


# Effect of immunocastration and caponization on fatty acid composition of male chicken meat

I. C. Antunes,<sup>\*,†</sup> M. A. G. Quaresma,<sup>\*,1</sup> M. F. Ribeiro,<sup>‡</sup> S. P. Alves,<sup>\*</sup> P. Martins da Costa,<sup>§</sup>  
and R. J. B. Bessa <sup>\*</sup>

*\*Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Pólo Universitário Alto da Ajuda, 1300-477 Lisboa, Portugal; †Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; ‡Escola Superior Agrária de Santarém, Instituto Politécnico de Santarém, Quinta do Galinheiro - S. Pedro, 2001-904 Santarém, Portugal; and §ICBAS-Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Largo Prof Abel Salazar 2, 4099-003 Porto, Portugal*

**ABSTRACT** Recently, immunocastration with Improvac (**ImmC**) has been tested in broilers and a considerable reduction in serum testosterone concentration (reduced by 79% compared to roosters) was observed. The aim of this study was to evaluate the effect of immunocastration on meat fatty acid (**FA**) composition and its comparison with caponized and intact males (roosters). The study was conducted with 3 experimental groups: control group (roosters), the group of birds submitted to surgical caponization (**SurgC**), and the group of birds submitted to immunocastration with Improvac. The comparison of breast meat partial FA sums of castrated (SurgC and ImmC) with control birds (roosters) revealed that castrated birds showed significantly higher content of n-3 polyunsaturated fatty acids (**n-3 PUFA**) than control birds (1.76 vs. 1.46 g/100 g of total FA; superiority of 20.2%), which has contributed to the occurrence of significant differences on both the n-6/n-3 ratio and the atherogenicity index (**AI**). In contrast, on leg meat portion, castrated birds

displayed higher contents of both total saturated and monounsaturated fatty acids (**SFA** and **MUFA**, with 2.2 and 4.1% more, respectively) and lower total n-6 PUFA content (8.3% less) than was observed in control birds, which contributed to significant differences in the AI index. On the other hand, the comparison of breast meat portion from SurgC with ImmC showed that immunocastration contributed to lower total SFA and higher total n-6 PUFA, which have contributed to significant differences on both Polyunsaturated/Saturated (P/S) and n-6/n-3 ratios. Whereas, on leg meat portion no significant differences were observed on partial sums and a single difference was observed on the thrombogenicity index. Immunocastration of broilers has contributed to minor changes in the FA profile, but has improved the overall lipid quality indexes in both breast and leg meat portions. Therefore, immunocastration could be applied as an alternative method to caponization without negative consequences in meat FA profile.

**Key words:** capon, fatty acids, Improvac, immunocastration

2019 Poultry Science 98:2823–2829  
<http://dx.doi.org/10.3382/ps/pez034>

## INTRODUCTION

Capon production is an ancient practice, with records dating back to more than 2,000 yr ago (Winter and Funk, 1960; Symeon et al., 2010), that has survived worldwide until now (Sirri et al., 2009; Symeon et al., 2010; Calik, 2014; Sokolowicz et al., 2016). Capon production is done in small scale, representing just a market niche, but encloses a great growth potential because capon meat has specific sensorial attributes valorized by consumers (Amorim et al., 2016). In Portugal, capon

production has strong tradition, particularly in Freixo County. Such tradition has recently resulted in the establishment of a certification label (the protected geographical indication—PGI), which is under the supervision of European Union (ACCF, 2011).

Caponization consists of testectomy (the surgical removal of the testes) (Chen et al., 2007a,b; Lin et al., 2012; Duan et al., 2013), leading to androgen deficiency and consequent phenotypic and behavioral changes, such as, reduced development of comb and wattles, abolished vocalization (Mast et al., 1981), loss of aggressiveness, and reduced activity (Calik, 2014). Therefore, the energy that is normally expended in fighting and territorial dominance is abolished, providing an extra energy for growth and fat deposition (Rikimaru et al., 2011).

© 2019 Poultry Science Association Inc.

Received May 9, 2018.

Accepted January 23, 2019.

<sup>1</sup>Corresponding author: [mquaresma@fmv.ulisboa.pt](mailto:mquaresma@fmv.ulisboa.pt)

Caponization increases intramuscular fat content enhancing meat sensorial attributes, such as tenderness and flavor; within this experimental trial, an increase in the intramuscular fat of 0.21 and 2.15 g/100 g edible portion was observed, in breast and leg meat portions, which was responsible for a rise of 10.8 and 29.1%. The influence of caponization has been previously observed by other authors (Chen et al., 2007a,b; Sinanoglou et al., 2011), and it was observed that it has a positive influence on consumer's preferences (Tor et al., 2002).

However, dietary fat might favor the occurrence of cardiovascular and other chronic diseases when consumed in excess (FAO, 2010; McAfee et al., 2010). Therefore, the World Health Organization has developed specific nutritional guidelines regarding fat intake, encouraging an increase of n-3 polyunsaturated fatty acids (**n-3 PUFA**) intake and a reduction in total fat intake, particularly the saturated fatty acids (**SFA**) (FAO, 2010).

