Effect of immunocastration and housing conditions on pig carcass and meat quality traits[1]

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ABSTRACT: The present study investigated the effects of immunocastration and housing conditions on carcass, meat, and fat quality traits. Immunocastrates (IC, $n = 48$), entire (EM, $n = 48$), and surgical castrates (SC, $n = 48$) male pigs were reared under three different housing conditions. The conditions were standard ($n = 36$), enriched ($n = 36$, twice as much space as standard and additional outdoor access), or standard with repeated social mixing ($n = 72$). Pigs of the IC group were vaccinated at the age of 12 and 22 wk. The animals were slaughtered in four batches, balanced for sex category and housing, at the age of 27 wk reaching 124.7 ± 1.0 kg. Immunocastration led to increased fat deposition (i.e., thicker subcutaneous fat at different anatomical locations, more leaf fat, fatter belly in IC than EM, $P < 0.05$) but did not affect muscularity traits. As a result, EM exhibited higher and SC lower ($P < 0.05$) carcass leanness than IC. Fatty acids composition of either subcutaneous or intramuscular fat (IMF) agreed with general adiposity, that is, IC were intermediate between EM and SC exhibiting the lowest and highest fat saturation ($P < 0.05$), respectively. Compared to SC, EM exhibited higher ($P < 0.05$) levels of muscle oxidation and collagen content than SC, with IC taking an intermediate position in the case of the level of peroxidation and collagen content, or closer to SC as regards to oxidation of muscle proteins (i.e., carbonyl groups). Meat quality (including marbling score, cooking loss, subjective color redness, and chroma) of IC was similar to EM, and both differed ($P < 0.05$) from SC. However, IC and SC had less ($P < 0.05$) tough meat than EM, consistent with protein oxidation. The effect of housing was less evident. Mixing of pigs resulted in lower ($P < 0.05$) carcass weight and fatness in all sex categories with lower ($P < 0.05$) oleic and higher ($P < 0.05$) arachidonic acid in IMF of EM.

Key words: carcass traits, entire males, housing, immunocastrates, meat quality, surgical castrates

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INTRODUCTION

Surgical castration of male piglets without pain relief is currently a wide-spread practice, but it has been lately severely criticized due to its welfare drawbacks. Many actors in the European pig production are, thus, considering alternatives like immunocastration or rearing of entire males (EM; Backus et al., 2018). Despite several advantages, the introduction of the alternatives may also bring up important management (Bonneau and Weiler, 2019) and quality issues (Čandek-Potokar et al., 2015). In the case of EM, the risk is not only higher boar taint incidence but also other quality issues, including extremely lean carcasses [lack of tissue cohesion, undesired softness due to increased fat unsaturation, and low amount of intramuscular fat (IMF)], increased meat toughness (Lundström et al., 2009; Pauly et al., 2012), and indications for reduced water holding capacity (Škrlep et al., 2012a; Batorek et al., 2012b; Aluwé et al., 2013). In the case of immunocastrates (IC), the quality concerns are fewer. To trigger a physiological response, the procedure requires two consecutive vaccinations against gonadotropin-releasing hormone (Bonneau and Weiler, 2019). If vaccinated according to the standard protocol [4–6-wk delay from second vaccination (V2) to slaughter], IC are generally more similar to surgical castrates (SC) and superior to EM in terms of technological meat quality, (Čandek-Potokar et al., 2015). With regard to meat quality, for both alternatives, published results are not consistent and need further confirmation (Channon et al., 2017; Škrlep and Čandek-Potokar, 2018).

Another important issue is rearing management of different alternatives. Before V2, the IC are physiologically and behaviorally equal to EM, whereas boar-like behavior, as well as injuries, markedly decline in IC shortly after V2 (von Borell et al., 2009) in accordance with intensive hormonal and metabolic changes after V2 (Claus et al., 2007; Batorek-Lukač et al., 2016). The EM show more aggressive and sexual behavior, which may increase the intensity of fighting and injuries. Aggressive behavior is also more pronounced when unfamiliar animals are mixed (von Borell et al., 2009). The result of such interactions may result in chronic stress affecting performance (Hyun et al., 1998) and carcass and meat quality traits (Yoshioka et al., 2005; Škrlep et al., 2009). Appropriate housing and management conditions to accommodate the typical behavior of alternatives are not well known (Holinger et al., 2018).

In the present study, three sex categories (SC, IC, and EM) were compared under different housing conditions. Whereas the first part of the research (Kress et al., 2020) aimed to investigate possible impact of sex category and housing on growth performance and immune response, the present paper considers the effects on carcass properties and meat quality traits and relates them to physical-chemical traits of muscle and adipose tissue.

MATERIALS AND METHODS

Animals and Sampling

The experiment was approved by the ethical committee for animal experiments of the regional authority of Tübingen, Germany (ID HOH 47/17TH), and conducted in accordance with the German law on animal protection (Federal Republic of Germany, 2019). The animals used in the study originated from a trial conducted by the University of Hohenheim as a part of the project SuSI (ERA-NET SusAn) described in detail by Kress et al. (2020). Briefly, the study was performed in two replicates, each with 72 animals. Crossbred pigs (German Landrace × Pietrain) were allocated to three sex categories, that is, EM (n = 48), SC (n = 48), and IC (n = 48). Pigs assigned to the IC group received two doses of vaccine against GnRH (IMPROVAC, Zoetis Deutschland GmbH, Berlin, Germany) at the age of 12 and 22 wk, while animals from the SC group were castrated surgically during the first week of life. Within each sex category, animals were equally distributed to pens (each pen consisting of six pigs) with different housing conditions to simulate different levels of stress during

