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1	Intramuscular fat content in different muscles, locations, weights and
2	genotype-sexes and its prediction in live pigs with computed tomography
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8	Short title Intramuscular fat by muscle, weight & genotype-sex
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19 Abstract

20 Intramuscular (IMF) fat content depends on sex, genotype and diet, and varies with pig growth. The aim of the present work was to determine the evolution of 21 22 IMF by genotype-sex, muscle, and muscle location, to determine relationships between IMF content of different muscles and to predict IMF in live pigs with 23 computed tomography (CT). For this purpose, 155 pigs of seven combinations 24 25 of genotype-sex were CT scanned and slaughtered at 70, 100 and 120 kg. From the carcasses fat thickness was measured at several locations along the 26 midline. Loin samples from 3 anatomical positions (between the 8<sup>th</sup> and 9<sup>th</sup> last 27 ribs, between the the 3<sup>rd</sup> and 4<sup>th</sup> last ribs, and between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar 28 vertebrae) and three ham muscles (biceps femoris, semimembranous and 29 gluteus medius) were extracted, weighed and IMF was determined with near-30 infrared equipment. From CT images the distribution of volume by Hounsfield 31 value (unit related with the density) was obtained for each muscle and 32 33 anatomical location. Marbling was evaluated in the three loin locations. The effects of genotype-sex and live weight and their interaction were included in 34 the statistical model. For prediction of IMF with CT images, partial least square 35 regression was used. The results show differences in IMF content by genotype-36 sex and muscle. In general, the most cranial part of the loin presented higher 37 IMF content, as well as the *biceps femoris* muscle of the ham. Depending on 38 the genotype-sex, IMF content increased during all growth or increased until 39 100 kg and then became constant. Correlation coefficients between IMF content 40 41 by muscle/location were between 0.74 and 0.83 within loin locations and between 0.53 and 0.70 for ham muscles. Correlation coefficients between 42 marbling and IMF content evaluated at the same location varied between 0.51 43

and 0.66. Prediction of IMF content from CT images is not accurate enough
(residual predictive deviation statistical values lower than 1.3). Muscle weight
increase with animal growth and allometric coefficients varied between 0.89 and
0.97 for the muscles evaluated. The conclusions of the present work are that
IMF content differs between and within muscle, during growth and by genotypesex and that prediction of IMF in CT images of live pigs is not accurate.

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## 51 Keywords

swine; marbling; loin; ham; growth

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## 54 Implications

Intramuscular fat content is related to the eating quality of the meat and it is an
important characteristic to improve meat quality. To know the pattern of muscle
growth and deposition of intramuscular fat by genotype, sex and weight is
important for breeding companies, producers and the meat industry to choose
the best slaughter time to obtain the desired product regarding this
characteristic. This work presents the intramuscular fat content and muscle
weight by genotype-sex, muscle, muscle location and weight of the pig.

# 63 Introduction

64 Intramuscular fat (IMF) is an adipose tissue deposited within the lean tissue.

65 Some previous works showed that its content depends on the breed or

genotype (Plastow et al., 2005, Tyra et al., 2013), sex (Gispert et al., 2010,

Jeong et al., 2012, Škrlep et al., 2012), diet (Huang et al., 2008, Cordero et al., 67 68 2010, Lambe et al., 2013) and age/weight (Bosch et al., 2012, Tyra et al., 2013). However, some of these factors have not had a clear effect in other 69 70 works; for instance, Jeong et al. (2012) did not find an effect of the diet and weight and Lambe et al. (2013) did not find an effect of weight. Additionally, 71 there are differences in IMF content depending on the exact muscle (Lambe et 72 73 al., 2013, Tyra et al., 2013) and within the same muscle (Faucitano et al., 2004). Furthermore, some works show a relationship between IMF and meat 74 palatability and eating quality and on consumers' acceptability (Brewer et al., 75 76 2001, Font-i-Furnols et al., 2012) although this has not been confirmed in other works (Channon et al., 2004, Moeller et al., 2010). Because of the inconsistency 77 in previous findings, it is important to increase knowledge about the growth of 78 79 IMF tissue and its content depending on the muscle, genotype, sex and weight of the animals. 80

The knowledge about IMF content in live pigs is important mainly for breeding 81 82 and nutritional purposes, and several technologies mainly based on ultrasound have been evaluated in live pigs and in carcasses with variable results (Newcom 83 et al., 2002, Lakshmanan et al., 2012, Kvam and Kongsro, 2017). Computed 84 tomography (CT) is a non-invasive technology based on X-rays used for breeding 85 purposes. X-ray pass through the object and are attenuated in different degree 86 depending on the density of the tissues they cross by and, this attenuation, is 87 measured in Hounsfield units (HU). Lean has positive HU values from 0 to +140 88 89 and fat has negative HU values from 0 to -149 approximately (Font-i-Furnols et al., 2015), although the limits can change between works. As far as the authors 90 know, only two works have studied the possibility of using CT to estimate IMF 91

content in live pigs, those from Kongsro and Gjerlaug-Enger (2013) that
concluded that CT is not feasible for this purpose, and those from Lambe *et al.*(2013) that concluded that CT has a great potential to determine IMF in live pigs.
Thus, further work is needed to evaluate the feasibility of CT to predict IMF in live
pigs. The aim of the present work was to determine the patterns of IMF by
genotype-sex, muscle and muscle location, to evaluate relationships between
IMF content of different muscles and to predict IMF in live pigs with CT.