A recent study developed by our team (Quaresma et al., 2017) has showed that capon production can be achieved by immunocastration of male chickens using Improvac, a vaccine used for immunocastration of pigs. However, in male chickens, there is no information regarding the effect of immunocastration on the composition of meat lipid fraction. Therefore, this study's aim was to evaluate the effect of immunocastration on meat FA composition and its comparison with caponized and intact males (roosters).

## MATERIAL AND METHODS

### *Experimental Design, Animal Management and Sample Collection*

The experimental design, animal management, and sample collection are described in detail by Quaresma et al. (2017). Briefly, the study was conducted with 3 experimental groups: control group (n = 14 roosters); surgical caponization (**SurgC**) group (birds submitted to SurgC; n = 7); and immunocastration with Improvac (**ImmC**) group (birds submitted to the Improvac protocol; n = 15). The birds from SurgC group were submitted to SurgC, at the age of 28 d old, following a commercial poultry industry protocol, and the effectiveness of the SurgC was double checked: at slaughter during the evisceration procedure and by the absence of plasma testosterone. The Improvac (Parsippany-Troy Hills, Zoetis, New Jersey, USA) administration was performed 2 times, at the age of 28 and 91 d old (day of caponization and 63 d before slaughter, respectively). All groups were subjected to the same feeding (feed and water were available ad libitum), environmental, and sanitary conditions. The slaughter was performed at 154 d old at an accredited slaughterhouse.

The sample preparation was performed in the laboratory. First, the breast and leg meat portions of each bird were trimmed of skin, bones, and major visible

fat and connective tissue, but the leg and breast meat portions of each bird were always independently processed. Afterward, the muscle tissues from each meat portion (breast and leg portions) were kept apart, cut in small pieces (square cuts with 15 to 20 mm and 20 to 25 mm thickness) with a bistoury blade and homogenized in a domestic food processor (5 × 5 s; Moulinex D56, France). Meat samples were individually identified accordingly, the experimental group, bird's number, and meat portion; and vacuum packed in polyethylene bags with 90 μm (Tecnopack, Venda do Pinheiro, Portugal), with a permeability to O<sub>2</sub> and CO<sub>2</sub> of <65 and <200 cm<sup>3</sup>/m<sup>2</sup>/bar/day at 23°C and 0% relative humidity, respectively. Vacuum packed samples were stored in freezer (-20°C) until analysis (a period ranging between 30 and 44 d).

### *Fatty Acids and Dimethyl Acetals Analysis*

The reagents, namely pellet potassium hydroxide (KOH), methanol, and sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) were of pro analysis grade; whereas, n-hexane was for gas chromatography and were all purchased from Merck Biosciences (Darmstadt, Germany). Fatty acid (**FA**) methyl esters standards (FAME mix 37 components) were acquired from Supelco Inc. (Bellefont, PA, USA).

Meat FA composition from both breast and leg meat portions was assessed according to O'Fallon et al. (2007). Briefly, 1 g of wet sample was weighted into a 16 × 125 mm screw-cap Pyrex culture tube, to which 0.5 mL of the internal standard solution (C19:0 at a concentration of 1.0 mg/mL in Methanol), 0.7 mL of 10 N KOH, and 5.3 mL of Methanol were added. After incubation in a water bath (55°C during 1.5 h with vigorous hand-shaking for 5 s every 20 min) and cooling in a cold tap water bath, 0.58 mL of 24 N H<sub>2</sub>SO<sub>4</sub> was added; the tube was mixed by inversion and incubated again in the same conditions as previously described. After the FA methyl esters synthesis, the tube was cooled in a tap water bath. A total of 3 mL of n-hexane was added, and the tube was vortex-mixed (5 min) on a multi-tube vortex (Heidolph, Nuremberg, Germany). Afterwards, the tube was centrifuged (5 min) in a tabletop centrifuge, and the n-hexane layer, containing the FA methyl esters, was placed into a 2.0 mL GC vial, which was capped and placed at -20°C until GC analysis.

Fatty acid methyl esters were analyzed by fast gas-liquid chromatography using a Shimadzu GC-2010 Plus (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a SupraWax-280 capillary column (10 m, 0.10 mm i.d., 0.10 μm film thickness; Teknokroma, Barcelona, Spain). The injector and detector temperatures were maintained at 250 and 280°C, respectively. The column oven parameters were as follows: initial temperature of 120°C was increased at 35°C/min to 175°C and held for 0.5 min, then it was increased at 70°C/min to 260°C and held for 6 min,

with a total run time of 9.29 min. Helium was used as carrier gas at a flow rate of 1 mL/min, and 1  $\mu$ L of sample was injected. Identification of FA methyl esters was achieved by comparison of the FA methyl esters retention times with those of authentic standards (FAME mix 37 components) and by electron impact mass spectrometry using a Shimadzu GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan). Results for each FA were expressed as a percentage of the sum of detected FA and dimethylacetals (**DMA**) (g/100 g of total FA+DMA).