the fattening period. The housing was either standard (STD, n = 36) or enriched (ENR, n = 36) with twice as much space as STD and additional outdoor access. For the third treatment (MIX, n = 72), two pens of pigs (same sex category) were mixed at the time of vaccinations. As described by Kress et al. (2020), mixing consisted of an exchange of two out of six animals per pen with two animals from another pen of a similar sex group every third day of the mixing phase. For this reason, the animal number of the mixing scenario was twice as high as in the two other housing conditions. All other rearing factors, including the diet, were equal for all treatments. All groups of pigs were fed the same diet ad libitum (three phase feeding, diet based on the recommendations for intact boars; see Supplementary Appendix 1) as described in Kress and Verhaagh (2020). Reaching the age of 27 wk and average live weight of 124.7 ± 1.0 kg, the pigs were slaughtered in experimental abattoir (LSZ Boxberg, Boxberg, Germany) according to the routine procedure (CO_2 stunning, exsanguination, vapor scalding, and dehairing). The slaughter was performed in four batches; each batch was balanced with regard to treatment groups (sex and housing).

Carcass Traits

On the slaughter line, carcasses were eviscerated and split apart. Subperitoneal (i.e., leaf) fat was removed and weighed. Left sides of the carcasses were used for all further measurements and sampling. Longissimus thoracis (LT) muscle and subcutaneous fat thickness was measured using a Fat-O-Meat'er device (Frontmatec, Kloding, Denmark) at the level between the second and third last rib, 7 cm laterally from the carcass split line, allowing the calculation of lean percentage by the equation approved for German slaughter plants (European Commission, 2011). Dressing was calculated as a percentage of warm carcass weight relative to live weight. At the end of the slaughter line, additional measures of backfat thickness were taken at withers (at the level between last cervical and the first thoracic vertebra) and at the thinnest point over gluteus medius (GM) muscle. Additional muscle thickness indicator was measured at the carcass split line defined as the shortest distance between the dorsal edge of vertebral canal and cranial edge of GM. Measurement of pH was taken in longissimus lumborum (LL) muscle at the level of last rib and semimembranosus muscle (SM) at 45 min postmortem using pH-STAR (Matthäus GmbH, Co. KG, Eckelsheim, Germany). Carcass length was measured as the distance between the cranial edge of pubic bone and cranial edge first cervical vertebra. The carcasses were placed in a cooling room overnight at 0–2 °C to reach internal temperature below 7 °C.

Meat Quality Traits and Chemical Composition of Muscle and Fat Tissue

A day after slaughter, ultimate pH and conductivity (CoTrol, RWi Handels OG, St. Georgen b. Obernberg, Austria) were measured in LL and SM muscle. The hind leg was removed from the carcass with a cut between the sixth and seventh lumbar vertebra. The shank was cut off at the level of the knee joint and the ham was weighed prior and after the removal of the skin and subcutaneous fat to assess ham leanness (meat with bones, %). The carcasses were cut perpendicularly to the spine at the level of last rib, enabling the measurements of loin eye area, area of corresponding fat, belly leanness percentage, objective color parameters (CIE L*, a*, and b*) and evaluation of LL marbling, subjective color, and belly leanness score (Batorek et al., 2012b). Objective color parameters were measured in triplicate in the central area of a freshly cut LL surface (without blooming). Additional color parameters hue angle (h°) and chroma (C*) were calculated as $h° = tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$, respectively. Subjective LL color was assessed using a 1–6-point Japanese color scale (Nakai et al., 1975). Two LL chops were cleaned of external connective and fatty tissue to be used for the determination of shear force, water holding capacity parameters (drip, thawing, and cooking loss), and chemical composition. In addition, samples of LL and overlying subcutaneous fat were also taken for the determination of fatty acid composition. All chemical analyses were performed in duplicates; the quality criteria for the methods have been previously reported (Tomažin et al., 2019). Chemical composition of LL (protein, moisture, and IMF content) was assessed using near-infrared spectroscopy (NIR Systems 6500 Monochromator, Foss NIR System, Silver Spring, MD) and internal calibrations developed at Agricultural Institute of Slovenia. The determination of drip loss was performed according to EZ method by Christensen (2003) with two cylindrical samples of approximately 10 g being excised from the center of LL, weighed, placed in plastic containers, and stored at 4 °C for 48 h and then reweighed. For the determination of thawing loss, cooking loss, and shear force, a LL chop was trimmed to

exact dimensions (8 × 5 × 4 cm), weighed, vacuum-packed, and stored at −20 °C prior to analysis. For thawing loss determination, the samples were thawed at 4 °C overnight, superficially dried with a paper towel and reweighed. The same samples were then cooked in a thermostatic bath (ONE 7–45, Memmert GmbH, Schwarzenbach, Germany) to the internal temperature of 72 °C, cooled overnight at 4 °C, and reweighed. To measure shear force, four or five 1.27-cm cylindrical cores were excised from the middle of the cooked samples. The cores were cut perpendicular to the direction of muscle fibers using a texture analyzer (TA Plus, Ametek Lloyd Instruments Ltd., Fareham, UK), fitted with a 60° V-shaped rectangular-edged blade at the speed of 3.3 mm/s.

