Myofibrillar fragmentation in entire male, immunocastrated or surgically castrated pigs

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Abstract. In order to better characterise differences in meat quality traits between the alternatives to surgical castration, myofibrillar fragment length was investigated in longissimus dorsi muscle of entire (n=12), immunocastrated (n=12) and surgically castrated (n=12) male pigs. Higher myofibrillar fragment length was observed in meat from entire pigs than in surgically castrated and immunocastrated male pigs after two days of post mortem storage (38% and 19%, respectively). There were no differences between the groups after 7 days of post mortem storage. Although this change in myofibrillar fragment length indicates a higher proteolytic potential of muscle from entire male pigs than the other pigs studied, it could not be associated with the meat quality traits of meat tenderness or water holding capacity, suggesting the importance of other influential factors over the proteolysis.

1. Introduction

Introduction of alternatives to surgical castration like rearing of entire males (EM) or immunocastrates (IC) is becoming a reality in the European pig sector. In spite of several positive aspects such as better feed conversion, lean deposition and cost-effectiveness [1], rearing of alternatives brings certain disadvantages. In addition to a higher risk of boar taint, these include reduced meat quality, either in terms of lower intramuscular fat (IMF) content, increased meat toughness or reduced water holding capacity in EM and IC compared to surgical castrates (SC) [2]. The literature reports on the differences between the sex categories are inconsistent and need to be further substantiated, as does the aetiology behind the associated biochemical processes. Both EM and IC are metabolically very different categories from SC, due to the presence (in EM) or sudden drop (in IC) of androgen potential [3], which likely affects muscle proteolytic properties known to be associated with meat quality traits like tenderness and water holding capacity (WHC) [4,5]. One of the methods used to indicate post-mortem proteolysis in meat is the measurement of myofibrillar fragmentation [6], which has been applied in the present study in an attempt to characterise changes in EM and IC muscle and is associated with these selected meat quality traits.

2. Materials and Methods

The material for the study originated from 36 pigs (12 EM, 12 IC and 12 SC), commercial Landrace × Pietrain crossbred pigs. IC pigs were vaccinated at the ages of 12 and 22 weeks. All pigs were slaughtered at the age of 27 weeks, when their average live weight was 128.9±1.5 kg. A day after slaughter, samples of longissimus dorsi (LD) muscles were taken from the cooled carcasses and the next day (day 2), meat quality traits (drip loss, thawing loss, cooking loss, shear force (SF) and IMF)
were measured as described in Batorek et al. [7]. On day 2, the length of myofibrillar fragments (MFL) was assessed, and samples of LD were vacuum packed and stored at 4°C for MFL determination 7 days post mortem (day 7).

For MFL measurements, a small amount (2.5 g) of LD was excised, cut to small pieces with a scalpel, added to 25 ml of isolation buffer (consisting of 4 mM KH₂PO₄, 16 mM K₂HPO₄, 1 mM EDTA, 1 mM NaN₃ and 100 mM KCl, at pH 7.0) and homogenised using Ultra-Turrax T25 (IKA Werke GmbH & CO.KG, Staufen, Germany) for 60 seconds at 10,000 rpm. The homogenate was centrifuged at 2°C for 15 min at 1000 g, and the supernatant discharged. Isolation buffer (12.5 ml) was added to the pellet and stirred well; 1 ml of the suspension was further diluted with 12.5 ml of isolation buffer and stirred again. A drop of the suspension was put on a glass slide and examined under a Zeiss Axio Imager Z1 microscope (Carl Zeiss AG, Oberkochen, Germany) with differential interference contrast (DIC) illumination, equipped with Zeiss AxioCam MRc5 digital camera supported by the AxioVision 4.8.2.0 imaging software. For each sample, image analysis (determination of MFL) was performed on 5 different pictures with 40 to 60 fragments measured in each. Relative changes of average MFL (MFL index) between days 2 and 7 were calculated and expressed as %.

Data were analysed with SAS statistical software (SAS Institute Inc., Cary, NC, USA). The effect of sex category was tested using the GLM procedure; when significant differences (p < 0.05) were observed, least squared means were compared using Tukey’s test. Furthermore, factor analysis (method=prin) was conducted to investigate the relationships between the MFL and the measured meat quality traits.