### Lipid Quality Ratios and Indexes

The nutritional ratios Polyunsaturated/Saturated (P/S) and n-6/n-3 were calculated as previously established by the British Department of Health (1994):

$$P/S = [(C18:2n-6 + C18:3n-3) / (C14:0 + C16:0 + C18:0)];$$

$$n-6/n-3 = \left[ \left( \sum n-6PUFA \right) / \left( \sum n-3PUFA \right) \right].$$

The indexes of atherogenicity (**AI**) and thrombogenicity (**TI**), were estimated as proposed by Ulbricht and Southgate (1991):

$$AI = (C12:0+4 \times C14:0+C16:0) / \left[ \left( \sum MUFA + \sum (n-6) + \sum (n-3) \right) \right];$$

$$TI = (C14:0+C16:0 + C18:0) / \left[ \left( 0.5 \times \sum MUFA + 0.5 \times (n-6) + 3 \times (n-3) + (n-3) / (n-6) \right) \right].$$

### Statistical Analysis

The statistical analysis was accomplished using the GLM procedure of SAS (SAS Inst., Cary, NC, USA), version 9.3, with a model considering the experimental groups as the single effect. A total of 2 orthogonal contrasts were used to compare: (1) control birds (rooster) vs. castrated birds [surgical caponized (SurgC) and immunocastrated (ImmC) birds; (C vs. SI); and 2] surgical castrated vs. immunocastrated birds (S vs. I). Least square means and residual standard deviation (RSD) are presented in the tables. Variables presenting a significant difference for any of the contrasts tested were submitted to a complementary analysis with a model that included the total FA plus dimethylacetals (**DMA**) (expressed as g/kg of meat) as covariate.

## RESULTS

### Breast Meat Portion

Total FA and DMA content of breast meat ranged between 20.6 g/kg of meat in control birds and 28.6 g/kg of meat in SurgC, but no differences were found between castrated (SurgC and ImmC) and control birds, neither between castration methods (Table 1). The total DMA content averaged 4.3% of the sum FA and DMA, and no differences ( $P > 0.05$ ) were found in the contrasts tested for both individual DMA and its sum. The C18:1*cis*-9, C16:0, and C18:2n-6 were the predominant FA and together comprised nearly 79% of total FA. Breast meat from castrated birds presented higher proportions of C14:0 ( $P = 0.022$ ), C18:3n-3 ( $P = 0.006$ ), and the polyunsaturated FA of the n-3 family (n-3 PUFA;  $P = 0.012$ ), but lower proportions of C18:0 ( $P = 0.006$ ) than breast meat from control birds. Moreover, the C16:0 tended to be higher ( $P = 0.069$ ) in castrated than in the control counterparts. The breast meat from ImmC birds presented higher proportions of C18:2n-6 ( $P = 0.020$ ) and the polyunsaturated FA of the n-6 family (n-6 PUFA;  $P = 0.035$ ) and lower proportions of C14:1*cis*-9 ( $P = 0.018$ ), C20:5n-3 ( $P = 0.048$ ), and total saturated FA (SFA;  $P = 0.029$ ) than SurgC birds. No other differences on FA were observed for the contrasts tested.

The effect of intramuscular FA content variation was tested, as a possible explanation for the differences observed in the FA profile, using total FA and DMA (expressed as g/kg of meat) as covariate. The differences observed on breast meat portion between control and castrated birds on C14:0, C16:0, C18:0, C18:3n-3, and n-3 PUFA ( $P < 0.05$  for all these variables) persisted after covariate adjustment (adjusted least square means not shown). Moreover, the difference observed for C14:1*cis*-9 and C18:2n-6 on breast between SurgC and ImmC have also persisted ( $P < 0.05$ ) after the covariate adjustment, whereas the difference observed on C20:5n-3, sum of SFA and sum of n-6 PUFA tended to persist ( $0.05 < P < 0.10$ ).

Regarding nutritional evaluation of breast meat lipid FA profile, it was observed that breast meat from castrated birds presented lower n-6/n-3 ratio ( $P = 0.002$ ) but higher AI ( $P = 0.019$ ) than the control, whereas ImmC presented higher P/S ( $P = 0.005$ ) and n-6/n-3 ratios ( $P = 0.023$ ), and lower TI ( $P = 0.004$ ) than SurgC.

### Leg Meat Portion

Total FA and DMA content of leg meat ranged between 58.0 g/kg of meat in control and 88.8 g/kg of meat in SurgC (Table 2). Castration tended ( $P = 0.058$ ) to increase the FA and DMA content when compared to control, whereas the castration method had no effect. The DMA averaged 1.5% of the sum FA and DMA, and no differences were observed between contrasted

**Table 1.** Fatty acid (FA), dimethyl acetals (DMA) composition and nutritional FA ratios, and lipid quality indexes of breast meat of roosters (control birds), surgical castrated birds (SurgC), and immunocastrated birds (ImmC).