To determine collagen (total and soluble), myoglobin content, and meat oxidation (protein carbonyl group content, thiobarbituric acid reactive substances—TBARS), the samples of LL were powdered in liquid nitrogen. Meat oxidation parameters were determined in both fresh (taken 24 h postmortem) and cooked samples. Collagen (total and soluble) was analyzed as described in Tomažin et al. (2019) and based on hydroxyproline content [determined according to ISO (1994)] multiplied by a factor of 8. Briefly, LL samples (thermally treated at 77 °C for 90 min) were hydrolyzed in sulphuric acid at 105 °C for 16 h. Filtered hydrolyzate was incubated with p-dimethylaminobenzaldehyde and chloramine-T in propan-2-ol and perchloric acid. Absorbance at 558 nm was measured (using BioSpectrometer Fluorescence, Eppendorf GmbH, Wesseling-Berzdorf, Germany) and used for the calculation of hydroxyproline content. The insoluble collagen fraction was determined by heating LL sample (77 °C for 90 min) in 25% Ringer's solution, centrifuged, and the supernatant discarded. The pellet was analyzed further as in the case of soluble collagen. Soluble collagen was determined by subtracting the insoluble from total collagen content and used for the calculation of collagen solubility. Myoglobin content was determined according to the method of Trout (1991). After homogenization of muscle samples in potassium phosphate buffer, sodium nitrite and Triton X-100 were added to the filtered solution. Myoglobin concentration was calculated from the absorbance measured at 370 and 409 nm (using BioSpectrometer Fluorescence). Protein carbonyl group content was determined in isolated myofibrils as described in Rezar et al. (2017). Shortly, myofibrillar isolates were treated with 2.4-dinotrophenylhydrazine (DNPH) dissolved in HCl and the proteins precipitated by trichloroacetic acid. Precipitate was washed with ethyl acetate and ethanol (eliminating residual DNPH) and dissolved in guanidine hydrochloride solution. Absorbance at 370 nm was measured (BioSpectrometer Fluorescence), enabling the calculation of protein carbonyl groups content. The concentration of TBARS in LL was determined according to the method of Lynch and Frei (1993). For the analysis, samples were homogenized in KCl buffer with added butylated hydroxytoluene to prevent further oxidation. The homogenates were centrifuged and the resulting supernatant incubated in a thermostatic heating block with 2-thiobarbituric acid in NaOH and trichloroacetic acid ensuing in the development of pink chromagen. After the extraction of the chromagen with n-butanol, its absorbance was measured at 535 nm (BioSpectrometer Fluorescence), enabling the calculation of TBARS concentration.

Fatty acids composition of subcutaneous fat and IMF was determined with gas chromatography following lipid transesterification. Tissue samples were ground in liquid nitrogen (approximately 0.1 g of backfat at the level of the last rib and 0.5 g of LL muscle) and transmethylated in situ [according to Park and Goins (1994)] in methanol solution of 0.5 M NaOH and dichloromethane (at 90 °C for 50 min), followed by the addition of 14% BF_3 in methanol (at 90 °C for 10 min). The resulting fatty acid methyl esters (FAME) were extracted by hexane. In the case of backfat, the samples were additionally diluted 10-fold with hexane. FAME were separated by Hewlett Packard 6890 GC (Agilent, Santa Clara, CA), fitted with a Supelco SP-2560 Capillary GC Column (100 m × 0.25 mm i.d. × 0.20 µm; Supelco, Bellefonte, PA) with nitrogen carrier gas and flame ionization detector (FID). Agilent GC ChemStation software (Agilent, Santa Clara, CA) was used for the acquisition and processing of data. The initial temperature was set at 80 °C, increasing in the following order: to 160 °C at a rate of 20 °C/min, to 198 °C at a rate of 1 °C/min, and to 250 °C at a rate of 1 °C/min. The total run time was 94.4 min. The injector temperature was set at 220 °C and the temperature of FID at 300 °C. Specific FAME was identified by the use of standard mixtures (Supelco 37 Component FAME mix). Nonadecanoic acid (C19:0, Sigma Aldrich, St. Louis, MO) was used as an internal standard to determine adipose tissue fatty acid concentrations.

Statistical Analysis

Data were analyzed by SAS statistical software (SAS Institute Inc., Cary, NC) using the procedure

MIXED. The model included the fixed effects of sex category, housing conditions, and their interaction. The slaughter batch was included as a random effect. When a significant effect ($P < 0.05$) was observed, least square means were compared using PDIFF option. Pearson correlation coefficients between the selected LL meat quality and chemical composition traits were calculated using CORR procedure of SAS.

RESULTS

Regardless of the trait examined, no significant interactions were observed between sex category and housing conditions, indicating a similar effect of rearing stress regardless of the animal sex. The only exception was the fatty acid composition of IMF lipids (Fig. 1; Supplementary Appendix 2), which is described and discussed below.

Carcass Properties

A significant ($P < 0.05$) effect of sex category was observed on live weight, warm carcass weight, ham weight, and dressing percentage and on all properties associated with fat deposition (Table 1). Immunocastrates and SC had similar live weight, both groups were heavier compared to EM; the same was observed for ham weight. In the case of warm carcass weight, IC were intermediate between the SC (the heaviest) and EM (the lightest), not differing from either of the groups. Dressing percentage was similar in IC and EM, whereas SC exhibited higher values. Intermediate positioning of IC with respect to EM (the leanest) and SC (the fattest) was also noted for subcutaneous backfat thickness (either at the level of withers, second and third last rib, or above GM), fat surface above loin eye area, leaf fat, belly (leanness percentage and score), ham leanness, and carcass meat percentage. In all of the mentioned fatness traits, significant differences among all three sex categories were observed, except for backfat at withers (with EM not differing from IC). Traits directly associated to muscle growth (muscle thickness at the level of GM, loin eye area, ham muscle with bones) were not affected by sex category, except for muscle thickness at the level of second and third last rib that was similar in IC and SC, with EM exhibiting lower values.