99

#### 100 Materials and Methods

101 Animals

102 A total of 182 pigs were used that came from two experiments. Pigs were from 103 seven combinations of genotype and sex: Pietrain  $\times$  (Landrace  $\times$  Large White) gilts (PL-F), Landrace × Large White gilts (LL-F), Duroc × (Landrace × Large 104 105 White) gilts (DL-F), Pietrain × (Landrace × Duroc) gilts (PD-F), Pietrain × (Landrace x Duroc) entire males (PD-M), Pietrain x (Landrace x Duroc) 106 surgically castrated males (PD-C) and Pietrain × (Landrace × Duroc) 107 108 immunocastrated males (PD-I) (2 vaccines, one at 12 weeks and the other one at 18 weeks of age) (Carabús et al., 2015). There were no parental 109 110 relationships within the breeds, except for PD-F, PD-M, PD-C and PD-I. Pigs were managed a standard way, fed a commercial diet ad libitum 111 (Supplementary Material S1) and when they reach the desired live target body 112 113 weight (TBW) they were CT scanned and, the same day after the scan, they were slaughtered. No withdrawal period was necessary because meat from pigs 114 was not used for consumption. A total of 27 pigs (4–5 per genotype-sex, except 115

PD-I because at 30 kg immunocastration was not performed yet and they were
the same as PD-M) were slaughtered at 30 kg (TBW), 31 pigs (4–5 per
genotype-sex) were slaughtered at 70 kg TBW, 32 pigs (4–5 per genotype-sex)
at 100 kg TBW and 92 pigs (11–16 per genotype-sex) at 120 kg (TBW) (Table
Slaughter was performed at the experimental abattoir at IRTA Monells
following standard commercial procedures and after stunning with CO<sub>2</sub>.

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## 123 Computed tomography scanning and image analysis

Before slaughter live pigs of 70, 100 and 120 kg TBW were scanned with the 124 General Electric HiSpeed Zx/I CT equipment placed at IRTA-CENTA (Monells). 125 Scanning conditions were 140 kV, 145 mA, axials 1 s, 10 mm thick (Font-i-126 127 Furnols et al., 2015). Before scanning pigs were anaesthetized by intramuscular injection of azaperone at 0.1 mg/kg and ketamine at 0.2 mg/kg, or intravenous 128 injection of propofol at 0.2 mg/kg only at 100 and 120 kg TBW. 129 For the image analysis an in-house program of the software Matlab (Matlab 130 Version 7.5.0.342 (R2007b) © The MathWorks, Inc.) was used. A region of 131 interest (ROI) was selected for each of the studied muscles and/or locations 132 (Figure 1). Three ROI's were studied for the longissimus muscle in three 133 images, one between the 3<sup>rd</sup> and 4<sup>th</sup> *lumbar vertebrae*, one between the 3rd and 134 4th last ribs and one between the 8<sup>th</sup> and 9<sup>th</sup> last ribs. Furthermore, from two 135 images of the ham that allow to visualize the 3 muscles of interest, 3 different 136 ROI's were performed; one image at the joint between the femur and the pubis 137 138 bones for the muscle gluteus medius (GM), and one image in the middle of the femur for the muscles semimembranosus (SM) and biceps femoris (BF). For 139 each ROI (n=923), the volume associated with each HU value was obtained by 140

means of the thickness of the image, the matrix size and the displayed field of
view (Font i Furnols *et al.*, 2009) and its distribution was used for further
analysis.

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145 Carcass measures, muscle sampling and intramuscular fat analysis

After slaughter fat thickness (plus skin) of the 70, 100 and 120 kg TBW pigs was measured perpendicularly to the skin with a ruler on the midline at three different levels of the loin (between the 3<sup>rd</sup> and 4<sup>th</sup> *lumbar vertebrae*—LD34LV, between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs—LD34LR and between the 8<sup>th</sup> and 9<sup>th</sup> last ribs—LD89LR). Moreover, the minimum backfat and skin thickness over the GM muscle was also measured in the ham region.

152 Between 24 and 48 h after slaughter, the *longissimus* muscle and SM, GM and BF muscles of the ham were removed by trained butchers and weighed. Three 153 154 samples of 4 cm thickness were obtained at different levels of the loin (LD34LV, 155 LD34LR and LD89LR), minced, homogenized and a subsample of approximately 200 g was used for IMF analysis. IMF was also determined in the 156 muscles SM, GM and BF from the ham using a subsample of approximately 157 158 200 g obtained from mincing and homogenizing all the muscle. In the three loin samples, marbling was measured by two trained technicians according to the 159 National Pork Producers Council (NPPC, 1999) scale (from 1=very low IMF to 160 161 10=high IMF). Intramuscular fat of all of the muscles was determined using near infrared FoodScan equipment (Foss Analytical, Denmark). IMF for the 30 kg 162 163 TBW pigs was not considered because the measures were not reliable.