|                                | Treatments |       |      | RSD <sup>1</sup> | Contrasts <sup>2</sup> |         |
|--------------------------------|------------|-------|------|------------------|------------------------|---------|
|                                | Control    | SurgC | ImmC |                  | C vs. SI               | S vs. I |
| FA + DMA content <sup>3</sup>  | 20.6       | 28.6  | 23.1 | 10.97            | 0.260                  | 0.263   |
| FA profile <sup>4</sup>        |            |       |      |                  |                        |         |
| C14:0                          | 0.51       | 0.60  | 0.60 | 0.092            | 0.022                  | 0.956   |
| C15:0                          | 0.06       | 0.05  | 0.06 | 0.023            | 0.167                  | 0.393   |
| C16:0                          | 22.6       | 23.8  | 23.0 | 1.09             | 0.069                  | 0.117   |
| C17:0                          | 0.13       | 0.15  | 0.15 | 0.079            | 0.515                  | 0.930   |
| C18:0                          | 7.58       | 6.48  | 6.35 | 0.930            | 0.006                  | 0.768   |
| C20:0                          | 0.42       | 0.35  | 0.38 | 0.110            | 0.301                  | 0.467   |
| ∑ SFA                          | 31.3       | 31.4  | 30.6 | 0.85             | 0.477                  | 0.029   |
| C14:1 <i>cis</i> -9            | 0.11       | 0.19  | 0.11 | 0.068            | 0.141                  | 0.018   |
| C16:1 <i>cis</i> -9            | 3.87       | 4.27  | 4.30 | 0.935            | 0.294                  | 0.945   |
| C17:1 <i>cis</i> -9            | 0.10       | 0.11  | 0.09 | 0.028            | 0.955                  | 0.211   |
| C18:1 <i>cis</i> -9            | 35.9       | 35.8  | 34.1 | 2.496            | 0.378                  | 0.136   |
| ∑ MUFA                         | 40.0       | 40.4  | 38.7 | 3.149            | 0.722                  | 0.217   |
| C18:2n-6                       | 16.9       | 16.4  | 17.7 | 1.227            | 0.730                  | 0.020   |
| C20:2n-6                       | 0.20       | 0.21  | 0.22 | 0.062            | 0.560                  | 0.563   |
| C20:4n-6                       | 4.60       | 4.29  | 4.98 | 1.586            | 0.959                  | 0.330   |
| C22:4n-6                       | 1.08       | 1.06  | 0.96 | 0.317            | 0.610                  | 0.450   |
| C22:5n-6                       | 0.36       | 0.44  | 0.37 | 0.141            | 0.415                  | 0.237   |
| ∑ n-6 PUFA                     | 23.1       | 22.4  | 24.2 | 1.909            | 0.796                  | 0.035   |
| C18:3n-3                       | 0.50       | 0.59  | 0.63 | 0.094            | 0.006                  | 0.314   |
| C20:5n-3                       | 0.03       | 0.07  | 0.03 | 0.047            | 0.272                  | 0.048   |
| C22:5n-3                       | 0.46       | 0.56  | 0.50 | 0.150            | 0.246                  | 0.422   |
| C22:6n-3                       | 0.47       | 0.58  | 0.54 | 0.165            | 0.215                  | 0.562   |
| ∑ n-3 PUFA                     | 1.46       | 1.80  | 1.71 | 0.268            | 0.012                  | 0.421   |
| DMA profile <sup>4</sup>       |            |       |      |                  |                        |         |
| DMA-16:0                       | 3.02       | 2.67  | 3.39 | 1.080            | 0.974                  | 0.140   |
| DMA-18:0                       | 0.69       | 0.79  | 0.87 | 0.313            | 0.285                  | 0.573   |
| DMA-18:1                       | 0.46       | 0.50  | 0.55 | 0.179            | 0.429                  | 0.535   |
| ∑ DMA                          | 4.17       | 3.96  | 4.81 | 1.525            | 0.734                  | 0.216   |
| Nutritional ratios and indexes |            |       |      |                  |                        |         |
| P/S <sup>5</sup>               | 0.57       | 0.55  | 0.61 | 0.046            | 0.481                  | 0.005   |
| n-6/n-3                        | 16.1       | 12.6  | 14.4 | 1.725            | 0.002                  | 0.023   |
| AI <sup>6</sup>                | 0.38       | 0.40  | 0.39 | 0.018            | 0.019                  | 0.128   |
| TI <sup>7</sup>                | 0.60       | 0.63  | 0.59 | 0.027            | 0.168                  | 0.004   |

<sup>1</sup>Residual standard deviation.<sup>2</sup>C vs. SI = Control vs. (SurgC + ImmC); S vs. I = SurgC vs. ImmC.<sup>3</sup>Expressed in g/kg of meat.<sup>4</sup>Expressed in percentage of total FA plus DMA.<sup>5</sup>Polyunsaturated/Saturated.<sup>6</sup>Atherogenicity index.<sup>7</sup>Thrombogenicity index.

groups, in neither individual DMA nor its sum. As previously observed on breast, the C18:1*cis*-9, C16:0, and C18:2n-6 were prime FA in leg and together comprised approximately 81 to 83% of total FA. Leg meat from castrated birds presented higher proportions of C16:0 ( $P = 0.004$ ), and lower proportions of C20:2n-6 ( $P = 0.002$ ), C20:4n-6 ( $P = 0.002$ ), and n-6 PUFA ( $P = 0.013$ ) than leg meat from control birds. Moreover, C18:1*cis*-9 ( $P = 0.075$ ), sum of MUFA ( $P = 0.053$ ), and the sum of SFA (0.067) tended to be higher; whereas, C18:0 ( $P = 0.059$ ) tended to be lower in castrated than in control birds. The ImmC birds presented a similar FA profile to SurgC birds, except for the C16:0 content that was lower in ImmC than in SurgC treatments ( $P = 0.019$ ).

