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1 **The effect of immunocastration of male and female Duroc pigs on the**
2 **morphological, mechanical and compositional characteristics of pork**
3 **belly**

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14

15 **Abstract**

16 Pork belly is an important and heterogeneous cut, characterized by its fat
17 content. Immunocastration is an alternative to surgical castration that can
18 modify the composition of carcasses and cuts and it can affect at its processing.
19 This work compares the morphological, mechanical and compositional
20 characteristics of pork belly of (1) pure Duroc pigs from surgically castrated
21 males (CM), entire females (EF) and immunocastrated females (IF), and (2)
22 Duroc crossbred pigs from immunocastrated males (IM) and entire males
23 (EM). Two trials were carried out: Trial 1, in which 36 bellies were evaluated, 12
24 from each sexual type, CM, EF and IF; and Trial 2, where 30 bellies were used,
25 15 from each sexual type, IM and EM. Results show few differences in bellies
26 from EF and IF, while those from CM were fatter and firmer and with lower
27 polyunsaturated fat. Bellies from IM were longer and firmer than those from EM,
28 and their skin was thinner. IM bellies had higher saturated and lower
29 polyunsaturated fat than those from EM. To conclude, the sex of the pigs affects
30 belly characteristics and this could be a criterion for determining the destination
31 of the bellies in the cutting plant. Immunocastration of pure Duroc females had a
32 lower effect on the belly characteristics when compared to those from entire
33 females, but some differences could be found in the fat distribution.
34 Immunocastration of Duroc crossbred males produces firmer and thicker bellies,
35 with a thinner skin, that could be advantageous for slicing and further
36 processing.

37 **Keywords:** computed tomography, firmness, flop, fatness, fatty acids

38

40 **1. Introduction**

41 Pork belly is an important fatty cut of pig carcasses, which accounts for
42 approximately 10% of its weight (Zomeño et al., 2022) although this percentage
43 can vary depending on how the cuts are made. The quality of the belly is related
44 to the lean and fat content and distribution, thickness, fat characteristics
45 (softness/firmness) and mechanical properties and it varies across anatomical
46 region (Trusell et al., 2011). In general, leaner bellies are thinner (Averette
47 Gatlin et al., 2003), while thicker bellies are fatter. While leaner bellies are in
48 general preferred by most consumers, thinner bellies are not beneficial for
49 producers due to the low processing yield and the possibility of having softer fat
50 (Averette Gatlin et al., 2003). Thus, it is necessary to find an equilibrium that
51 satisfies consumer demand without compromising productivity. The
52 characteristics of the belly are mainly related to the genotype, sex and diet,
53 thus, by modifying these factors, it is possible to modify belly quality (Apple et
54 al., 2011; Duziński et al., 2015; Soladoye et al., 2015).

55 The Duroc breed and crossbreeds are characterized by their high carcass and
56 intramuscular fat content (Font-i-Furnols et al., 2012), but with a tendency to
57 have high levels of boar taint (Xue et al., 1996) and undesired odour and flavour
58 present in meat from some entire male pigs. Because of this, pure Duroc males
59 (and some crossbreeds) are usually castrated. Immunocastration is an animal-
60 friendly alternative to surgical castration that can be used in males and females,
61 but it can affect the fatness of the carcasses and the quality of the fat.
62 Immunocastrated males behave like entire males until the second vaccine (4-10
63 weeks before slaughter) at which point they tend towards the behaviour of those

64 castrated (Dunshea et al., 2001; Gispert et al., 2010), *i.e.* by increasing fat and
65 reducing boar taint. Thus, carcasses from immunocastrated males have a fat
66 content in between those of entire males and surgically castrated males
67 (Carabús et al., 2017; Gispert et al., 2010; Zomeño et al., 2022). The
68 immunocastration of females has been less studied than that of males and is
69 more common in females from fatty breeds to obtain carcasses closer to those
70 of castrated males since it increases the carcass fat content (Daza et al., 2014;
71 Pérez-Ciria et al., 2021). The quality of the fat is also affected by the sex. In
72 general, entire males have higher unsaturated fat, which can result in visual
73 defects and lower oxidative stability, and lower saturated fat than surgically
74 castrated pigs, immunocastrated pigs being in between the two (Škrlep et al.,
75 2020). Regarding fat from females, some research shows they have lower
76 monounsaturated fat than surgically castrated males (Zomeño et al., 2023)
77 while in other research, no differences were reported (Font-i-Furnols et al.,
78 2012).

79 Regarding bellies characteristics, Kyle et al. (2015) reported that those from
80 immunocastrated males had similar length and were thicker and firmer than
81 those from entire males. Bellies from immunocastrated males had also higher
82 saturated and monounsaturated fatty acids and lower polyunsaturated and
83 iodine value than those from entire males. Bellies from immunocastrated males
84 were also wider and less thick and firm than those from surgically castrated pigs
85 and had lower monounsaturated fatty acids and polyunsaturated fatty acids and
86 iodine value (Boler et al., 2012). Nevertheless, there are few studies in which
87 the effect of immunocastration of gilts on bellies characteristics has been
88 evaluated. In this sense, Palma-Granados et al. (2021) reported higher

89 proportion of belly in Iberian immunocastrated female pigs than in surgically
90 castrated and immunocastrated male pigs. On the other side, Rodriguez et al.
91 (2019) did not find significant differences in the bellies weight and fat thickness
92 from entire and immunocastrated females of a white crossbreed. Thus, a more
93 in depth study of bellies characteristics from these sexual types of pigs is
94 needed.

95 The distribution of the fat along the belly slice is an important characteristic
96 because in sliced bellies is what the consumers see and what can make them
97 taken the decision to purchase or not the belly. However, as far as the authors
98 known, there are not studies that look at the fat distribution in the belly slice.
99 The fat distribution can be evaluated without cutting or destructing the belly, by
100 means of a computed tomography device that is based on X-rays and allows to
101 obtain images from the interior part of the belly and to quantify the fat and lean
102 content of the slice or portion of the slice.

103 According to this, it is important to find out the effect of the immunocastration on
104 bellies morphological, mechanical and compositional characteristics, including
105 the fat distribution along the slice. This would allow to see whether
106 immunocastration of females of fatty breeds increases their fatness and brings
107 them closer to castrated males or whether immunocastration of males, as an
108 alternative to entire males, has an effect on bellies' characteristics since very
109 few information is available in the literature about it. This is a way to enables us
110 to understand the changes in belly characteristics due to the sex type in order to
111 optimize the destination of the bellies and its processing. Thus, the main
112 objective of this work is to compare the morphological, mechanical, and
113 compositional characteristics of bellies from (1) surgically castrated males and

114 entire and immunocastrated females of a pure Duroc pig line, and (2)
115 immunocastrated and entire males of a Duroc pig crossbreed.