Regarding the effect of housing conditions, mixing resulted in lower live weight, warm carcass weight, and ham weight, in addition to thinner backfat (measured at different positions within carcass) than STD or ENR, with the latter two groups not differing from each other. The same trend was, however, not observed for leaf fat, which was the heaviest in STD compared to ENR or MIX group. In addition, lower belly leanness was also noted in STD than MIX or ENR pigs. In both cases, ENR and MIX groups did not differ from each other. Housing conditions tended to affect ($P < 0.09$) the belly score, ham leanness, and muscle thickness at second and third last rib with the biggest difference between STD and MIX group.

Chemical Composition

Fatty acids profile of subcutaneous (Table 2; Supplementary Appendix 2) fat was significantly affected by sex category. Compared to SC (the most saturated) and EM (the most unsaturated), the IC pigs took intermediate position in the case of total

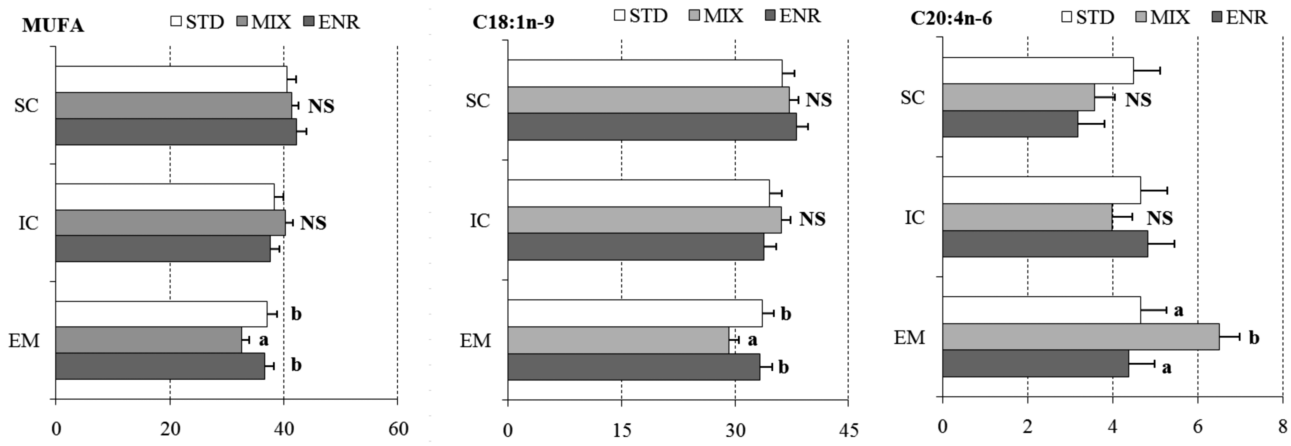

Figure 1. Content (g/100 g fat) of total monounsaturated (MUFA), oleic (C18:1n9), and arachidonic (C20:4n-6) fatty acids in the intramuscular fat of LL muscle in regard to the sex category and housing conditions. STD = standard housing; MIX = pigs mixed during rearing; ENR = enriched housing; EM = entire males; IC = immunocastrates, SC = surgical castrates. Significant differences ($P < 0.05$) between groups reared in different housing conditions within a sex category are indicated by different letters (a, b); NS = no significant effect ($P > 0.10$) of housing conditions within specific sex category.

Table 1. Carcass properties according to sex category (EM, IC, and SC) and housing conditions (STD, ENR, and MIX)

	Sex			Housing			*P*-value		
Item	EM	IC	SC	ENR	MIX	STD	Sex	Housing	RMSE
No. of animals	48	48	48	36	72	36			
Live weight, kg	122.1[a]	127.6[b]	127.8[b]	127.7[b]	121.4[a]	128.4[b]	0.021	0.001	9.11
Warm carcass, kg	98.6[a]	101.9[ab]	104.7[b]	103.9[b]	98.7[a]	102.5[b]	0.003	0.004	8.12
Dressing, %	80.5[a]	79.9[a]	82.1[b]	81.1	80.6	80.8	<0.001	0.650	1.96
Carcass length, cm	106.1	105.4	105.4	105.7	105.4	105.8	0.408	0.736	2.74
Ham, kg	12.09[a]	12.65[b]	13.11[b]	12.84[b]	12.28[a]	12.72[b]	<0.001	0.021	0.086
Fatness traits									
BFT withers, mm	31.9[a]	33.9[a]	36.8[b]	35.1[b]	32.6[a]	34.9[b]	<0.001	0.028	5.22
BFT at second to third last rib, mm	12.7[a]	14.3[b]	16.6[c]	14.8[b]	13.7[a]	15.2[b]	<0.001	0.015	2.69
BFT above GM, mm	8.6[a]	13.2[b]	17.4[c]	13.5[b]	12.1[a]	13.6[b]	<0.001	0.018	3.09
LEA fat, cm^2	11.5[a]	14.0[b]	17.2[c]	14.7[b]	13.3[a]	14.8[b]	<0.001	0.010	7.72
Leaf fat, g	657[a]	1007[b]	1544[c]	1014[a]	1036[a]	1159[b]	<0.001	0.048	275.5
Muscularity traits									
Muscle at second to third last rib, mm	62.7[a]	65.9[b]	66.7[b]	65.4	63.5	66.3	0.010	0.080	6.43
Muscle GM, mm	78.4	80.6	79.2	80.2	79.5	78.4	0.247	0.311	6.07
LEA, cm^2	56.3	57.3	56.9	57.8	55.6	57.1	0.815	0.282	6.95
Ham muscle and bone, kg	10.90	11.09	10.92	11.14	10.76	11.01	0.625	0.150	1.008
Leanness traits									
Carcass meat, %	60.7[c]	59.5[b]	58.0[a]	59.3	59.7	59.2	<0.001	0.561	2.34
Belly score, 1–7	5.8[c]	5.1[b]	4.0[a]	5.0	5.1	4.8	<0.001	0.087	0.55
Belly leanness, %	77.5[c]	70.0[b]	60.0[a]	69.7[b]	71.4[b]	66.4[a]	<0.001	0.002	6.83
Ham leanness, %	90.1[c]	87.6[b]	83.4[a]	86.9	87.7	86.6	<0.001	0.060	2.39

BFT, back fat; LEA fat, fat corresponding to loin eye area; muscle GM, the shortest distance between the dorsal edge of vertebral canal and cranial edge of gluteus medius muscle at the carcass split line; RMSE, root mean square error.