As described for breast meat, the eventual effect of intramuscular FA content variation was tested, as a possible explanation for the differences observed in the FA profile, using the total FA and DMA (expressed as g/kg of meat) as covariate. The differences observed between control and castrated animals for C16:0, total SFA, C20:2n-6, C20:4n-6, and total n-6 PUFA persisted ( $P < 0.05$ ); whereas, those of C18:0 ( $P = 0.383$ ), C18:1*cis*-9 ( $P = 0.299$ ), and total MUFA ( $P = 0.280$ ) disappeared after covariate adjustment (adjusted least square means not shown). The difference on C16:0 concentration in leg meat detected between SurgC and ImmC also persisted ( $P = 0.028$ ) after covariate adjustment.

Regarding nutritional evaluation of leg meat FA composition, it was observed that castrated birds presented

**Table 2.** Fatty acid (FA), dimethyl acetals (DMA) composition and nutritional FA ratios, and lipid quality indexes of leg meat of roosters (control birds), surgical castrated birds (SurgC), and immunocastrated birds (ImmC).

|                                | Treatments |       |      | RSD <sup>1</sup> | Contrasts <sup>2</sup> |         |
|--------------------------------|------------|-------|------|------------------|------------------------|---------|
|                                | Control    | SurgC | ImmC |                  | C vs. SI               | S vs. I |
| FA + DMA content <sup>3</sup>  | 58.0       | 88.8  | 77.2 | 34.66            | 0.058                  | 0.463   |
| FA profile <sup>4</sup>        |            |       |      |                  |                        |         |
| C14:0                          | 0.64       | 0.66  | 0.68 | 0.077            | 0.261                  | 0.627   |
| C15:0                          | 0.10       | 0.09  | 0.09 | 0.028            | 0.444                  | 0.617   |
| C16:0                          | 22.7       | 24.6  | 23.4 | 1.35             | 0.004                  | 0.019   |
| C17:0                          | 0.14       | 0.12  | 0.14 | 0.046            | 0.727                  | 0.371   |
| C18:0                          | 6.24       | 5.27  | 5.80 | 0.950            | 0.059                  | 0.228   |
| C20:0                          | 0.43       | 0.34  | 0.50 | 0.389            | 0.942                  | 0.370   |
| ∑ SFA                          | 30.2       | 31.1  | 30.6 | 0.931            | 0.067                  | 0.204   |
| C14:1 <i>cis</i> -9            | 0.17       | 0.16  | 0.16 | 0.049            | 0.603                  | 0.893   |
| C16:1 <i>cis</i> -9            | 5.53       | 6.10  | 5.89 | 1.064            | 0.257                  | 0.660   |
| C17:1 <i>cis</i> -9            | 0.11       | 0.10  | 0.13 | 0.077            | 0.889                  | 0.354   |
| C18:1 <i>cis</i> -9            | 36.7       | 38.6  | 37.5 | 1.93             | 0.075                  | 0.213   |
| ∑ MUFA                         | 42.6       | 45.0  | 43.7 | 2.38             | 0.053                  | 0.238   |
| C18:2n-6                       | 20.2       | 19.2  | 19.6 | 1.59             | 0.183                  | 0.517   |
| C20:2n-6                       | 0.23       | 0.15  | 0.19 | 0.047            | 0.002                  | 0.119   |
| C20:4n-6                       | 2.67       | 1.65  | 2.01 | 0.659            | 0.002                  | 0.232   |
| C22:4n-6                       | 0.67       | 0.52  | 0.71 | 0.446            | 0.749                  | 0.367   |
| C22:5n-6                       | 0.27       | 0.18  | 0.30 | 0.235            | 0.721                  | 0.267   |
| ∑ n-6 PUFA                     | 24.1       | 21.7  | 22.8 | 1.82             | 0.013                  | 0.164   |
| C18:3n-3                       | 0.73       | 0.74  | 0.70 | 0.106            | 0.873                  | 0.469   |
| C20:5n-3                       | 0.07       | 0.03  | 0.06 | 0.048            | 0.321                  | 0.206   |
| C22:5n-3                       | 0.25       | 0.20  | 0.21 | 0.076            | 0.112                  | 0.849   |
| C22:6n-3                       | 0.35       | 0.26  | 0.30 | 0.122            | 0.150                  | 0.520   |
| ∑ n-3 PUFA                     | 1.40       | 1.24  | 1.27 | 0.234            | 0.125                  | 0.739   |
| DMA profile                    |            |       |      |                  |                        |         |
| DMA-16:0                       | 1.19       | 0.66  | 1.10 | 0.727            | 0.292                  | 0.214   |
| DMA-18:0                       | 0.43       | 0.19  | 0.34 | 0.295            | 0.154                  | 0.291   |
| DMA-18:1                       | 0.16       | 0.10  | 0.15 | 0.091            | 0.301                  | 0.227   |
| ∑ DMA                          | 1.79       | 0.96  | 1.67 | 1.15             | 0.289                  | 0.184   |
| Nutritional ratios and indexes |            |       |      |                  |                        |         |
| P/S <sup>5</sup>               | 0.71       | 0.65  | 0.68 | 0.061            | 0.075                  | 0.294   |
| n-6/n-3                        | 17.5       | 18.0  | 18.3 | 2.76             | 0.547                  | 0.819   |
| AI <sup>6</sup>                | 0.37       | 0.40  | 0.38 | 0.018            | 0.002                  | 0.034   |
| TI <sup>7</sup>                | 0.54       | 0.56  | 0.54 | 0.033            | 0.357                  | 0.273   |