116 **2. Material and Methods**

117 **2.1. Animals and bellies**

118 **2.1.1. Trial 1**

119 A total of 36 bellies were used, 12 from each sexual type: castrated male (CM),
120 entire female (EF) and immunocastrated female (IF). The bellies came from
121 pure Duroc commercial pigs, all from the same farm and fed *ad libitum* the
122 same diet (2317 kCal/kg net energy, 14.7% crude protein and 5.03% crude fibre
123 between 60 and 90 kg BW; 2395 kCal/kg net energy, 13.7% crude protein and
124 5.07% crude fibre between 90 and 125 kg BW). Immunocastration of females
125 was carried out with Improvac® (Zoetis, Madrid, Spain) in two doses, the first
126 (V1) at 132 days and the second (V2) after 4 weeks, at 159 days. Surgical
127 castration of male piglets was performed within the first week of life. Pigs were
128 slaughtered in a commercial abattoir after stunning with CO₂ when they reached
129 the target body weight (approximately to obtain carcasses with 95 kg weight).
130 Thus, they were slaughtered at fixed weight. In the case of immunocastrated
131 pigs, they were slaughtered 11 weeks after V2 (at approximately 235 days of
132 life). Carcass weight was recorded and bellies were collected in the cutting plant
133 24 h *post mortem*.

134 **2.1.2. Trial 2.**

135 A total of 30 bellies were tested, 15 from each sexual type: immunocastrated
136 male (IM) and entire male (EM). The bellies came from commercial Duroc
137 crossbred pigs, all from the same farm and fed *ad libitum* the same diet (2321.5
138 kCal/kg net energy, 14.9% crude protein and 3.60% crude fibre between 25 and

139 80 kg BW and 2316.7 kCal/kg net energy, 14.62% crude protein and 3.54%
140 crude fibre between 80 kg BW and slaughter). Immunocastration was carried
141 out with Improvac® (Zoetis, Madrid, Spain) in two doses, V1 at 56 days of age
142 and V2 after 10 weeks, at 126 days of life approximately. All the pigs were
143 slaughtered across 3 different days at 5-6 weeks after V2 at IRTA's abattoir,
144 after stunning with CO₂, and thus, they were slaughtered at fixed age. Carcass
145 weight was recorded, and bellies were collected in the cutting room 24 h *post*
146 *mortem*.

147 Bellies from both trials were cut from the left half of the carcass, at the dorsal
148 section, 1 cm from the end of the vertebrae, at the caudal section, 6 cm from
149 the last rib, at the ventral section along the breast line and at the cranial section
150 between the 4th and 5th ribs. Then, ribs were removed from the cut.

151 **2.2. Morphological and mechanical measurements of the bellies**

152 Bellies in both trials were weighed and the proportion of belly with respect to the
153 carcass weight was calculated. For the description of the bellies, 15 areas were
154 identified, similar to those defined by Trusell et al. (2011). From cranial to
155 caudal part, 5 sections were identified as 1, 2, 3, 4 and 5, and from dorsal to
156 ventral, 3 sections named A, B and C. Thus, the combination of all of these
157 allowed 15 different areas, A1, A2,..., C4 and C5 (Fig. 1), to be identified.

158 The length and width of the bellies were measured at the central section, i.e.
159 length from B1 to B5 and width from A3 to C3 (Fig. 2a). The thickness of each
160 belly was measured skin side-up in the centre of sections B1, B5, A3 and C3.
161 Then the average thickness was calculated. After that, the skin was stretched
162 using tweezers until the base of the belly lifted and the height was measured

163 (Fig. 2b). The difference between the initial height (thickness) and final height
164 was calculated and used as a measure of firmness in terms of subcutaneous fat
165 and skin separation (*i.e.* cohesiveness). Firmness was also determined by
166 means of the flop distance and angle measured skin-side up and skin-side
167 down (Fig. 2c) by means of the bar-suspension method (Thiel-Cooper et al.,
168 2001) using a horizontal stainless steel bar of 20 mm of diameter. Bellies were
169 suspended from the central part (between A3 and C3) and the distance
170 between the extremes of the dorsal section (from A1 to A5) was determined.
171 The angle was determined using this distance and the length of the belly.
172 Subsequently, the firmness of the subcutaneous fat of the bellies, was also
173 measured, having previously removed the skin, by two trained technicians
174 applying pressure with a finger (Fig. 2d) and using a 5-point scale as defined by
175 Soladoye et al. (2017) from the firmest to the softest: *"1-Firm fat, no finger*
176 *depression, almost horizontal; 2-Firm fat, no finger depression, partly floppy; 3-*
177 *Soft spongy fat, finger depression remains, floppy, roll over with resistance; 4-*
178 *Soft spongy fat, finger depression remains, very floppy, roll over easily; 5-Soft*
179 *spongy fat, finger depression remains, very floppy, roll over easily, oily"*. The
180 average score from both technicians was used.
181 A square of 4x4 cm² of skin from the central part of the belly (B3) was used to
182 measure skin thickness with a "Quick mini 25" (Mituyoto, Kanagawa, JP)
183 micrometer. Then, skin was placed and fixed in a support with the external part
184 of the skin oriented upwards and a puncture test was carried out using a 6 mm
185 cylinder probe fixed in a conical base, and a TA.HD.PLUS Texture Analyser
186 (Stable Micro Systems, Surrey, UK) texturometer equipped with a 250 kg load-
187 cell. A force-time deformation curve was recorded with trigger force of 50 g until

188 the skin broken to measure: the maximum value force (Peak force), leading to
189 the maximum force required to penetrate the sample; the area under the force-
190 time deformation curve (Total force), indicating the total energy required to
191 penetrate the sample; the slope of the force-time deformation curve (Slope)
192 between 75% and 95% of the maximum force, providing the elasticity.

193 **2.3. Physical and chemical compositional measurements of the bellies**

194 Deboned bellies with skin from both trials were subject to computed tomography
195 (CT) scanning using the HiSpeed Zx/I CT device (GE Healthcare, Madrid, ES).
196 Acquisition conditions were helical, pitch 1, 140 kV, 145 mA, 10 mm thickness,
197 displayed field of view 300 mm. The image of the central slice of each belly was
198 analysed as follows: the slice was divided into 5 regions, proportional to the
199 belly width. For each region the proportion of fat was calculated as the volume
200 associated with Hounsfield values from -200 to -20 (Romvári et al., 2005) and
201 using segmentation techniques that consider the neighbouring values to
202 establish the final limit.

203 A sample of 10 g of subcutaneous fat of the central part of the belly (B3) was
204 removed, vacuum packed and frozen at -20°C for chemical analysis. The
205 remainder of the belly was minced with the cutter. After homogenization of the
206 minced belly, a sample was vacuum packed and frozen at -20°C for further
207 processing.

208 Moisture (oven air-drying method), protein (Kjeldahl nitrogen), and ash (muffle
209 furnace) of the minced bellies were analyzed following official methods (AOAC,
210 2000). Lipids from the minced bellies were extracted, using
211 chloroform/methanol (1:2, v/v), and quantified according to the method

212 described by Bligh and Dyer (1959). Lipids from subcutaneous fat samples
213 (10g) of the central part of the belly were extracted in a microwave oven
214 following the method described by De Pedro et al. (1997).