[a,b,c]Values within a row and specific treatment (sex or housing) with different superscript letter differ ($P < 0.05$).

polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA), differing significantly from both groups. Content of monounsaturated fatty acids (MUFA) in the subcutaneous fat was similar in EM and IC but lower in SC. As for the fatty acid composition of IMF, EM tended ($P < 0.08$) to have less SFA than SC. Regarding MUFA and PUFA, sex-related distinctions were more pronounced. Again, an intermediate position of IC was found, with all three sex groups significantly differing from each other. As regards other LL chemical traits (Table 2), IMF and protein content was higher in IC and SC, whereas moisture content was lower compared to EM. Oxidation of muscle lipids (TBARS) in fresh LL was below the detection limit of the method, regardless of sex category (results not shown). Thiobarbituric acid reactive substances could only be detected in thermally treated samples; the values were higher in EM compared to SC, whereas IC samples were positioned in between, not differing from either EM or SC. The concentration of carbonyls (indicators of protein oxidation) in fresh LL was not affected by sex category, whereas, in cooked samples, their concentration was higher in EM than IC or SC. Concerning LL collagen content, the highest concentrations were observed in EM, followed by IC and SC, with all sex groups significantly differing from each other. Collagen solubility and myoglobin content did not differ between sex categories.

There was no effect of the housing system on examined chemical traits except for subcutaneous fat SFA content (mainly due to palmitic acid—C16:0; Supplementary Appendix 2), which was lower in ENR than MIX or STD pigs. However, in the case of LL IMF, a significant interaction between housing and sex was noted (Fig. 1); the EM reared in MIX housing had lower MUFA than STD or ENR housing, whereas SC and IC did not differ with regard to the housing. These differences were mainly a reflectance of decreased proportion of oleic acid (C18:1n-9) and arachidonic acid (C20:4n-6), which was higher in EM held in MIX housing (Fig. 1).

Meat Quality

Sex category significantly affected conductivity in SM and LL muscles, in addition to LL color score, color parameters a*, C*, and H°, marbling

Table 2. Backfat and LL muscle chemical traits according to sex category (EM, IC, and SC) and housing conditions (STD, ENR, and MIX)

	Sex			Housing			*P*-value		
Item	EM	IC	SC	ENR	MIX	STD	Sex	Housing	RMSE
Subcutaneous fat									
SFA, %	38.51[a]	40.27[b]	41.45[c]	39.34[a]	40.31[b]	40.59[b]	<0.001	0.040	1.531
MUFA, %	42.31[a]	43.77[b]	44.45[b]	44.21	43.26	43.06	0.001	0.129	1.837
PUFA, %	19.18[c]	15.96[b]	14.10[a]	16.45	16.44	16.35	<0.001	0.986	1.971
LL muscle									
SFA, %	38.62	39.19	39.88	39.41	39.18	39.12	0.075	0.870	1.772
MUFA, %	35.40[a]	38.75[b]	41.36[c]	38.81	38.07	38.62	<0.001	0.748	3.654
PUFA, %	25.98[c]	22.07[b]	18.75[a]	21.77	22.77	22.25	<0.001	0.734	4.426
IMF, %	1.3[a]	1.9[b]	2.1[b]	1.6	1.8	1.9	<0.001	0.234	0.78
Moisture, %	74.4[b]	73.5[a]	73.3[a]	73.7	73.9	73.6	<0.001	0.190	0.78
Protein, %	24.1[a]	24.7[b]	24.4[b]	24.5	24.3	24.3	0.002	0.293	0.69
TBARS cooked, µg MDA/kg	5.30[a]	4.72[ab]	4.06[b]	4.65	4.51	4.91	0.015	0.593	1.346
Carbonyl, fresh meat, nmol/mg protein	1.30	1.27	1.25	1.29	1.30	1.23	0.675	0.410	0.179
Carbonyl, cooked meat, nmol/mg protein	1.83[b]	1.60[a]	1.60[a]	1.69	1.73	1.62	0.010	0.393	0.267
Myoglobin, mg/g	1.35	1.32	1.31	1.27	1.39	1.32	0.853	0.310	0.275
Collagen, mg/g	4.59[a]	4.24[b]	3.85[c]	4.25	4.06	4.27	<0.001	0.198	0.467
Collagen solubility, %	16.6	15.0	15.4	16.8	14.9	15.3	0.427	0.275	3.98

Values for fatty acids composition are presented as percentage of fat; other chemical traits are presented on wet basis.

MDA, malondialdehyde; RMSE, root mean square error.

[a,b,c]Values within a row and specific treatment (sex or housing) with different superscript letter differ ($P < 0.05$).

score, cooking loss, and shear force and tended ($P < 0.07$) to affect color parameter b*, whereas pH, subjective color parameter L*, drip, and thawing loss were not affected (Table 3). Concerning the traits where significant effect of sex was noted (obviously, in these traits, EM differed from SC), IC were similar to SC in the case of conductivity, color score, color hue (h°), and shear force and similar to EM in the case of CIE parameters a*, C*, marbling, and cooking loss.