165 Statistical analysis

Statistical analyses were performed with SAS software (version 9.3; SAS 166 Institute Inc., Cary, NC, USA). The MEAN procedure was used to obtain mean 167 168 and standard deviation values and the CORR procedure to determine Pearson's correlation between different variables. The analysis of variance was 169 performed with the General Linear Model (GLM) procedure to determine the 170 effect of the different factors on the IMF content. The model included genotype-171 sex, muscle and TBW as fixed effects. All of the double interactions and the 172 triple interaction were added to the model. However, the triple interaction and 173 174 the double interaction between muscle and TBW were removed because they were not significant (Supplementary Material S2). The GLM procedure was also 175 used to determine the effect of the genotype-sex on the muscle weight. In this 176 case, the model included the genotype-sex and the TBW as well as their 177 interaction as fixed effects. To avoid the effect of differences in carcass weight 178 179 within TBW, the difference between the average left carcass weight (within TBW) and the individual left carcass weight was included as a covariate 180 (Supplementary Material S2). In all of the cases, significant differences between 181 182 least square means were obtained after applying a Tukey test (P < 0.05).

A prediction equation was obtained by partial least square regression (PLS) to predict the IMF content from the CT images of live pigs. Prediction was performed considering all of the muscles together and the muscles of the loin and the muscles of the ham separately. For this purpose, the PLS procedure was used and the volumes associated with the HU values of the ROIs were used as predictors. The smallest number of factors was selected to provide an error not significantly different (P>0.1) from the minimum error as suggested by SAS. The root mean square error of prediction was calculated by crossvalidation leave-one-out (RMSEP<sub>CV</sub>) following a modified version of the macro
developed by Causeur *et al.* (2003) (see Supplementary Material S2).
Additionally, to evaluate the predictive ability of the model, the residual
predictive deviation statistics (RPD) was calculated as the standard deviation
divided by the RMSEP<sub>CV</sub>.

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197 **Results** 

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## 199 Intramuscular fat content by genotype-sex, live weight and muscle

The averaged IMF content by muscle/location, genotype-sex and TBW is

201 presented in Table 2. In the loin, it is possible to see that the mean IMF content

at the LD34LR location varied between 1.02% (PL-F at 70 kg) and 2.22% (DL-F

at 100 kg), at the LD34VL location the mean IMF content varied between 0.98%

204 (PD-M at 70 kg) and 2.42% (DL-F at 100 kg), and at the LD89LR location the

205 mean IMF content was higher in general and ranged between 1.34% (PD-F at

100 kg) and 3.76% (DL-F at 100 kg). Regarding the ham muscles, SM

presented mean IMF values between 1.11% (PD-M at 70 kg) and 2.60% (DL-F

at 100 kg), BF between 1.31% (PD-M at 70 kg), and 2.73% (DL-F at 100 kg),

and GM presented in general the lowest mean values of IMF, which varied

210 between 1.10% (PD-M at 70 kg) and 2.27% (DL-F at 100 kg).

211 When the analysis of variance was performed for IMF content, a significant

interaction was found between genotype-sex and muscle (*P*<0.0001) and

between genotype-sex and live TBW (*P*=0.0144). The least squared means of

these interactions are shown in Table 3. It is possible to see that the highest 214 IMF content was found in the LD89LR in DL-F, LL-F and PL-F, while in the 215 other genotype-sexes this was not significantly different from those of BF and 216 SM (and also GM in PD-M). Loin at location LD34LR presented the lowest IMF 217 content in all of the genotype-sexes, although in some of them it was not 218 significantly different from other locations. PD-C did not present significant 219 differences in IMF content by TBW. However, for PD-F, PD-I, PD-M and PL-F, 220 IMF content was increasing with the live weight of the animal. For DL-F pigs, 221 IMF content was significantly higher in 100 and 120 kg than in 70 kg live weight 222 223 and in LL-F, IMF was higher in 120 kg than in 100 kg live weight, being in between at 70 kg live weight. In fact, according to Table 2, were the raw mean 224 and SD are presented, in LL-F, this occurred numerically with all of the muscles 225 226 except BF and GM.

227

# 228 Relationship between intramuscular fat content and marbling by muscle

Correlations of IMF content by muscle/location are presented in Table 4, with all 229 of the correlations being significant (P<0.0001). The highest correlations were 230 between IMF content at different locations of the loin (0.74 to 0.83). Correlations 231 of IMF content between loin locations and ham muscles (0.53-0.70) were 232 233 similar to those between ham muscles (0.63–0.67), being the lowest between LD34LR and SM (0.56) and between LD34LR and BF (0.53) and the highest 234 between LD89LR and GM (0.70). The correlations by target body weight are 235 presented in Supplementary Table S1 and Supplementary Table S2. These 236 tables show that the correlations were lower in 70 kg live weight pigs than in 237

100 and 120 kg live weight pigs, maybe because the amount of IMF is lower atthis weight for most of the genotype-sexes studied.