215 Fatty-acid composition of subcutaneous fat samples was determined, after lipid
216 extraction, by acidic trans-esterification in the presence of sodium metal (0.1 N)
217 and sulfuric acid (5% sulfuric acid in methanol) according to Sandler and Karo
218 (1992). The fatty acid methyl esters were analysed by gas chromatography,
219 using a Hewlett-Packard HP-4890 Series II gas chromatograph equipped with a
220 split/splitless injector and a flame ionization detector (FID). Separation was
221 carried out on a polyethylene glycol capillary column (HP-INNOWax 30 m long,
222 0.25 mm id, 0.25 µm film thickness; Hewlett-Packard, Palo Alto, CA, USA)
223 maintained at 200 °C for 25 min. Injector and detector temperatures were held
224 at 250 °C. The carrier gas was nitrogen at 1.8 mL/min. The individual fatty acids
225 were identified by comparison of their retention times with those of commercial
226 reference standard mixtures (Supelco 37 Component FAME Mix Ref. CRM
227 47885, Sigma Chemical Co., St. Louis, MO, USA). Results were expressed as
228 mg/100g of total fat.

229 Iodine value (IV) was determined according to the modified AOCS equation
230 obtained by Lo Fiego et al. (2016), by including all the unsaturated fatty acids
231 detected by gas chromatography:

$$\begin{aligned} 232 \quad IV = & [C16: 1] \times 0.950 + [C17: 1] \times 0.903 + [C18: 1] \times 0.860 + [C18: 2] \times 1.732 \\ 233 & + [C18: 3] \times 2.615 + [C20: 1] \times 0.785 + [C20: 2] \times 1.580 \\ 234 & + [C20: 3] \times 2.386 + [C20: 4] \times 3.201 \end{aligned}$$

235 **2.4. Statistical analysis**

236 The general linear model (GLM) procedure of SAS software (Ver. 9.4, SAS
237 Institute Inc., Cary, NC, USA) was used. Analysis was performed independently
238 for each trial and animal was the experimental unit. The experimental design is
239 factorial with one factor, sex. Thus, the model included the sexual type as a
240 fixed effect. For Trail 2, since animals were slaughtered at a fixed age, the
241 weight was not included as covariate to avoid an underestimation of differences
242 in those characters that grow linearly with the carcass weight. Non other
243 additional covariances were included (the full model can be found in the
244 Supplementary Material). Differences between sexual types have been
245 obtained after and were considered significant if the *P*-value was lower than
246 0.05. In Trial 1, because comparisons were between more than 2 sexes, Tukey
247 test was applied.

248 **3. Results and Discussion**

249 Results are detailed and discussed conjointly for both trials to avoid repetition.
250 However, it is important to take into account that comparison among sexual
251 types is only possible within the trial, because other factors such as genotype,
252 feeding, immunocastration vaccine protocol and management differ between
253 trials. Thus, the comparisons are between CM, EF and IF of pure Duroc pigs
254 slaughtered at fixed weight (approximately 95 kg carcass weight), which IF
255 received the second dose of the vaccine 11 weeks before slaughter (Trial 1),
256 and between EM and IM Duroc crossbreed pigs slaughtered at fixed age (23-24
257 weeks), which IM received the second dose 5-6 weeks before slaughter (Trial
258 2). In the present work, the diet was the same for all the pigs within the same
259 trial, thus, besides the effect of diet on bellies composition and fatty acid profile,
260 differences in belly characteristics (within Trial) were mainly due to sex.

261 **3.1. Morphological characteristics of the bellies**

262 Pork belly is an important cut for the meat industry and its proportion depends
263 on the type of cutting. The tendency in Europe is to increase the carcass
264 weight. Usually, the higher the carcass weight, the higher the belly weight and
265 belly dimensions, although differences at carcass level may not always be
266 significant at the level of the cut.

267 Logically, because animals were slaughtered at the same weight, no differences
268 in carcass weight between CM, EF and IF pigs from Trial 1 was found (Table 1).
269 Accordingly, no differences in belly weight were detected between sexes.

270 However, the proportion of bellies from CM pigs were 0.73% higher than those
271 from IF pigs ($P < 0.05$), indicating they have a higher yield, and not significantly
272 different from those of EF pigs, which were in between the two. Similar belly
273 proportions between CM and EF were also reported by Gispert et al. (2010) and
274 Zomeño et al. (2022) whereas Duziński et al. (2015) found a higher proportion
275 of belly in CM pigs than in EF pigs. On the other hand, belly weight and
276 proportion in IF and EF pigs were similar, in agreement with Rodrigues et al.
277 (2019) when comparing heavy weighted gilts. In Iberian pigs, Palma-Granados
278 et al. (2021) reported higher belly proportion for IF than CM. Thus, the effect of
279 immunocastration of gilts on belly weight and proportion is not conclusive and it
280 is probably dependent on the animal genotype, the vaccination pattern, the
281 feeding and other management strategies and the weight at slaughter.

282 Carcasses of IM pigs from Trial 2 were 5.0 kg heavier than those of EM (Table
283 1). This result is in line with that reported by Gispert et al. (2010), although, in
284 some research, differences in carcass weight between both sexes were not
285 significant (Zomeño et al., 2022), nor were they when different times between

286 V2 and slaughter were studied (Zoels et al., 2020). Similarly, although the IM
287 carcasses from Trial 2 were heavier than EM carcasses, belly weight and
288 proportion were not significantly different, in agreement with Zomeño et al.
289 (2022), but not in agreement with Gispert et al. (2010) which reported higher
290 belly proportion in IM carcasses than EM. Thus, as well as with females,
291 differences in carcass weight were not translated into differences in belly weight
292 and proportion.

293 Belly dimensions are important, especially when bellies are sliced. A greater
294 length provides a greater number of slices. Thicker and wider bellies provide
295 bigger slices. Dimensions depend on the characteristics of the belly and the
296 type of cutting. Moreover, production strategies such as genotype, sex and diet,
297 can modify belly characteristics (Soladoye et al., 2015) and it is therefore
298 important to take these into account in order to obtain the desired product.

299 Regardless of the lack of differences in belly weight and proportion, EF bellies
300 from Trial 1 tended to be wider than CM bellies, IF being in between the two
301 (Table 1). Nevertheless, length was not significantly different between CM, EF
302 and IF bellies. Lowell et al. (2019) reported longer bellies of CM than those of
303 EF, although not being different in width. However, in Trial 2, bellies of IM were
304 on average 2.9 cm longer than those of EM, but no significant differences in
305 width were detected (Table 1).

306 Belly thickness is difficult to measure because it is very variable and depends
307 on where it is measured (Trusell et al., 2011). Moreover, differences in
308 thickness were not consistent in the various places measured (Fig. 3) and, on
309 average, were not significantly different between sexes (Table 1). For instance,
310 in Trial 1, CM bellies were thicker than EF only at the centre of the dorsal

311 section (A3) (Fig. 3a), while Lowell et al. (2019) and Kyle et al. (2014) reported
312 thicker CM bellies than EF bellies when the average thickness obtained at 8
313 different locations was measured. Moreover, IF bellies tend to be thicker than
314 CM at the ventral section (C3). On the other hand, IM bellies from Trial 2 were
315 0.38 cm thicker than those of EM only in the centre of the caudal section (B5)
316 (Fig. 3b). On average there were no differences in thickness between IM and
317 EM, while in heavier animals (130 kg), Kyle et al. (2014) reported thicker bellies
318 of IM than EM. Thus, considering that thicker is considered better,
319 immunocastration of females or males did not seem to negatively affect the
320 thickness of the bellies when compared to entire females/surgically castrated
321 males or to entire males, respectively.