Only a minor effect of housing system was observed on meat quality traits. Color parameter b* tended ($P < 0.08$) to be lower in STD treatment and cooking loss tended ($P < 0.06$) to be lower in MIX treatment.

DISCUSSION

Carcass

As already reported for the same animals (Kress et al., 2020), faster growth of SC in the early fattening period and increased growth rate of IC after the effective immunization were the reason for higher live weight of IC and SC than EM at slaughter. This was not confirmed for warm carcass weight (IC taking intermediate position between EM and SC), which may be explained with the differences in dressing percentage. Lower dressing percentage of EM and IC than SC confirms previous findings (Batorek et al., 2012a) and could be attributed to the weight of reproductive tract, removed during slaughtering process. As the reproductive organs are regressed in IC (Kress et al., 2020), other factors like higher gut fill due to higher feed intake (Dunshea et al., 2001) and higher weight of abdominal organs and leaf fat could explain similar killing out percentage in EM and IC (Batorek et al., 2012a; Boler et al., 2014). Indeed, in the last fattening phase, feed intake was higher in IC than EM (3.0 vs. 2.8 kg/d, respectively). Higher carcass fatness of castrated (SC and IC) compared to EM (Table 1) has been firmly established by three meta-analytical studies (Pauly et al., 2012; Batorek et al., 2012a; Trefan et al., 2013) and is coherent with the well-known effects of castration on increased fat deposition in pigs (Kouba and Sellier, 2011). These changes may be explained by the reduction of testosterone, which negatively affects lipogenesis and promotes lipid expenditure (Kelly and Jones, 2013). As for IC, previous studies have shown that the effect of immunocastration is manifested shortly after V2 due to a rapid increase in GnRH antibody titers causing a marked decline of testicular steroids (Claus et al., 2007; Kress et al., 2020) and rapid change of metabolism from boar- to more castrate-like. As shown by the meta-analysis of Batorek et al. (2012a), IC increase voluntary feed intake after V2, which together with

Table 3. Meat quality traits according to sex category (EM, IC, and SC) and housing conditions (STD, ENR, and MIX)

	Sex			Housing			*P*-value		
Item[a]	EM	IC	SC	ENR	MIX	STD	Sex	Housing	RMSE
Semimembranosus muscle									
pH1	6.52	6.57	6.61	6.6	6.57	6.52	0.181	0.348	0.233
pHu	5.50	5.48	5.47	5.47	5.48	5.51	0.223	0.120	0.080
Conductivity	4.82[b]	3.74[a]	3.69[a]	4.15	3.97	4.13	<0.001	0.616	1.051
LL muscle									
pH1	6.56	6.59	6.62	6.59	6.60	6.57	0.396	0.739	0.200
pHu	5.52	5.50	5.53	5.50	5.52	5.53	0.389	0.416	0.096
Conductivity	3.30[b]	3.07[a]	3.00[a]	3.13	3.18	3.06	0.050	0.597	0.576
Color score, 1–6	3.1[b]	2.7[a]	2.9[ab]	2.8	2.9	2.9	0.020	0.913	0.75
L*	50.1	51.2	51.0	51.4	50.8	50.1	0.128	0.136	2.78
a*	7.4[b]	7.1[b]	6.6[a]	7.0	7.1	7.0	0.001	0.845	1.05
b*	4.9	5.3	4.9	5.1	5.1	4.8	0.072	0.075	0.81
C*	8.9[b]	8.9[b]	8.2[a]	8.7	8.8	8.5	0.007	0.550	1.12
h°	33.6[a]	36.6[b]	36.6[b]	36.4	35.9	34.4	0.005	0.160	4.84
Marbling score, 1–7	1.3[a]	1.3[a]	1.5[b]	1.3	1.3	1.5	0.009	0.196	0.45
Drip loss, %	5.17	4.99	4.48	5.20	4.69	4.76	0.453	0.628	2.622
Thawing loss, %	10.0	9.8	9.5	10.1	9.4	9.8	0.653	0.435	2.72
Cooking loss, %	32.3[b]	31.6[b]	29.4[a]	31.7	30.5	31.1	<0.001	0.064	2.59
Shear force, N	70.3[b]	63.9[a]	61.9[a]	65.2	66.1	64.8	<0.001	0.809	9.89

RMSE, root mean square error.

[a,b]Values within a row and specific treatment (sex or housing) with different superscript letter differ ($P < 0.05$).

[a]pH1 = pH measured 45 min after slaughter; pHu = ultimate pH; L*, a*, b* = objective color parameters denoting lightness, redness and yellowness, respectively; C* = chroma; h° = hue angle.

lower basal metabolic rate increases lipid deposition while not affecting the deposition of proteins (Batorek-Lukač et al., 2016). The results on carcass quality of the present study corroborate with these metabolic features as the effect of immunocastration (namely the difference between IC and EM) is evident for various fat depots and not for traits associated with muscle growth (Table 1). It has been shown that backfat thickness increase is proportional to the interval between V2 and slaughter (Lealiifano et al., 2011). This is not the case for muscle growth, as similar muscularity traits (loin eye area, ham muscles) between EM and IC were observed even at longer delay (8 wk) between V2 and slaughter (Škrlep et al., 2012b). Other studies (Boler et al., 2012; Harris et al., 2017) also failed to find any differences in longissimus muscle area or depth regardless of the V2 to slaughter interval.