<sup>1</sup>Residual standard deviation.

<sup>2</sup>C vs. SI = Control vs. (SurgC + ImmC); S vs. I = SurgC vs. ImmC.

<sup>3</sup>Expressed in g/kg of meat.

<sup>4</sup>Expressed in percentage of total FA plus DMA.

<sup>5</sup>Polyunsaturated/Saturated.

<sup>6</sup>Atherogenicity index (AI).

<sup>7</sup>Thrombogenicity index (TI).

higher AI ( $P = 0.002$ ) and tended to have lower P/S ratio ( $P = 0.075$ ) than control birds. Leg meat from ImmC presented lower AI than SurgC ( $P = 0.034$ ).

## DISCUSSION

The most important metabolic effect of caponization is an increase in body fatness throughout all body parts and compartments (Tor et al., 2002, 2005). The increase of the intramuscular fat content contributes to enhanced meat sensorial characteristics (Amorim et al., 2016), but it may also change the intramuscular FA profile and its nutritional quality, an issue of global health concern (FAO, 2010).

It was recently shown that the administration of Improvac to broilers was associated with a large de-

crease of serum testosterone concentration, as it was reduced by 79% compared to the average serum testosterone of roosters (Quaresma et al., 2017). Nevertheless, immunocastrated birds still possess detectable serum testosterone concentration, which is not the case in birds submitted to SurgC. Decreased serum testosterone has been associated with increased hepatic lipogenesis (Chen et al., 2007b), due to the upregulation of some genes involved in hepatic lipogenesis (Duan et al., 2013; Guo et al., 2015). Moreover, energy that is normally expended in fighting and territorial protection in roosters is greatly reduced in capons, due to absence of testosterone, allowing a more efficient feed conversion and increased fat deposition (Calik, 2014). Therefore, absent or diminished testosterone synthesis should increase de novo synthesis of FA or decrease  $\beta$ -oxidation of FA or both to produce energy. Despite the expected

general stimulation of lipogenesis, in the present experiment, castration (SurgC and ImmC) had no effect on the total FA content of the breast meat. Nevertheless, the leg meat of castrated birds was fatter ( $P = 0.058$ ) than the leg meat of control birds. Moreover, no difference was observed on leg meat total FA content between castration methods, which suggest that immunocastration is equally effective as surgical castration in increasing leg intramuscular fat. The leg meat accumulated higher lipid content than breast meat, which is in agreement with the results previously shown in roosters and capons (Tor et al., 2002; Díaz et al., 2012).

In birds, lipogenesis occurs predominantly in the liver, and little or no FA synthesis occurs in the adipose tissue (Leveille, 1966; Griffin et al., 1992). Therefore, differences observed in the FA content and composition between breast and leg meat portions should reflect differences in the lipid uptake and catabolism between meat portions, as well as differences in the muscle fiber composition and differences in the proportion of muscle and intermuscular tissues between these two portions, which could explain differences in the amount of intramuscular (Xiong, 1994) and intermuscular lipids (Tor et al., 2002). The increased fat content of meat is associated with the dilution of membrane phospholipids by triacylglycerols, which might explain some differences in FA profile (Sahasrabudhe et al., 1985; Betti et al., 2009). De novo FA synthesis pathways produce mainly C16:0 and C18:1*cis*-9 as end products that are then predominantly stored in triacylglycerols. Thus, when increased lipogenesis is observed, it is expectable that these FA accumulate. On the other hand, in broiler meat, C18:0 is predominantly found in phospholipids, and its content in meat is inversely related with the amount of total lipid in meat (Betti et al., 2009). Nevertheless, and despite the absence of effects on total FA content in the breast meat portion, some differences between treatments in FA profile were still observable. In fact, the SurgC and ImmC animals presented or tended to present higher C16:0 and C18:1*cis*-9 in meat portions and lower C18:0 than control birds. Notably, the SurgC and ImmC animals presented higher C18:3*n*-3 and total *n*-3 PUFA in breast than control birds and these differences persist after covariate adjustment to FA + DMA. Similar results were found by Mašek et al. (2014), who reported higher content of *n*-3 PUFA in meat and adipose tissue from caponized broilers than from roosters. It is not clear how it occurs, but we can speculate that suppressed or diminished testosterone dependent metabolic pathways and physiological responses can spare *n*-3 PUFA. Independent of the method, castrated animals presented lower C20:4*n*-6 in the leg than control birds. The reduction in the meat contents of C20:4*n*-6 has been previously observed in breast (Sinanoglou et al., 2011), in thigh (Rikimaru et al., 2009), and in both meat portions (Sirri et al., 2009) of caponized broilers compared to roosters, and the suggested explanation for such differences is an eventual reduction of the  $\Delta_6$

