengthened when high skatole concentrations are found simultaneously, seeming to have a synergetic effect [39] The intensity of pork flavor was also the same for all the studied groups. As was observed in Aroma, Flavor perception of boar taint was concordant with the urine attribute. The SC and L3 groups provided the best results compared to Bo ($p < 0.05$), while the panelists did not identify manure when tasting the samples. Several authors attest that boar taint can be more easily perceived when cooking instead of eating, which might explain the differences between Aroma and Flavor perception of the studied attributes [93–95].

Juiciness, tenderness, and chewiness were significantly different between E2 and A2 groups ($p < 0.05$). Group A2 had the lowest scores for these descriptors, which is in accordance with the meat quality traits studied, more specifically shear force. Although we were not able to find a reasonable explanation for those values, the panelists corroborated these findings. Excluding the results from the adult immunocastration protocol (A2), our findings are in accordance with those from two meta-analyses, which found no differences between the meat from entire male, immuno- and surgically castrated males [60,61]. Some studies were done with consumers [51,62,63,89] and others with a trained panel [63,64,72]. There are not many notable differences between the results from consumers and trained panelists. Trained panels tend to find meat from entire males less tender than castrated groups [63,64,72]. In contrast, consumers tend to find no differences at all [62] or only differences between surgically castrated and entire males, with immunocastrated males in-between [51,89]. Aluwé et al. [63] also did a study with consumers from six different countries, where half of the countries found no differences in tenderness. Of the other three, two found the meat from surgically castrated males different from boars, and two found the meat from immunocastrated more tender than the boar. In terms of juiciness, no differences were found in studies from four countries, whereas one found the meat from boars similar to both surgically castrated and immunocastrated, and the other found that meat from boars was similar to only immunocastrated males. Trained panels also found meat from surgically castrated pigs more tender than the other studied groups. Still, one found no differences between immunocastrated and entire males [31] and the other observed that boars with intermediate levels and immunocastrated were considered the less juicy [63]. Consumers found no differences in juiciness between boars, immuno- and surgically castrated pigs [51,62]. All prior investigations have exclusively utilized pre-pubertal pigs, facilitating a direct correlation between their outcomes and the E2 and L3 protocols, thereby aligning closely with our own research findings. Seeing as the A2 protocol was executed in adult animals, with significantly different shear force values when compared to the other groups, differences in juiciness, tenderness, and chewiness were expected.

4. Conclusions

Immunocastration has proven itself as a safe alternative to surgical castration, not only in industrial breeds but also in autochthonous breeds. The case of the Bísaro pig had yet to be explored, but the present study confirmed that, with specific protocols, immunocastration can be used. Despite significant differences in some meat quality parameters, immunocastration did not affect the overall meat quality negatively concerning the consumer's view. The pre-pubertal protocol L3 exhibited characteristics closer to surgically castrated males, rendering it the most effective protocol, whereas the other two protocols had intermediate results between surgically castrated and entire males. The post-pubertal protocol also presented positive results; nonetheless, a third inoculation might be considered. When it is not possible to perform surgical castration, or considering that this practice could be banned in Europe, immunocastration presents itself as a viable alternative to produce traditional and cured meat products.

Author contributions

Conceptualization, S.B.-F, R.P.-C. and A. E.; methodology, A.S., L.P., M.A.P., J.M.L., R.P.-P. and M.V.V.; investigation, S.B.-F., and A.S.; data curation, S.B.-F, A.S. and A.E.; writing—original draft preparation, S.B.-F, G.P. and R.P.-P.; writing—review and editing, S.B.-F, A.S. and A.E.; supervision, A.E.; project administration, A.E.; funding acquisition, R.P.-C and A.E. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

Data is available upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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