322 **3.2. Mechanical characteristics of bellies**

323 The firmness of the bellies from Trial 1, measured as the flop distance and
324 angle, is presented in Table 1. Flop distance and angle skin below were higher
325 (higher firmness) in bellies of CM than in those of FE and IF. Flop distance skin
326 above was higher in CM than in IF and flop angle was higher in CM than in EF.
327 Firmness was also measured as the separation of the skin plus fat when
328 stretched with tweezers (Fig. 3), showing higher firmness (less height increase)
329 in bellies of CM than EF and IF in A3. CM also tended ($P < 0.10$) to be firmer
330 than EF in B1, and firmer than IF in B5. Differences in the increased height
331 were not significant in C3 position. Another measurement of firmness was
332 carried out by trained operators scoring the resistance when applying pressure
333 with a finger. The higher the score, the lower the firmness. Results show that
334 firmness was lower in the ventral section (C) and higher in the dorsal section
335 (A), central section (B) being in between the two. In general, firmness was also

336 higher in the cranial than in the caudal section. This in general agrees with the
337 texture measured by Trusell et al. (2011) except in the cranial-central and
338 cranial-dorsal positions. Moreover, when comparing between sexual types, no
339 difference in firmness was found in the ventral region (C), where all the bellies
340 were scored close to 5 (maximum softness). Bellies from IF and EF pigs were
341 not significantly different in firmness in any position, with the exception of B1
342 where IF bellies tended to be firmer than EF ($P < 0.10$). With respect to CM
343 bellies, they were firmer than IF and EF bellies in all the positions of the dorsal
344 region (A) and in B2, B3 and B5, and firmer than IF in B4. In B1 region, bellies
345 of CM and IF were similar and tended to be firmer than EF ($P < 0.10$) (Fig. 4).
346 According to all the measurements of firmness, no significant differences can be
347 found between bellies of IF and EF, indicating that immunocastration may not
348 negatively affect the firmness of the bellies, but also that immunocastration may
349 not improve the firmness of female bellies compared to those surgically
350 castrated. This result was also confirmed by the lack of difference obtained in
351 the texture analysis of the belly (results not shown).

352 When immunocastration is applied to male pigs, differences in belly firmness
353 are more significant and bellies of IM were firmer than those of EM when flop
354 distance and angle were evaluated (Table 1). However, the firmness of the
355 subcutaneous fat evaluated by stretching the skin was only significantly higher
356 (less increase) in IM than EM bellies in B1 position (Fig. 4). Similarly, firmness,
357 scored by the trained operators applying pressure with a finger at the 15
358 different zones (Fig. 4b), was significantly higher in IM than EM bellies in B4, C2
359 and C3 positions and tended to be firmer ($P < 0.10$) at B1, B5 and C1 positions.
360 Thus, especially in the central and ventral section, bellies of IM pigs were firmer

361 than those of EM pigs, while there was no difference in all the dorsal positions.
362 The increase in firmness in bellies of IM might mean they are more suitable for
363 bacon processing and for export requirements (Uttaro et al., 2020). Moreover,
364 at industrial level, it is difficult to slice soft bellies or bellies with low
365 cohesiveness between skin and subcutaneous fat layer and they must be given
366 a cold shock to facilitate the cutting process. Thus, increasing the firmness
367 could help to slice the fresh bellies.

368 Skin thickness and firmness was not significantly different between CM, IF and
369 EF (Trial 1; Table 1). However, when males are immunocastrated, significant
370 differences can be found in skin properties, skin from EM being thicker and
371 harder than that from IM (Trial 2; Table 1). A thicker skin might reduce the yield
372 of skinless bellies. Moreover, consumer acceptability of the belly might also be
373 affected by the thickness of the skin.

374 **3.3. Compositional characteristics**

375 Belly fatness is an important parameter that also influences firmness (Soladoye
376 et al., 2017). The tendency is to increase the leanness of the carcasses and the
377 leanness of bellies due to consumer demand for leaner meat (Lebret & Čandek-
378 Potokar, 2022). Leanness is related to genotype and diet, but sex also has an
379 important effect. It is well known that EM carcasses are leaner than EF and that
380 these are leaner than CM (Pauly et al., 2012). Moreover, at the beginning, IM
381 are similar to EM, but after V2 they increase the fat content and become closer
382 to EF (Carabús et al., 2017).

383 Results show that the protein and ash content of minced bellies was higher in IF
384 than in CM bellies and intermediate in EF ones (Trial 1). Furthermore, dry

385 matter was higher and moisture lower in CM than in EF and IF bellies.
386 Regarding fat content, bellies from CM pigs were fatter than those from IF pigs,
387 EF bellies being in between (Table 1). When IM and EM were compared, no
388 significant differences in any of the proximate composition parameters were
389 observed (Table 1). Thus, immunocastration, whether of females (Trial 1; Table
390 1) or males (Trial 2; Table 1) did not have an influence on the fat and lean
391 composition of the bellies. This result is surprising since, in general,
392 immunocastration increases the fat thickness, both in females (Daza et al.,
393 2014; Pérez-Ciria et al., 2021) and probably even more clearly in males
394 (Batorek et al., 2012; Dunshea et al., 2001) especially when the time between
395 V2 and slaughter is long (Allison et al., 2021; Poulsen Nautrup et al., 2018).
396 However, in some research, mainly concerning females, this effect is not so
397 clear (Di Martino et al., 2018; Rodrigues et al., 2019), probably due to genetics,
398 the diet or the time between V2 and slaughter. Besides, it has been reported
399 that the fat allometric growth coefficient of IM is higher than that of EM (Carabús
400 et al., 2017), thus, fat deposition speed of IM is higher than that of EM. On the
401 other hand, the allometric coefficient for belly weight was not significantly
402 different between EM and IM (Carabús et al., 2017). This finding might explain
403 the lack of differences in belly composition between IM and EM. As far as the
404 authors know, no information regarding the allometric growth of fat of IF has
405 been reported.

406 When looking in more detail at the fat distribution in the central slice of the belly
407 (Fib. 4) it is possible to see that, as is well known, the fat content of bellies is
408 higher in the dorsal section than in the ventral (Trusell et al., 2011). When
409 comparing the distribution of fat depending on the sex type, studied in Trial 1, it

410 is possible to see that CM presented higher fat content in the central and ventral
411 sections of the belly than EF and IF. Moreover, although there were no
412 differences in total fat content between IF and EF (Table 1), in the more dorsal
413 section of the belly, IF presented higher fat content than EF, with IF fat content
414 being similar to those of CM bellies (Fig. 5a). This variation in the distribution of
415 fat in the different anatomical positions of the belly slice could explain the lack of
416 differences in total fat content between bellies of IF and of EF. Also, looking in
417 detail at the fat distribution in the central slice between bellies of IM and EM, it is
418 possible to see that, although there were no differences in the total fat content
419 (Table 1), EM bellies are leaner ($P < 0.05$) than IM bellies in the dorsal and
420 ventral sections, with no significant differences in the central section (Fig. 5b).
421 Thus, although the global fat content is significant, it is also very important to
422 evaluate the distribution of the fat in the different zones of a slice since some
423 differences can be found that might influence processing suitability and
424 consumer preference for the bellies.