When correlations between marbling and IMF content are considered (Table 5) it is possible to see that the highest correlations were between marbling scores in the loin and IMF of the loin. The correlations between marbling and IMF in the same loin locations were between 0.51 and 0.66. As expected, the correlations were lower between marbling in the loin and IMF in the ham muscles.

Correlations between subcutaneous fat thickness at the three loin locations and 245 at the ham with IMF content are also presented in Table 5. Although significant 246 247 (P<0.0001), the correlations are moderate, since except for those of IMF in GM muscle, all are lower than 0.50. Correlations of IMF in GM and the different 248 subcutaneous fat thicknesses were between 0.52 and 0.54, being the highest 249 between GM and subcutaneous fat thickness of the ham, which makes sense 250 251 since it was measured over the GM muscle. These correlations for each target 252 body weight are presented in Supplementary Table S3 to Supplementary Table 253 S5. It is possible to see that in general, the highest correlations were found for 100 kg live weight pigs. 254

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# 256 Prediction of intramuscular fat content with computed tomography

<sup>257</sup> Prediction of IMF from CT images of live pigs had a RMSEP of 0.56–0.66,

which according to a mean of 1.79–1.78% implies a coefficient of variation of

- 259 31-37%. The prediction when all of the muscles are considered together
- 260 (RPD=1.09, R<sup>2</sup>=0.17) is worse than those when only loin muscles are
- considered (RPD=1.28, R<sup>2</sup>=0.42). The worst prediction is when only ham

muscles are considered in the calculations (RPD= 1.03, R<sup>2</sup>= 0.07).

263 Nevertheless, in all the cases RPD was much lower than 3, which is the recommended value for a suitable prediction model (Williams, 2001) (Table 6). 264 In fact, the coefficient of determination was also very low, except for when only 265 loin muscles were considered. The accuracy of the prediction was almost the 266 same when only the Hounsfield values with variance importance for projection 267 268 (VIP) higher than 0.8 were considered as predictors (results not shown) and, because of that, only the results of prediction using Hounsfield values between 269 -50 and -120 are presented. 270

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## 272 Muscles' weight by genotype-sex and target body weight

Muscle growth with the live weight of the pig by genotype-sex is presented in 273 Figure 2. In general, LL-F and DL-F genotypes-sex presented a lower weight of 274 all the studied muscles than the other genotype-sex studied, especially at 275 higher body weights. This difference can be seen also at early weights and it is 276 maintained throughout growth. This is only a tendency in the muscle GM 277 because no significant differences among genotypes-sexes can be seen in this 278 muscle. The highest weights of the muscles were usually by the PD-F 279 genotype-sex, although in most of the muscles/locations, not significantly 280 281 different than those from the other genotype lines with Pietrain. The average allometric coefficient of the total muscle weight with respect to the live weight of 282 the pig was 0.97 for the loin, 0.89 for SM, 0.95 for BF and 0.92 for GM, although 283 there were some differences between genotypes-sex, with DL-F, LL-F and PL-F 284 having the lowest allometric coefficients (results not shown). 285

287 Discussion

Intramuscular fat content varied between muscles (Table 3). Furthermore, 288 within some muscles, such as *longissimus dorsi*, IMF also varied depending on 289 its anatomical position. In this work, we confirmed what was presented by 290 Faucitano et al. (2004) for longissimus muscle. The IMF content was higher 291 between the 8<sup>th</sup> and 9<sup>th</sup> last ribs, then it decreased at the level of the 3<sup>rd</sup>-4<sup>th</sup> last 292 ribs and then it increased again, between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae. 293 Thus, IMF content was much higher at the most cranial part of the loin (8<sup>th</sup>-9<sup>th</sup> 294 last ribs). Regarding IMF in three ham muscles, there were also some 295 differences and, in general, BF presented the highest IMF values. Lambe et al. 296 (2013) found higher levels of IMF in SM muscle compared with LT muscle at the 297 last rib level (1.79 vs 1.18%, respectively), which is in agreement with the 298 299 present results, but Tyra et al. (2013) found the opposite, high IMF levels in LT 300 (at the last rib level) compared to SM (1.95 vs. 1.76%). Intramuscular fat content for all of the muscles was higher in PD-C compared 301 with PD-F, PD-I and PD-M, indicating a higher IMF content for castrated pigs, in 302 accordance with Faucitano et al. (2004), Gispert et al. (2010) and Jeong et al. 303 (2012), among others. Furthermore, IMF content depended on the genotype, 304 305 with crosses with Duroc higher (DL-F) than those from Landrace-Large White crosses (LL-F) and crosses with Pietrain (PL-F). This is in accordance with the 306 results found by Plastow et al. (2005) and Gil et al. (2008) studying pure breeds. 307 308 Pietrain are animals with high muscularity (Plastow et al., 2005, Gispert et al., 2010) and from very lean breed. This high muscularity is related with a low IMF 309 content (Hocquette et al., 2010). 310