3. Results and Discussion

As presented in Figures 1 and 2, MFL in the LD muscles on day 2 was 38% higher (p < 0.05) in EM than in SC, while IC had MFL in the intermediate position (19% lower values than in EM), not differing (p > 0.05) from either EM or SC. There were no differences (p > 0.10) in MFL between the sex groups on day 7. The differences in MFL during the post mortem storage (expressed as the MFL index) tended to be higher (p < 0.10) in EM (38% MFL reduction) than in SC (24% MFL reduction), with IC being positioned in between (31% MFL reduction).

![Figure 1. Microscopic images of myofibrillar fragments in longissimus dorsi muscle of entire male (EM), immunocastrated (IC) and surgically castrated (SC) pigs after 2 and 7 days of post mortem storage.](image-url)
A positive association between the extent of myofibrillar fragmentation and meat tenderness (i.e. lower shear force) was established long ago [8] and is commonly ascribed to *post mortem* proteolytic degradation of the main myofibrillar structural proteins, leading to Z-line disruption [5]. The structural detachments could also release tensions caused by muscular *post mortem* shortening and, thus, improve WHC [4]. The results obtained for the MFL index in the present study indicate the highest proteolytic potential of the LD muscle can be attributed to EM pigs. On the other hand, the difference in MFL on day 2 between EM and SC pig muscle was not accompanied by increases in meat tenderness (SF) or selected WHC traits (Table 1). Moreover, drip loss, thawing loss and shear force did not differ (*p* > 0.10) between our pig castration categories. Only cooking loss was higher (*p* < 0.05) in LD from IC than in SC pigs. Factor analysis (Figure 3) confirmed a lack of association between MFI and our measured meat quality traits, while WHC traits were positively related to each other and negatively related to IMF. Similarly to the present research, our recent study on EM and SC differences in muscle physical-chemical traits and proteomic profile [9] also indicated a higher degree of proteolysis in EM (based on the higher abundance of myofibrillar protein fragments) and no correlation with meat toughness or WHC. This suggested that other traits, like IMF and protein oxidation, could be the main factors in explaining the differences in meat quality of LD muscle derived from EM and SC pigs.

### Table 1. Meat quality traits measured in *longissimus dorsi* muscle

| Trait          | EM<sup>a</sup> | IC<sup>b</sup> | SC<sup>c</sup> | RMSE<sup>d</sup> | *p*-value |
|----------------|---------------|---------------|---------------|-----------------|----------|
| Intramuscular fat | 1.2<sup>a</sup> | 2.0<sup>y</sup> | 2.2<sup>y</sup> | 0.66            | 0.001    |
| Drip loss, %    | 3.8           | 4.1           | 3.8           | 2.51            | 0.450    |
| Thawing loss, % | 10.6          | 8.7           | 8.0           | 3.02            | 0.123    |
| Cooking loss, % | 30.7<sup>y</sup> | 32.7<sup>y</sup> | 29.3<sup>x</sup> | 2.68            | 0.015    |
| Shear force, N  | 63.8          | 68.3          | 60.0          | 10.39           | 0.166    |

<sup>a</sup>Entire males;  
<sup>b</sup>Immunocastrates;  
<sup>c</sup>Surgical castrates;  
<sup>d</sup>Root-mean-square error;  
<sup>x</sup>, <sup>y</sup>, <sup>z</sup>Within a row, values with a different superscript differ significantly (*p* < 0.05).
Figure 3. Factor analysis plot showing associations between selected meat quality traits (shear force – SF, intramuscular fat – IMF, drip loss – DL, thawing loss – TL, cooking loss – TL) and myofibrillar length index (MFI)

4. Conclusions
Based on the myofibrillar fragment length, the present study indicates that LD from EM pigs possesses the highest proteolytic potential among the pig castration categories studied. The results on myofibrillar fragment length, however, could not be associated with meat tenderness or water holding capacity, implying that other factors have more impact. Further research on characteristics like protein oxidation and enzyme activities are needed to explain the nature of the underlying processes.

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