425 Fatty acid composition of the subcutaneous fat of the centre of the belly (B3
426 zone) is different depending on the sex type. Although, globally, SFA were not
427 significantly different between sexual types (CM, EF and IF; Trial 1; Table 2),
428 some minor individual SFA like C14:0 (myristic) and C20:0 (arachidic) were
429 higher in the subcutaneous fat of CM than EF bellies, with IF being in between
430 the two. Also, the major individual SFA, C16:0 (palmitic) tended ($P < 0.10$) to be
431 higher in CM than in EF. MUFA globally were also not significantly different
432 between sexes, while C20:1(n-9) was significantly higher in bellies from CM
433 than IF pigs, the subcutaneous fat of EF bellies falling in between. When PUFA
434 are considered globally, they were higher in fat from EF and IF than in those

435 from CM bellies, probably due to the fact that this difference can be observed in
436 the major individual PUFA such as C18:2(n-6) (linoleic). This fatty acid is the
437 major ω 6 and probably because of this, the same pattern can be seen, both in
438 ω 6 and in the ratio ω 6/ ω 3. Significant differences in IV between sexual types
439 were also found with a higher value in EF than in CM pigs, with IF in between.
440 This differences in PUFA and IV might explain that bellies from EF and IF were
441 less firm than those from CM, when firmness was evaluated as the separation
442 between subcutaneous fat and skin, pressing with a finger the subcutaneous fat
443 and using the flop angle and distance. According to these results, the
444 immunocastration of females does not seem to affect the fatty acid composition
445 of the subcutaneous fat of the belly, when compared to entire females. This is in
446 disagreement with the results reported by Pérez-Ciria et al. (2021) and Daza et
447 al. (2014), in which IF had higher SFA and a lower PUFA, PUFA/SFA ratio, ω 6,
448 ω 3 and ω 6/ ω 3 ratio, when Duroc crossbreed gilts were studied. Also in a meta-
449 analysis, Poulsen Nautrup et al. (2020) reported that IF had lower IV than EF, in
450 disagreement with the results of the present study. Differences between studies
451 might be due to the diet administered to the pigs, different metabolism due to
452 breed differences, the region from which the subcutaneous fat was obtained
453 (ham or belly) or the methodology used for the FA analysis. According to the
454 present results, immunocastration of Duroc females does not affect the quality
455 of the subcutaneous fat, which is good both from the nutritional and from the
456 technological point of views.

457 When the immunocastration of males is studied (Trial 2; Table 2), subcutaneous
458 fat from IM bellies had higher SFA and lower PUFA, PUFA/SFA ratio, ω 6, ω 3
459 and IV than that of EM pigs and this might explain the fact that bellies from IM

460 were firmer than those from EM. Similar results were reported by Kyle et al.
461 (2014), Pauly et al. (2012) and Škrlep et al. (2020). This agrees with the fact
462 that fat from immunocastrated males is firmer than that from EM, improving its
463 technological properties when processed (Škrlep et al., 2020) and being less
464 prone to lipid oxidation (Lebret & Čandek-Potokar, 2022), even though it might
465 be less favourable for human health (Wood et al., 2004). In fact, the results
466 confirm that, in general, the higher the fat content, the higher the saturation.

467 The belly is a very heterogeneous cut of the carcass, probably due to the
468 different layers of fat and muscle that compose it. It is therefore very important
469 to define and standardise how and where the different quality measurements
470 are taken so that they would be comparable. However, due to the heterogeneity
471 it is difficult for an averaged measure to be representative enough for a whole
472 characterisation, so it makes sense to study the characteristics of the belly slice
473 by regions and non-destructive technologies such as those based on X-ray can
474 be helpful.

475 **4. Conclusions**

476 According to all the results, and under the conditions of the present work, it is
477 possible to conclude that the sex of the pig influences the morphological,
478 mechanical, compositional and chemical characteristics of the belly. This might
479 affect the processing properties and consumer acceptability. Thus, the sex
480 could be a criterion for pre-classifying bellies according to the desired final
481 product to be produced and/or the best processing to be applied. If fatter and
482 firmer bellies are demanded, bellies from CM would probably be more suitable
483 than those from EF and IF and bellies from IM more suitable than those from
484 EM. Furthermore, at similar fatness, the distribution of this fat can be different

485 depending on the sex type and, consequently, especially when bellies are
486 sliced, it is important that this is considered. For this purpose, non-destructive
487 technologies such as computed tomography have been proved to be useful.

488 **5. Ethical statement**

489 Not applicable.

490 **6. CRediT authorship contribution statement**

491 **M. Font-i-Furnols:** Conceptualization, Methodology, Formal analysis,
492 Investigation, Writing -Original draft, Writing -Review & Editing, Visualization,
493 Supervision, Project Administration, Funding Acquisition; **M. Albano:**
494 Investigation, Data curation, Writing -Review & Editing; **A. Brun:** Investigation,
495 Data curation, Writing -Review & Editing; **J.F. Tejeda:** Investigation, Data
496 curation, Writing -Review & Editing; **M. Gispert:** Investigation, Formal analysis,
497 Writing -Review & Editing; **B. Marcos:** Investigation, Writing -Review & Editing;
498 **C. Zomeño:** Investigation, Formal analysis, Writing -Review & Editing

499 **7. Declaration of competing interest**

500 The authors declare no conflict of interest associated with this research.

501 **8. Data and model availability statement**

502 None of the data was deposited in an official repository. Data available under
503 request.

504 **9. Acknowledgements**

505 The authors would like to thank the IRTA technicians Agustí Quintana, Albert
506 Rossell, Adrià Pacreu, Cristina Canals and Joel González, for their help in the
507 execution of the project. Thanks also given to José M. Martínez for their
508 contribution in the analysis of fatty acids. Also, the IRTA researcher Israel
509 Muñoz is thanked for his help in the treatment of the computed tomography
510 images. The CERCA programme from the Generalitat de Catalunya is also
511 acknowledged.

512 **10. Financial support statement**

513 This work has been partly funded by the Spanish Ministry of Science and
514 Innovation, project number RTI2018-096993-B-I00. Michela Albano received
515 funding from the Spanish National Institute of Agricultural Research (INIA)
516 (PRE2019-089669). Cristina Zomeño has received funding from the European
517 Union's Horizon 2020 research and innovation programme under grant
518 agreement No 801370 and the Beatriu de Pinós postdoctoral programme
519 funded by the Secretariat of Universities and Research (Government of
520 Catalonia)"

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682 **Tables**

683 **Table 1.** Morphological, mechanical and compositional measurements of pork
 684 bellies from surgically castrated males (CM), entire females (EF) and
 685 immunocastrated females (IF) (Trial 1) and from immunocastrated males (IM)
 686 and entire males (EM) (Trial 2).