For most of the genotype-sex groups (PD-F, PD-I, PD-M and PL-F) the IMF 311 312 increased with the growth of the pig, indicating that the highest IMF content is reached at 120 kg, with a content significantly higher than that found at 70 kg 313 314 (Table 3). These results are in agreement with those found by Bosch et al. (2012) when pigs of 95, 113 and 105 kg were studied. A tendency can also be 315 316 seen for PD-C but not significant differences between weights and, for DL-F, at 100 kg the IMF stops increasing. Bosch et al. (2012) did not find significant 317 differences in the IMF content of (Yorkshire × Landrace) × Duroc gilts and 318 barrows at 110, 125 and 138 kg. Thus, for these genotype-sexes, the early 319 320 development of IMF would avoid the necessity of increasing the weight of the pig to increase its IMF content in all of the muscles. However, there was an 321 opposite finding for LL-F, and the difference in IMF is very important (P<0.05) 322 323 between 100 and 120 kg. Lambe et al. (2013) for Pietrain × (Landrace × Large White) intact male pigs fed different diets found no important differences in IMF 324 325 content in pigs after 60 kg of weight, since the average IMF content was 1.14, 326 1.12 and 1.18% for pigs of 60, 85 and 115 kg, respectively. Thus, in general, in 327 the present study, the higher the weight (up to 120 kg) the higher the IMF 328 content with some exceptions. In this sense, it is important to know the genotype-sex of the pigs to optimize the IMF content of the final product and to 329 decide the optimal slaughter moment to obtain the desired product. 330 Since the IMF content is related to the tenderness and juiciness of the meat 331 (Enfält et al., 1997, Heyer and Lebret, 2007, Font-i-Furnols et al., 2012), it is 332

important to know the differences in this content between genotypes-sex and

within muscles, because this can help in understanding the eating

335 characteristics of the meat used for consumption. Even though there are

variations between and within muscles in the IMF content, correlations of IMF 336 337 content within the same muscle (longissimus) are high (0.74-0.83) (Table 4). Correlations between muscles of the same animal were always significant and 338 varied between 0.53 and 0.70 (Table 4). These correlations were higher when 339 only 100 kg live weight pigs were considered and lower when only 70 kg live 340 weight pigs were taken into account (Supplementary Table S1). This result may 341 indicate that the knowledge of the IMF content of one muscle can be related to 342 the other muscles, since when it increases, it increases for all of the muscles; 343 nevertheless, the relationship is not perfect and it would be difficult to estimate 344 345 the IMF content of one muscle from those of other muscles.

The correlation between marbling evaluated according to the NPPC scale and 346 IMF content is higher for the *longissimus* than for the muscles of the ham. This 347 is probably because the NPPC scale was measured in the *longissimus* muscle 348 and, consequently, the similarities are higher. These correlations were always 349 350 positive and significant, indicating the higher the IMF, the higher the marbling. 351 Nevertheless, the correlation is not perfect, varying from 0.51 to 0.66 in the loin and between 0.33 and 0.50 in the ham muscles. These correlations are lower 352 353 than those reported by Font-i-Furnols et al. (2012), which was 0.89, even in 100 kg live weight animals that presented the highest correlations (Supplementary 354 Tables S3 to S5). 355

The weight of the *longissimus* muscle was the highest followed by BF, SM and GM (Figure 2), but logically all of the muscles increase in weight when an animal grows. Except for the GM muscle, differences in weight for the same muscle between genotype-sex can be seen at 30 kg TBW and this became more important at 120 kg, where differences are greater. Crosses that do not

include Pietrain (LL-F and LL-F) presented in general a lower weight of the 361 362 muscles, especially at higher TBW. This is in agreement with the work of Fisher et al. (2003) and Carabús et al. (2014) where the ham muscles of several 363 genetic types were studied. This makes sense since Pietrain is a highly 364 conformed breed, whose carcasses have higher ham weight, ham lean content 365 366 and loin lean content, although not loin weight (Gispert et al., 2007). Thus, the 367 most adequate genotype-sex depends on the desired final product. Fisher et al. (2003) predicted ham muscle weights for carcasses of approximately 25 kg and 368 75 kg. For 25 kg carcasses, which would represent 31 kg live weight, the 369 370 average weight of the muscles SM (367 g) and GM (239 g) are similar to those found in the present project (367 g and 239 g, respectively). In 75 kg carcasses, 371 which would represent approximately 93 kg live weight, the average weight of 372 373 the muscles SM (833 g) and GM (617 g) are similar to those of 70 kg of the present work (844 g and 616 g, respectively). Thus, it seems that the growth of 374 375 these muscles, at higher weights, is higher in the present work, probably due to 376 differences in the genotypes used. Moreover, the allometric coefficients for ham 377 (0.89-0.95) and loin (0.97) muscle weight with respect to the live weight of the 378 pig were close to one in agreement with previous works that reported allometric coefficients between 0.87 and 1.13 for ham muscle and 1.01 and 1.03 for loin 379 muscle (Carabús et al., 2014, Carabús et al., 2017). 380