Lower warm carcass weight in MIX group compared to STD and ENR [in agreement with lower live weight and growth rate already reported for the same animals by Kress et al. (2020)] can be related to lower subcutaneous fat deposition (Table 1). Namely, using the adjustment to the same weight (i.e., by introducing carcass weight in the statistical model as a covariate), differences between housing groups for the examined fatness traits disappeared (results not shown). Reduced growth performance as a result of mixing of pigs during fattening has been demonstrated by previous studies (Stookey and Gonyou, 1994; Hyun et al., 1998) and ascribed to greater levels of aggression and increased activity and energy expenditure. In the situation of acute stress, body hormonal regulation induces lipolysis and proteolysis (Simmons et al., 1984; Nielsen et al., 2014), which may also explain the reduced fat deposition found in the current assay in the MIX group. Contrary to our results, a study of Holinger et al. (2018) reported no effect of mixing on performance, body weight, or hot carcass but indicated increased backfat thickness and lower carcass leanness in mixed pigs. Their results indicate a response characteristic for obesity due to chronic stress as described in humans (Scott et al., 2012). This contradiction also indicates the effect of experimental conditions, that is, the actual level and duration of the endured stress. The lipid accumulation response was not the same in all the examined fat depots as, for belly and leaf fat, lower amounts compared to STD were observed in MIX but also ENR group (Table 1). This may be related to differential development (Kouba and Sellier, 2011) or metabolic reactivity (Frühbeck et al., 2014) of various fat depots in addition to higher energy demands in pigs with

outdoor access (i.e., ENR) due to more exercise and thermoregulation (Bee et al., 2004; Hansen et al., 2006). Although higher animal activity and higher susceptibility to stress have been postulated for EM than other sex categories due to more aggressive behavior (Cronin et al., 2003; Fàbrega et al., 2010), the lack of interaction between sex category and housing system in the present study does not indicate any differential response at least not to a degree that could be detected on the carcass traits. In line with our results, Holinger et al. (2018) also showed no differential response to stress between EM and SC pigs in either of the examined performance, carcass meat and fat traits. Studies of physiological stress indicators (i.e., acute phase proteins) showed no differences between the three male sex categories (Fàbrega et al., 2010) or even indicated lower stress response of EM [when compared to females (Olivan et al., 2018)].

Chemical Composition

Higher SFA and MUFA but lower PUFA proportion in both castrated groups compared to EM (Table 2) is generally in line with the meta-analytical study of Pauly et al. (2012) comparing these three alternatives and is also consistent with the differences in carcass fatness. In pigs, an increase in fatness corresponds to higher amounts of de novo synthesized SFA and MUFA, while diluting PUFA, which cannot be synthesized by the body and depend on dietary supply (Wood et al., 2008). Regarding fatty acid composition, the IC vaccinated and slaughtered according to the classical protocol (i.e., 4–5-wk delay) generally take intermediate position between EM and SC (Grela et al., 2013; Mackay et al., 2013), while a further delay of slaughter quickly increases the fatty acids saturation (Harris et al., 2018). It was also indicated that EM had a higher proportion of PUFA than castrates even at the same fat thickness (Wood et al., 1989). The same was confirmed in the present study as the adjustment for the same subcutaneous fat thickness or IMF level did not change the proportions (results not shown). So, besides the differences in de novo synthesis, other factors should be considered, like enzyme selectivity, selective uptake and mobilization of fatty acids (as reviewed by Raclot, 2003), and differential fatty acid oxidation (Leyton et al., 1987) as combined to the metabolic effects of androgens (Kelly and Jones, 2013). Experiments on fatty acid utilization (Leyton et al., 1987; Reubsaet et al., 1989) indicated remarkably high oxidation rate for oleic acid (in this study, the most prominent MUFA, present in lower proportions in EM than in SC and IC in both analyzed fat depots; Supplementary Appendix 2) and relatively slow utilization of long-chain PUFA including arachidonic acid (the most abundant long chain PUFA in IMF of LL, present in higher proportions in EM; Supplementary Appendix 2). Taking into account lower de novo synthesis and higher metabolic use of fat due to hormonal differences between EM and both castrated categories, this could explain the differences. Differential utilization may also explain the interactions between sex category and housing observed for fatty acid composition (Supplementary Appendix 2; Fig. 1), namely a lower oleic and higher arachidonic and some less important PUFA proportions in IMF of EM that were mixed during the rearing. The combination of the two treatments is probably the one that elicited the highest level of physical activity, making the differences evident in the lipid depot, where fatty acids are the most readily utilized as an energy source. As reported by Daza et al. (2009), higher physical activity in pigs induces an increase of intramuscular PUFA.

Higher LL collagen content (Table 2) in EM and IC compared to SC indicates a more developed connective tissue of EM and IC. For IC, there are no comparable literature sources, except for the histological study on skin and subcutaneous adipose tissue using the same pigs as in the present study (Poklukar et al., 2019), showing intermediate position of IC regarding dermis thickness. As for the comparison of entire and castrated males, the differences are more firmly established, indicating that connective tissue development is positively related to the presence of testosterone (Petersen et al., 1997). Thus, in cattle, higher muscle collagen was shown for bulls compared to castrated steers (Dikeman et al., 1986). In pigs, higher LL collagen content was reported in EM compared to SC or females (Petersen et al., 1997; Nold et al., 1999), which is supported by the results on other body tissues, indicating thicker dermis and higher backfat collagen content in EM (Vold and Moen, 1972; Wood et al. 1989). In our recent research (Škrlep et al., 2019), higher LL collagen along with higher collagen solubility was observed in EM than SC. As collagen solubility is positively related to collagen synthesis (i.e., less cross-linking in newly formed immature collagen) and overall protein turnover (Bailey, 1985), lower overall growth rate (Kress et al., 2020) and lower muscle accretion of EM observed in the present study may have contributed to the absence of differences