	Trial 1					Trial 2			
	CM	EF	IF	RMSE	<i>P</i> -value	IM	EM	RMSE	<i>P</i> -value
Carcass weight (kg)	96.70	98.88	95.33	7.30	0.493	107.68 ^a	102.85 ^b	5.85	0.032
Belly weight (kg)	4.96	4.89	4.55	0.50	0.117	4.66	4.43	0.57	0.278
Belly proportion (%)	10.27 ^a	9.88 ^{ab}	9.54 ^b	0.54	0.008	8.64	8.60	0.87	0.907
<i>Belly dimensions (cm)</i>									
Length	46.01	46.33	44.50	2.27	0.126	45.20 ^a	42.32 ^b	2.84	0.009
Width	23.98	25.19	24.48	1.24	0.071	23.62	24.00	1.34	0.445
Average thickness	4.15	4.08	4.04	0.35	0.751	3.86	3.69	0.35	0.191
<i>Belly mechanical properties</i>									
Flop distance skin									
above (cm)	27.55 ^a	21.80 ^{ab}	21.21 ^b	5.93	0.024	18.58 ^a	13.44 ^b	4.81	0.007
Flop angle skin above									
(°)	75.43 ^a	56.74 ^b	57.35 ^{ab}	18.16	0.026	48.50 ^a	37.02 ^b	11.90	0.013
Flop distance skin									
below (cm)	29.72 ^a	20.92 ^b	22.98 ^b	6.27	0.004	22.11 ^a	15.81 ^b	6.25	0.010
Flop angle skin below									
(°)	82.62 ^a	54.37 ^b	62.79 ^b	19.41	0.004	59.45 ^a	44.08	17.97	0.027
<i>Minced belly chemical composition</i>									
Dry matter (%)	64.24 ^a	59.99 ^b	58.47 ^b	3.48	0.001	50.81	48.66	4.12	0.163
Moisture (%)	35.76 ^b	40.01 ^a	41.53 ^a	3.48	0.001	49.19	51.34	4.12	0.163
Fat (%)	49.25 ^a	47.24 ^{ab}	43.52 ^b	5.55	0.050	35.26	31.98	5.36	0.105
Protein (%)	9.67 ^b	10.97 ^{ab}	11.90 ^a	1.92	0.026	15.308	15.949	1.80	0.337
Ashes (%)	0.44 ^b	0.52 ^{ab}	0.54 ^a	0.08	0.013	0.679	0.687	0.09	0.823
<i>Skin properties</i>									
Thickness (mm)	3.20	2.89	3.07	0.58	0.417	1.99 ^b	2.57 ^a	0.44	0.001
Peak force (kg)	71.39	73.32	77.31	16.76	0.680	110.55 ^b	122.96 ^a	15.74	0.040
Total force (kg·s)	114.87	124.71	108.67	28.88	0.400	160.45	179.52	37.53	0.175
Slope (kg/s)	27.43	25.55	31.66	7.01	0.107	40.92	42.50	6.53	0.514

^{a,b} Different superscripts within row and trial indicate significant differences between sexes ($P < 0.05$).

687 **Table 2.** Fatty acids composition (mg/100g) of the subcutaneous fat of the
 688 central part of pork bellies from surgically castrated males (CM), entire females
 689 (EF) and immunocastrated females (IF) (Trial 1) and from immunocastrated
 690 males (IM) and entire males (EM) (Trial 2).

	Trial 1					Trial 2			
	CM	EF	IF	RMSE	P-value	IM	EM	RMSE	P-value
C14:0	1419 ^a	1324 ^b	1403 ^{ab}	86	0.024	1406	1408	107	0.959
C15:0	53	50	45	8	0.081	53	57	12	0.357
C16:0	23855	23218	23689	687	0.077	25018 ^a	24261 ^b	920	0.032
C17:0	285	282	240	50	0.061	284	330	70	0.081
C18:0	10038	10090	10051	784	0.986	12167	11529	967	0.082
C20:0	211 ^a	187 ^b	195 ^{ab}	23	0.039	239	225	41	0.375
<i>SFA</i>	35862	35152	35623	1210	0.355	39164 ^a	37811 ^b	1761	0.045
C16:1	2944	2770	2970	401	0.423	2808	2860	285	0.621
C17:1	363	356	318	46	0.053	327	377	79	0.095
C18:1(n-9)	46383	46318	45761	1239	0.411	44412	44354	1348	0.907
C20:1(n-9)	1119 ^a	1008 ^{ab}	958 ^b	118	0.007	921 ^a	831 ^b	92	0.012
<i>MUFA</i>	50808	50450	50005	1335	0.348	48471	48421	1390	0.922
C18:2(n-6)	11371 ^b	12382 ^a	12348 ^a	901	0.014	10871 ^b	12150 ^a	1193	0.007
C18:3(n-3)	783	811	846	63	0.067	513 ^b	580 ^a	61	0.006
C20:2(n-9)	662	673	651	50	0.547	521	533	57	0.572
C20:3(n-6)	113	119	113	18	0.584	119	128	64	0.715
C20:4(n-6)	228	245	246	29	0.239	223	247	43	0.135
C20:3(n-3)	176	168	166	15	0.238	117	129	24	0.177
<i>PUFA</i>	13331 ^b	14401 ^a	14371 ^a	997	0.019	12365 ^b	13766 ^a	1324	0.007
<i>PUFA/SFA</i>	0.37 ^b	0.41 ^a	0.40 ^{ab}	0.04	0.028	0.32 ^b	0.37 ^a	0.05	0.007
ω 3	958	980	1013	60	0.101	631 ^b	708 ^a	70	0.005
ω 6	11709 ^b	12748 ^a	12707 ^a	921	0.014	11213 ^b	12523 ^a	1228	0.007
ω 6/ ω 3	12.21 ^b	12.99 ^a	12.53 ^b	0.37	<0.001	17.79	17.67	0.85	0.704
Iodine Value	67.67 ^b	69.28 ^a	68.90 ^{ab}	1.60	0.049	63.88 ^b	66.35 ^a	2.50	0.011

SFA: saturated, MUFA: monounsaturated and PUFA: polyunsaturated fatty acids; ω 3: omega 3; ω 6: omega 6.

^{a,b} Different superscripts within row and Trial indicate significant differences between sexes ($P < 0.05$).

691

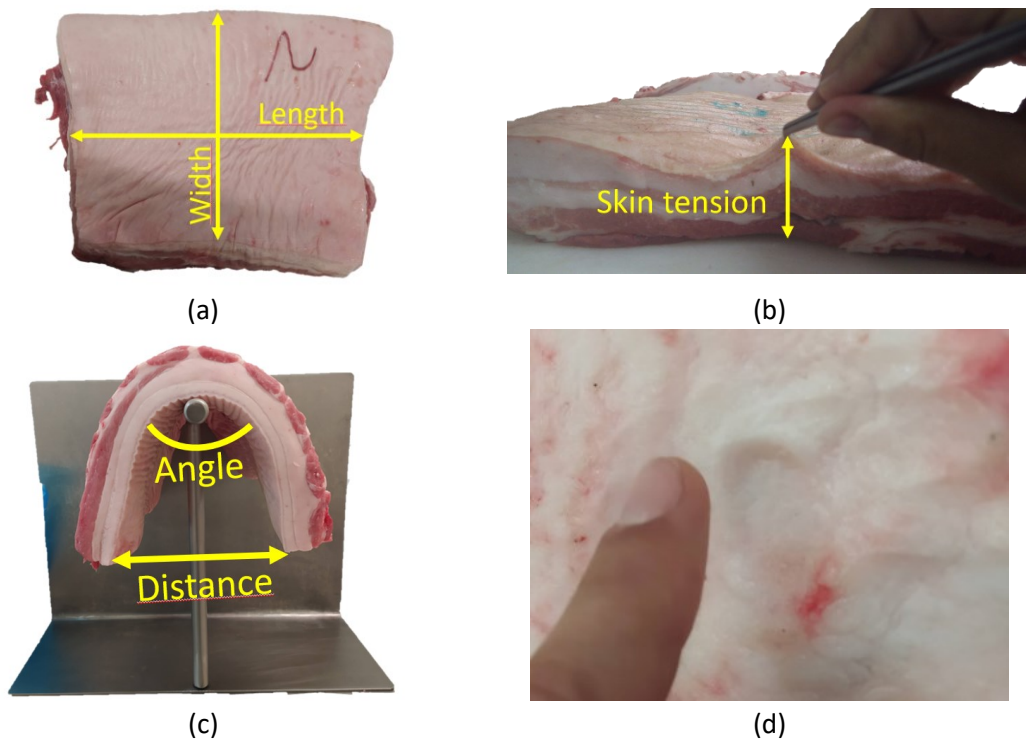
		Dorsal zone (spine)						
		1	2	3	4	5		
Cranial (shoulder)	A	A1	A2	A3	A4	A5		
	B	B1	B2	B3	B4	B5		
	C	C1	C2	C3	C4	C5		
		Ventral zone (breasts)						
							Caudal (ham)	