The estimation of IMF content in live pigs is very important to optimize the slaughter moment of the pig to yield the final desired product. However, this prediction in live pigs by means of CT, in the conditions of this experiment, was not accurate enough and had an unacceptable error (Table 6). This result is in accordance with those reported by Kongsro and Gjerlaug-Enger (2013) who

concluded that CT is not a feasible method for prediction of IMF from live pigs. 386 387 This is probably due to the respiratory movement of the pig and to the size of the voxel that is probably too big because the displayed field of view has to 388 cover all of the body of the pig and, since the IMF is small, it might have many 389 partial volume effect that makes it difficult to identify the IMF. These two 390 reasons can explain why, although still not good, the prediction of IMF in loins is 391 392 somewhat better (Font-i-Furnols *et al.*, 2013), since there is no breath movement and the displayed field of view can be adjusted to the loin area. 393 Furthermore, the prediction of IMF is better when only loin muscles images are 394 395 considered in the analysis compared with those of ham muscles. This is probably because for loin muscles, the sample analysed for IMF is almost the 396 397 same as those analysed in CT images. However, for ham muscles, the whole 398 muscle was minced and the IMF determined, while only one image of the muscle was analysed to predict IMF content. However, Lambe et al. (2013) 399 400 predicted IMF from CT images using the average muscle density as a predictor. 401 The error of prediction was not provided, but the correlation between this density and the chemical IMF was found to be around -0.44 for 60 kg pigs, 402 403 -0.63 for 85 kg pigs and -0.70 for 115 kg pigs. Thus, these correlations indicate that CT can predict IMF, but the error is probably guite high, especially in pigs of 404 lower weight. In the present project the correlation between averaged 405 Hounsfield value of the ROI of each muscle and IMF content was 0.12 (0.16 at 406 70 kg, 0.24 at 100 kg and 0.15 at 120 kg) and, because of that, this approach 407 for IMF prediction has not been considered. 408

In the conditions of the present experiment, it can be concluded that the IMFcontent differs between and within muscle, during growth and by genotype-sex.

To optimize the final product for IMF content and amount of muscle it is

important to know the growth of these tissues with the growth of the pig.

Nevertheless, this is difficult to obtain from live pigs by means of computed

tomography since the prediction of this parameter is not accurate enough.

415

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424

# 425 **Declaration of interest**

426 The authors have no conflicts of interest.

427

# 428 Ethics statement

429 The IRTA's ethical committee approved the protocol.

430

- 431 Software and data repository resources
- The data are not deposited in an official repository.

433

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- **Table 1** Number of pigs scanned, slaughtered and sampled (muscle weight and
- 545 intramuscular fat-IMF) by genotype-sex and target body weight (TBW), total
- number of muscles evaluated by genotype-sex and mean live weight and warm

	TBW					
Genotype-sex <sup>3</sup>	30 kg <sup>1</sup>	70 kg	100 kg	120 kg	Total <sup>2</sup>	
PD-C	4	4	4	12	120	
PD-F	4	4	4	12	120	
PD-I <sup>4</sup>	-	4	4	12	120	
PD-M	4	4	5	11	120	
DL-F	5	5	5	16	156	
LL-F	5	5	5	15	150	
PL-F	5	5	5	14	144	
TBW (kg) <sup>5</sup>	30.6 <u>+</u> 1.83	69.15 <u>+</u> 8.10	100.8 <u>+</u> 2.87	120.9 <u>+</u> 6.5		
Carcass weight (kg) <sup>5</sup>	23.4 <u>+</u> 1.60	56.5 <u>+</u> 2.23	81.8 <u>+</u> 2.52	98.4 <u>+</u> 2.85		

547 carcass weight by TBW.

<sup>1</sup>: At 30 kg only the weight of the muscles was measured.

<sup>2</sup>: Total numbers of samples evaluated for IMF considering that for each genotype-sex and TBW,

6 different muscles/locations were analysed.

<sup>3</sup>: PD-C: Pietrain × (Landrace × Duroc) surgically castrated males; PD-F: Pietrain × (Landrace × Duroc) gilts; PD-I: Pietrain × (Landrace × Duroc) immunocastrated males; PD-M: Pietrain × (Landrace × Duroc) entire males; DL-F: Duroc × (Landrace × Large White) gilts; LL-F: Landrace × Large White gilts; PL-F: Pietrain × (Landrace × Large White) gilts.

<sup>4</sup>: At 30 kg, the immunocastrated pigs were entire male pigs.

<sup>5</sup>: Mean <u>+</u> SD

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551 **Table 2** Mean and standard deviation (in italics) of the intramuscular fat content