desaturase activity as a consequence of caponization (Sirri et al., 2009).

The FA profile of meat portions was almost unaffected by the castration method, although ImmC birds contained more C18:2*n*-6 in breast and less C16:0 in leg than SurgC, which might somehow be related to the circulating serum testosterone. Nevertheless, these differences are desirable, as C16:0 is a hypercholesterolemic SFA and C18:2*n*-6 an essential PUFA.

The comparison of castrated with control birds showed minor changes in the FA profile, even so, castration contributed to the improvement of the AI in both breast and leg meat portions. In breast meat portion, it was also observed a significant reduction in the *n*-6/*n*-3 ratio due to the increase in the *n*-3 PUFA contents, whereas in the leg meat portion it worsened the P/S ratio. On the other hand, the comparison of castration methods showed different effect between meat portions. In the breast meat portion, the immunocastration contributed to the improvement of the P/S ratio and the TI but worsened the *n*-6/*n*-3 ratio; whereas, in the leg meat portion, immunocastration had a positive effect on AI.

## CONCLUSION

The intramuscular FA profile is of great importance to meat flavor and nutritional quality. Therefore, an alternative method to surgical castration should not drastically influence the FA profile. The results presented herein showed that the immunocastration with Improvac had minor influence on breast meat portion and some positive influence on leg meat portions.

## ACKNOWLEDGMENTS

We would like to thank the contribution of ACIVET (Rui Gonçalves for his assistance throughout sampling, and Ana Maria Reigado for laboratory support). The contribution of Campoaves (Rúben Fonseca, Fernando Moreira, Luís Valente, and Manuela Valente, who provided technical assistance and support throughout the experimental trial, supplied the birds, the production facilities to execute the trial, and slaughterhouse facilities). The contribution of Zoetis (Portugal) for supplying the Improvac used in the trial. The study received financial support from Fundação para a Ciência e Tecnologia under the CIISA Project UID/CVT/00276/2019 and also from Zoetis, Portugal.

## REFERENCES

- ACCF. 2011. Caderno de especificações Capão de Freamunde - IGP (Indicação Geográfica Protegida), <https://tradicional.dgadr.gov.pt/pt/cat/carne/carne-de-aves/591-capao-de-freamunde-igp>.
- Amorim, A., S. Rodrigues, E. Pereira, and A. Teixeira. 2016. Physicochemical composition and sensory quality evaluation of capon and rooster meat. *Poult. Sci.* 95:1211–1219.
- Betti, M., T. I. Perez, M. J. Zuidhof, and R. A. Renema. 2009. Omega-3-enriched broiler meat: 3. fatty acid distribution between