692

693 **Fig. 1.** Areas identified in the bellies.

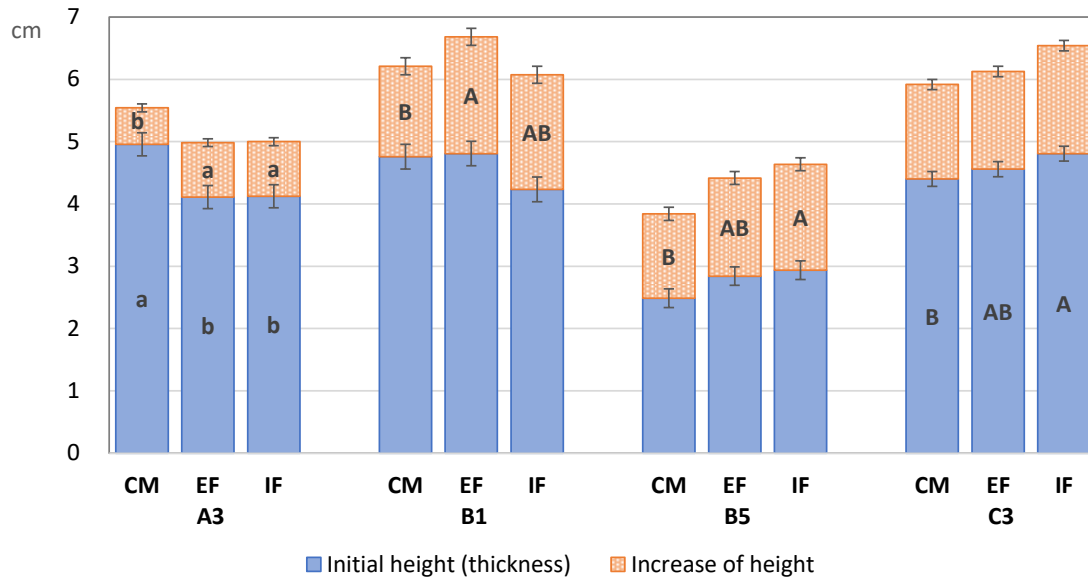
694

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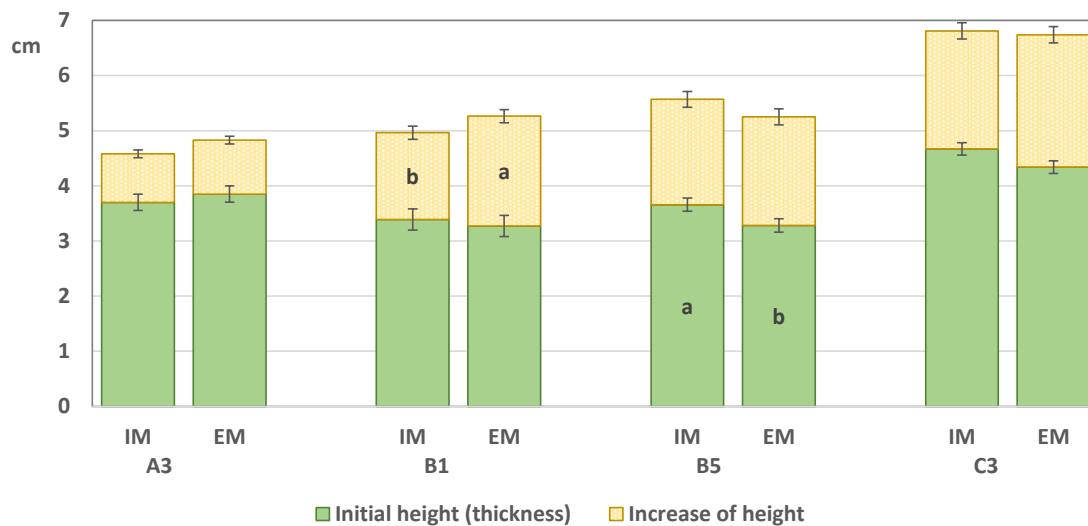
696

697 **Fig. 2.** Morphological and mechanical measurements in the bellies, a: length
 698 and width, b: flop angle and distance skin below, c: skin tension measurement;
 699 d: firmness measurement applying finger pressure.



700

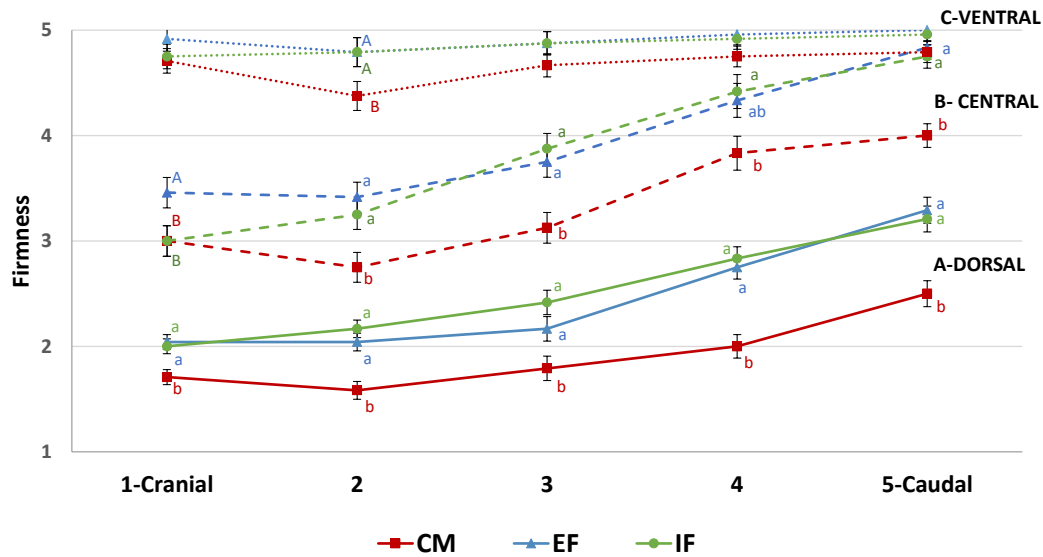
701 (a)