G-S <sup>2</sup>	TBW	LD89LR	LD34LR	LD34VL	SM	BF	GM
	70	1.95±0.74	1.35±0.27	1.44±0.18	1.83±0.54	1.98±0.36	1.51±0.16
PD-C	100	1.84±0.52	1.53±0.50	1.75±0.52	2.08±0.46	2.30±0.50	1.70± <i>0.5</i> 2
	120	2.31±0.81	1.40± <i>0.4</i> 2	1.58± <i>0.4</i> 3	1.69± <i>0.54</i>	2.14±0.39	1.68± <i>0.3</i> 5
	70	1.58±0.65	1.03± <i>0.12</i>	1.15±0.06	1.35±0.19	1.39±0.33	1.22±0.09
PD-F	100	1.34±0.41	1.04± <i>0.34</i>	1.12±0.32	1.78±0.50	1.93± <i>0.51</i>	1.23±0.15
	120	1.79±0.61	1.10± <i>0.</i> 26	1.22±0.27	1.75±0.35	2.07±0.51	1.43±0.25
	70	1.43±0.17	1.03±0.20	1.08± <i>0.16</i>	1.20± <i>0.15</i>	1.36± <i>0.25</i>	1.13±0.24
PD-I	100	1.75±0.48	1.11±0.23	1.28±0.36	1.33±0.24	1.76± <i>0.3</i> 9	1.28±0.32
	120	1.86± <i>0.7</i> 6	1.22±0.46	1.42±0.56	1.61± <i>0.28</i>	1.95± <i>0.41</i>	1.53±0.34
	70	1.36±0.67	1.09± <i>0.4</i> 7	0.98±0.34	1.11±0.32	1.31±0.35	1.10±0.35
PD-M	100	1.53±0.49	1.04± <i>0.34</i>	1.10±0.28	1.43±0.32	1.47±0.21	1.33±0.32
	120	1.68±0.50	1.08± <i>0.</i> 26	1.19± <i>0.22</i>	1.62± <i>0.3</i> 9	1.91± <i>0.4</i> 8	1.43± <i>0.41</i>
	70	3.27±0.13	2.20±0.13	1.88±0.36	1.30± <i>0.3</i> 6	1.72±0.51	1.34±0.18
DL-F	100	3.76±1.20	2.22±0.97	2.42±0.95	2.60±0.51	2.73±0.57	2.27±0.96
	120	3.41±1.01	2.09±0.74	2.38±0.72	2.37±0.75	2.49±0.73	2.20±0.55
	70	2.49±0.82	1.59±0.56	1.70± <i>0.12</i>	1.91±0.72	1.78±0.39	1.41±0.41
LL-F	100	2.46±0.51	1.48±0.65	1.27±0.61	1.55±0.47	2.00±0.13	1.57±0.92
	120	2.66±0.64	1.54± <i>0.4</i> 2	1.77±0.48	2.14±0.40	2.14±0.62	1.95± <i>0.4</i> 3
	70	2.12±0.54	1.02±0.20	1.47±0.25	1.27±0.35	1.53±0.77	1.17±0.42
PL-F	100	2.19±0.98	1.35±0.30	1.51±0.47	1.72±0.38	1.92± <i>0.81</i>	1.72±0.29
	120	2.91±0.76	1.34±0.64	1.60± <i>0.4</i> 2	1.76± <i>0.51</i>	1.87±0.61	1.84± <i>0.4</i> 9

552 (%) by pig genotype-sex (G-S), muscle/location<sup>1</sup> and target body weight (TBW).

<sup>1</sup>LD34LR: between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs; LD34VL: between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar *vertebrae*;

554 LD89LR: between the 8<sup>th</sup> and 9<sup>th</sup> last ribs; BF: *Biceps femoris*; GM: *Gluteus medius*; SM:

555 Semimembranosus

- 556 <sup>2</sup> PD-C: Pietrain × (Landrace × Duroc) surgically castrated males; PD-F: Pietrain × (Landrace ×
- 557 Duroc) gilts; PD-I: Pietrain × (Landrace × Duroc) immunocastrated males; PD-M: Pietrain ×
- 558 (Landrace x Duroc) entire males; DL-F: Duroc x (Landrace x Large White) gilts; LL-F: Landrace
- 559 × Large White gilts; PL-F: Pietrain × (Landrace × Large White) gilts.

# 560 **Table 3** Least square means of intramuscular content (%) for the interaction

- 561 between muscle or target body weight (TBW) with pig genotype-sex (RMSE =
- 562 *0.52%)*+.