in collagen solubility. A higher concentration of TBARS (Table 2) indicates that IMF of LL was more oxidized in EM, followed by IC and SC. The concentration of TBARS could only be detected in thermally processed samples and, even in this case, the levels were low [i.e., well below the threshold level of 0.50 mg/kg for sensory detection of rancidity (Dunshea et al., 2005)]. In general, higher levels of unsaturated fatty acids (preferentially PUFA), strongly affect meat oxidative stability (Wood et al., 2003). Although the TBARS concentrations (Table 2) agree with the differences in fatty acid composition, no significant correlations between this oxidation indicator and fatty acid composition could be observed in the present study. Carbonyls in fresh meat show the same numerical trend as TBARS but with no significant differences observed. After thermal processing, the differences were more pronounced and indicated higher protein oxidation in EM compared to IC and SC. Lipid oxidation is one of the main causes for oxidation of amino acids and proteins (Estévez, 2011). Both processes are generally positively correlated (in the present study, r = 0.43 between TBARS and carbonyls), although TBARS levels may not be exactly proportional to the amounts of protein carbonyls measured by DNPH method (Armenteros et al., 2010) as seen in the present case (i.e., both SC and IC samples exhibiting similarly lower carbonyl levels than EM).

Meat Quality

Higher subjective color score in EM, in addition to higher objective parameter a* (denoting higher redness) and lower H° (Table 3), may be related to lower IMF (Huff-Lonergan et al., 2002) or to higher myoglobin content (Kim et al., 2010); the latter being numerically (but not significantly) higher in the present study. Moreover, it could indicate more oxidative muscle metabolic profile of EM, which was demonstrated in our previous research (Škrlep et al., 2019). The least intensive meat subjective color of IC pigs is difficult to explain with color measurements or other influential factors like pH, IMF, or meat pigment. The present results, however, agree with the meta-analytical studies (Pauly et al., 2012; Batorek et al., 2012a; Trefan et al., 2013), confirming that IC exhibit the lowest color intensity of LL muscle among all compared sex categories, though the reported differences were small and likely without much practical importance. Regarding water holding capacity (WHC), no effect was observed for drip or thawing loss, whereas cooking loss was higher in EM than IC or SC (Table 3). The majority of studies (as reviewed by Lundström et al., 2009) show no differences between the sexes or indicate higher proportion of dark, firm, and dry meat in the case of EM. But recent investigations indicated lower WHC of EM than SC meat assessed either as drip loss (Batorek et al., 2012b; Aluwé et al., 2013; Škrlep et al., 2019) or cooking loss (Channon et al., 2016; Van den Broeke et al., 2016; Škrlep et al. 2019). Lower WHC assessed as drip loss was also reported for IC compared to both EM (Batorek et al., 2012a) and SC (Lowe et al., 2014; Harsh et al., 2017; Seiquer et al., 2019). In accordance with the present investigation, several studies demonstrated higher cooking loss in IC than SC (Boler et al., 2012) while being similar to EM (Batorek et al., 2012b; Van den Broeke et al., 2016), whereas some data (Aluwé et al., 2013; Akit et al., 2014) show even higher cooking loss of IC than EM. Reduced WHC can be related to the increased protein oxidation causing denaturation, loss of solubility, aggregation, cross-linking, and myofibrillar shrinkage, reducing the ability of muscular structures to bind water (Estévez, 2011). In the present study, this is supported by the positive correlation between cooking loss and carbonyls (r = 0.49), TBARS (r = 0.50), or intramuscular PUFA (r = 0.39). Moreover, cooking loss was significantly correlated with collagen content (r = 0.56) explaining greater water loss of EM and IC due to collagen shrinkage during thermal treatment (Lepetit, 2008). Higher muscle conductivity, in the present study observed in EM (Table 3), also indicates a greater proportion of free (extracellular) water (Pospiech et al., 2002) and can also be an indicator of reduced WHC. Tougher meat demonstrated in EM (Table 3) agrees with meta-analyses (Pauly et al., 2012; Batorek et al., 2012a) and some newer studies (Jones-Hamlow et al., 2015; Channon et al., 2016, Škrlep et al., 2019) and may be associated to increased protein oxidation and inferior WHC (Huff-Lonergan et al., 2010). In addition, lower IMF (Hocquette et al., 2010) and higher collagen content (Lepetit, 2008) could contribute to higher toughness in EM. However, correlation analysis showed that, in the present study, only protein oxidation (namely protein carbonyls in thermally processed LL) were significantly correlated (r = 0.44) to shear force, while other tested traits (TBARS, total collagen, collagen solubility, and IMF) did not show any significant correlation.

CONCLUSIONS

Compared to EM, immunocastration reduced carcass leanness due to greater fat deposition. Fatty acids composition (subcutaneous fat and IMF) of IC was intermediate between EM and SC, which had the least and the most saturated fatty acids, respectively. The inferior meat quality of EM (lower WHC and tenderness) could be related to higher protein oxidation. Although IC resembled EM in many meat quality traits, immunocastration still presented an advantage over EM, in particular, due to lower meat toughness. The effect of housing conditions was smaller and showed that mixing of pigs lead to lower fat deposition in all sexes. It can be concluded that, when considering the use of immunocastration as an alternative, the benefits to surgical castration can be expected for carcass quality, while, compared to EM, the main advantage is better quality of meat.

SUPPLEMENTARY DATA

Supplementary data are available at *Translational Animal Science* online.

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