702

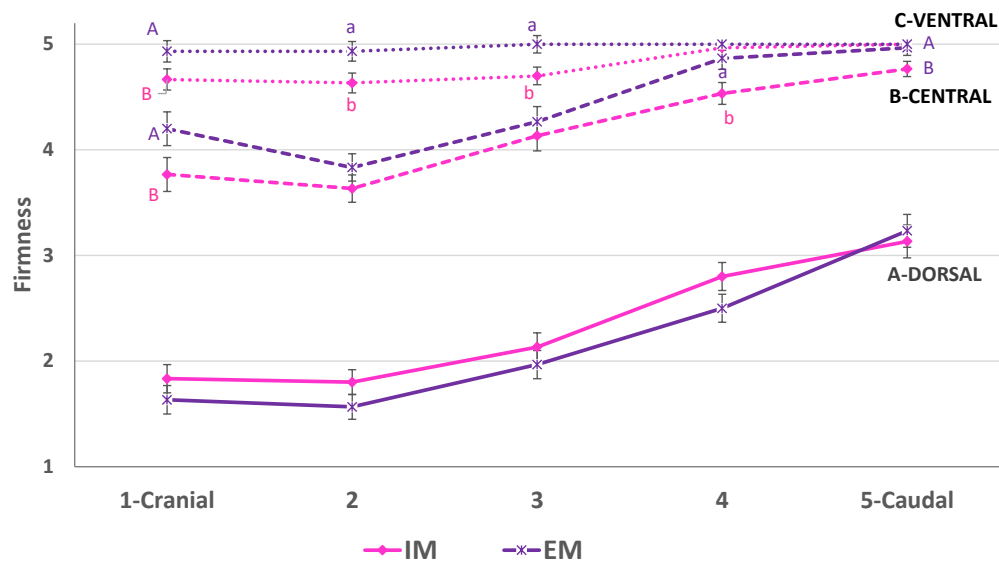
703 (b)

704 **Fig. 3.** Belly thickness, evaluated as the initial height (cm), and belly firmness,
 705 evaluated as the difference between the initial and final heights (after stretching
 706 the skin with tweezers until the base of the belly lifts up) measured in the centre
 707 of each of the four sides (see ¡Error! No se encuentra el origen de la referencia.) for
 708 bellies from (a) Trial 1 [Castrated males (CM); entire females (EF);
 709 immunocastrated females (IF)] and (b) Trial 2 [immunocastrated males (IM) and
 710 entire males (EM)]. Bars represent standard error of the mean. Different letters
 711 between sexual types [within the same location and measurement (initial or
 712 increase)] indicate significant differences ($P < 0.05$ lower-case letters; $P < 0.10$
 713 capital letters).



714 <

715 (a)

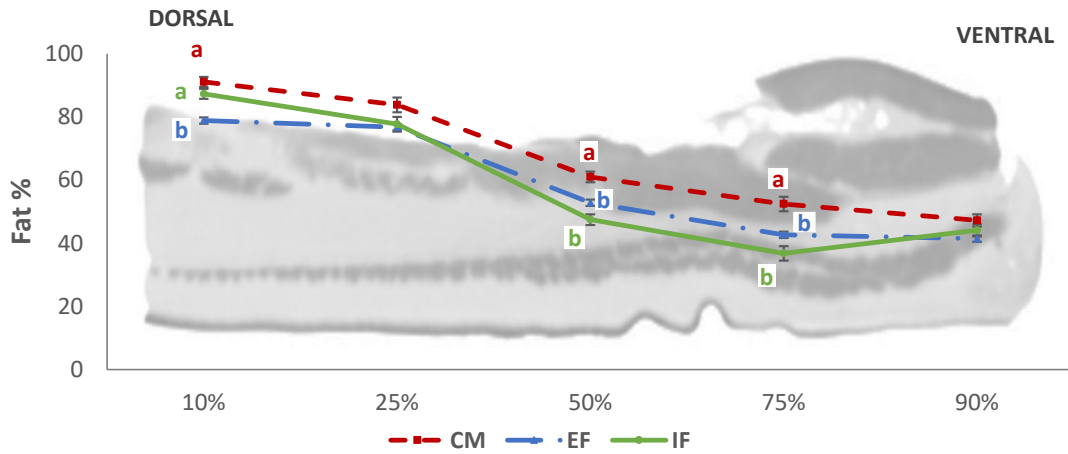


716

717 (b)

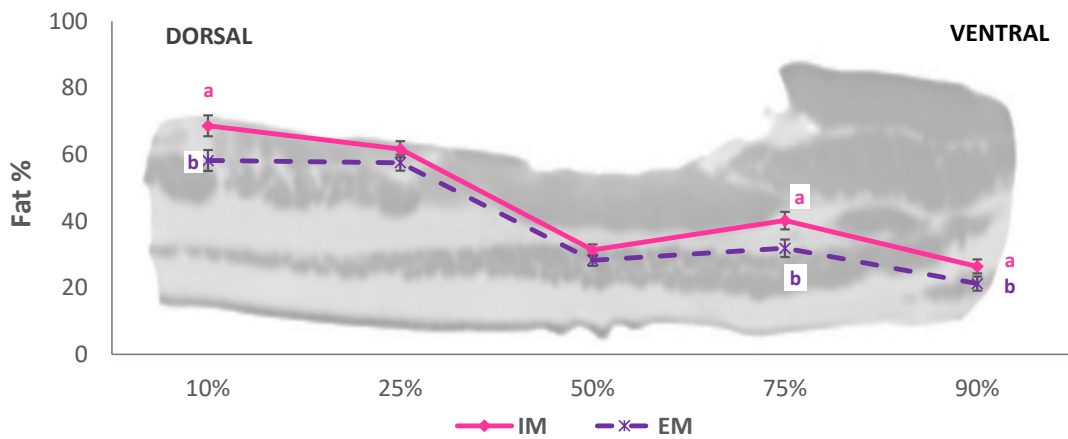
718 **Fig. 4.** Belly firmness, evaluated by two trained technicians applying pressure
 719 using a finger (from 1: firm fat to 5: soft spongy fat) in 15 different locations of
 720 the belly (see ¡Error! No se encuentra el origen de la referencia.) for bellies from (a)
 721 Trial 1 [Castrated males (CM); entire females (EF); immunocastrated females
 722 (IF)] and (b) Trial 2 [immunocastrated males (IM) and entire males (EM)]. Bars
 723 represent standard error of the mean. Different letters between sexual types
 724 [within the same location and measurement (initial or increase)] indicate
 725 significant differences ($P < 0.05$ lower-case letters; $P < 0.10$ capital letters).

726



727

728 (a)



729

730 (b)

731 **Fig. 5.** Fat percentage of the different regions (5 regions from dorsal to ventral,
 732 placed at 10, 25, 50, 75 and 90% of the total area) of the central slice of bellies
 733 from (a) Trial 1 [Castrated males (CM); entire females (EF); immunocastrated
 734 females (IF)] and (b) Trial 2 [immunocastrated males (IM) and entire males
 735 (EM)]. Bars represent standard error of the mean. Different letters between
 736 sexual types [within the same location and measurement (initial or increase)]
 737 indicate significant differences ($P < 0.05$).