- triacylglycerol and phospholipid classes. *Poult. Sci.* 88:1740–1754.
- British Department of Health. 1994. *Nutritional Aspects of Cardiovascular Disease*. London.
- Calik, J. 2014. Capon production – breeding stock, rooster castration and rearing methods, and meat quality – a review. *Ann. Anim. Sci.* 4:769–777.
- Chen, K., T. Chen, K. Lin, and P. W. Chiou. 2007a. The effects of caponization age on muscle characteristics in male chicken. *Asian-Australas. J. Anim. Sci.* 20:1684–1688.
- Chen, K. L., W. T. Chi, C. Chu, R. S. Chen, and P. W. S. Chiou. 2007b. Effect of caponization and testosterone implantation on hepatic lipids and lipogenic enzymes in male chickens. *Poult. Sci.* 86:1754–1759.
- Díaz, O., L. Rodríguez, A. Torres, and A. Cobos. 2012. Fatty acid composition of the meat from the Mos breed and commercial strain capons slaughtered at different ages. *Grasas y Aceites*. 63:296–302.
- Duan, J., F. Shao, Y. Shao, J. Li, Y. Ling, K. Teng, H. Li, and C. Wu. 2013. Androgen inhibits abdominal fat accumulation and negatively regulates the PCK1 gene in male chickens. *PLoS One* 8:e59636.
- FAO. 2010. *Fats and Fatty Acids in Human Nutrition: Report of an Expert Consultation*. 55. Food Agric. Organ. United Nations, [https://www.who.int/nutrition/publications/nutrientrequirements/fatsandfattyacids\\_humannutrition/en/](https://www.who.int/nutrition/publications/nutrientrequirements/fatsandfattyacids_humannutrition/en/).
- Griffin, H. D., K. Guo, D. Windsor, and S. C. Butterwith. 1992. Adipose tissue lipogenesis and fat deposition in leaner broiler chickens. *J. Nutr.* 122:363–368.
- Guo, X., H. Nan, D. Shi, J. Zhou, Y. Wan, B. Zhou, Z. Geng, X. Chen, and R. Jiang. 2016. Effects of caponization on growth, carcass, and meat characteristics and the mRNA expression of genes related to lipid metabolism in roosters of a Chinese indigenous breed. *Czech J. Anim. Sci.* 60:327–333.
- Leveille, G. A. 1966. Glycogen metabolism in meal-fed rats and chicks and the time sequence of lipogenic and enzymatic adaptive changes. *J. Nutr.* 90:449–460.
- Lin, C.-Y., J. Hsu, and T. Wan. 2012. Effect of age and caponization on blood parameters and bone development of male native chickens in Taiwan. *Asian-Australas. J. Anim. Sci.* 25:994–1002.
- Mašek, T., K. Starčević, N. Filipović, Z. Stojević, D. Brozić, Ž. Gottstein, and K. Severin. 2014. Tissue fatty acid composition and estimated  $\Delta$  desaturase activity after castration in chicken broilers fed with linseed or sunflower oil. *J. Anim. Physiol. Anim. Nutr.* 98:384–392.
- Mast, M. G., H. C. Jordan, and J. H. Macneil. 1981. The Effect of partial and complete caponization on growth rate, yield, and selected physical and sensory attributes of cockerels. *Poult. Sci.* 60:1827–1833.
- McAfee, A. J., E. M. McSorley, G. J. Cuskelly, B. W. Moss, J. M. W. Wallace, M. P. Bonham, and A. M. Fearon. 2010. Red meat consumption: an overview of the risks and benefits. *Meat Sci.* 84:1–13.
- O'Fallon, J. V., J. R. Busboom, M. L. Nelson, and C. T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *J. Anim. Sci.* 85:1511–1521.
- Quaresma, M. A. G., I. C. Antunes, M. F. Ribeiro, S. Prazeres, R. J. B. Bessa, and P. M. Martins da Costa. 2017. Immunocastration as an alternative to caponization: evaluation of its effect on body and bone development and on meat color and composition. *Poult. Sci.* 96:3608–3615.
- Rikimaru, K., S. Ogawa, M. Komastu, and J. Ishizuka. 2009. Effects of caponization on meat quality of Hinai-Jidori chicken. *J. Poult. Sci.* 46:345–350.
- Rikimaru, K., H. Takahashi, and M. A. Nichols. 2011. An efficient method of early caponization in slow-growing meat-type chickens. *Poult. Sci.* 90:1852–1857.
- Sahasrabudhe, M. R., N. F. Delorme, D. F. Wood, and C. J. Randall. 1985. Neutral and polar lipids in chicken parts and their fatty acid composition. *Poult. Sci.* 64:910–916.
- Sinanoglou, V. J., F. Mantis, S. Miniadis-Meimaroglou, G. K. Symeon, and I. A. Bizelis. 2011. Effects of caponisation on lipid and fatty acid composition of intramuscular and abdominal fat of medium-growth broilers. *Br. Poult. Sci.* 52:310–317.
- Sirri, F., M. Bianchi, M. Petracci, and A. Meluzzi. 2009. Influence of partial and complete caponization on chicken meat quality. *Poult. Sci.* 88:1466–1473.
- Sokołowicz, Z., J. Krawczyk, and S. Świątkiewicz. 2016. Quality of poultry meat from native chicken breeds – a Review. *Ann. Anim. Sci.* 16:347–368.
- Symeon, G. K., F. Mantis, I. Bizelis, A. Kominakis, and E. Rogdakis. 2010. Effects of caponization on growth performance, carcass composition, and meat quality of medium growth broilers. *Poult. Sci.* 89:1481–1489.
- Tor, M., J. Estany, and D. Villalba. 2002. Comparison of carcass composition by parts and tissues between cocks and capons. *Anim. Res.* 51:421–431.
- Tor, M., J. Estany, A. Francesch, and M. D. Cubiló. 2005. Comparison of fatty acid profiles of edible meat, adipose tissues and muscles between cocks and capons. *Anim. Res.* 54:413–424.
- Ulbricht, T. L. V., and D. A. T. Southgate. 1991. Coronary heart disease: seven dietary factors. *Lancet N. Am. Ed.* 338:985–992.
- Winter, A. R., and E. M. Funk. 1960. *Poultry Science and Practice*. J.B. Lippincott Co., New York, NY.
- Xiong, Y. L. 1994. Myofibrillar protein from different muscle fiber types: implications of biochemical and functional properties in meat processing? *Crit. Rev. Food Sci. Nutr.* 34:293–320.