	Genotype-sex						
	PD-C	PD-F	PD-I	PD-M	DL-F	LL-F	PL-F
Muscle (genotype-sex x muscle, P<0.0001)							
LD89LR	2.14 <sup>a</sup>	1.60 <sup>ab</sup>	1.68 <sup>ab</sup>	1.52 <sup>a</sup>	3.40 <sup>a</sup>	2.51ª	2.52ª
LD34LR	1.41 <sup>b</sup>	1.02 <sup>c</sup>	1.09 <sup>c</sup>	1.02 <sup>c</sup>	2.08 <sup>b</sup>	1.46 <sup>c</sup>	1.20 <sup>c</sup>
LD34VL	1.58 <sup>b</sup>	1.13 <sup>℃</sup>	1.25 <sup>c</sup>	1.06 <sup>bc</sup>	2.24 <sup>b</sup>	1.59 <sup>bc</sup>	1.48 <sup>bc</sup>
SM	1.79 <sup>ab</sup>	1.62 <sup>ab</sup>	1.39 <sup>abc</sup>	1.41 <sup>a</sup>	2.16 <sup>b</sup>	1.92 <sup>b</sup>	1.58 <sup>bc</sup>
BF	2.13 <sup>a</sup>	1.85 <sup>a</sup>	1.72 <sup>a</sup>	1.62 <sup>a</sup>	2.34 <sup>b</sup>	1.96 <sup>b</sup>	1.73 <sup>b</sup>
GM	1.64 <sup>b</sup>	1.29 <sup>bc</sup>	1.32 <sup>bc</sup>	1.28 <sup>abc</sup>	1.99 <sup>b</sup>	1.69 <sup>bc</sup>	1.60 <sup>bc</sup>
TBW (genotype-sex x TBW, <i>P</i> =0.0144)							
70 kg	1.68	1.29 <sup>b</sup>	1.20 <sup>b</sup>	1.16 <sup>b</sup>	1.95 <sup>b</sup>	1.81 <sup>ab</sup>	1.43 <sup>b</sup>
100 kg	1.86	1.41 <sup>ab</sup>	1.42 <sup>ab</sup>	1.32 <sup>ab</sup>	2.67ª	1.72 <sup>b</sup>	1.74 <sup>ab</sup>
120 kg	1.80	1.56 <sup>a</sup>	1.60 <sup>a</sup>	1.48 <sup>a</sup>	2.49 <sup>a</sup>	2.03 <sup>a</sup>	1.89 <sup>a</sup>

\*Different superscripts within genotype-sex indicate significant differences (*P*<0.05) between muscle or between TBW

LD34LR: between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs; LD34VL: between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar *vertebrae*; LD89LR: between the 8<sup>th</sup> and 9<sup>th</sup> last ribs; BF: *Biceps femoris*; GM: *Gluteus medius*; SM: *Semimembranosus* 

PL-F: Pietrain x (Landrace x Large White) gilts, LL-F: Landrace x Large White gilts, DL-F: Duroc x (Landrace x Large White) gilts, PD-F: Pietrain x (Landrace x Duroc) gilts, PD-M: Pietrain x (Landrace x Duroc) entire males, PD-C: Pietrain x (Landrace x Duroc) surgically castrated males, PD-I: Pietrain x (Landrace x Duroc) immunocastrated males

563

- 566 **Table 4** Pearson correlation coefficients between intramuscular fat content of
- 567 different muscles or anatomical positions for pigs from 70 to 120 kg live weight
- 568 (*n*=153–155).

		LD34LR	LD34VL	SM	BF	GM
LD8	39LR	0.74	0.78	0.63	0.60	0.70
LD3	34LR		0.83	0.56	0.53	0.62
LD3	34VL			0.65	0.62	0.66
S	M				0.66	0.67
E	3F					0.63

All the correlations have a *P*-value <0.0001

LD89LR: between the 8<sup>th</sup> and 9<sup>th</sup> last ribs; LD34LR: between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs;

LD34VL: between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar *vertebrae*; SM: *Semimembranosus*; BF: *Biceps femoris*; GM: *Gluteus medius*.

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570

571 **Table 5** Pearson correlation coefficients between marbling and subcutaneous fat

		Marbling (NPPC <sup>3</sup> )		Subcutaneous fat thickness (ruler)			
Muscles <sup>2</sup>	LD89LR	LD34LR	LD34VL	LD89LR	LD34LR	LD34VL	Ham <sup>4</sup>
LD89LR	0.51	0.50	0.57	0.44	0.48	0.39	0.49
LD34LR	0.62	0.58	0.60	0.37	0.35	0.28	0.37
LD34VL	0.59	0.57	0.66	0.44	0.41	0.33	0.46
SM	0.33	0.37	0.41	0.46	0.44	0.42	0.48
BF	0.44	0.38	0.43	0.46	0.47	0.40	0.43
GM	0.45	0.47	0.50	0.54	0.54	0.52	0.56

572 thickness with intramuscular fat (IMF) in several muscles of pigs (n=148–153)<sup>1</sup>

573 <sup>1</sup> All the correlations have a P value <0.0001

<sup>2</sup> LD34LR: between the 3rd and 4th last ribs; LD34VL: between the 3rd and 4th *lumbar* 

575 *vertebrae*; LD89LR: between the 8th and 9th last ribs; BF: *Biceps femoris*; GM: *Gluteus medius*;

576 SM: Semimembranosus

<sup>3</sup> National Pork Producers Council (NPPC, 1999) scale from 1: very low to 10: very high

578 <sup>4</sup> Fat thickness over the GM muscle

579

- **Table 6** Statistical parameters of the prediction of intramuscular fat content (%)
- in pig's muscles from computed tomography images by means of partial least
- 584 square regression (PLS).

Predictors	Factors	Mean	SD	RMSEP <sub>CV</sub>	R <sup>2</sup>	RPD
All the muscles						
HU -50 to +120	2	1.78	0.72	0.66	0.17	1.09
Loin muscles						
HU -50 to +120	2	1.77	0.83	0.65	0.42	1.28
Ham muscles						
HU -50 to +120	1	1.79	0.58	0.56	0.07	1.03

HU: Hounsfield values; Factors: Number of PLS factors; RMSEP<sub>CV</sub>: Root mean square error of prediction obtained with cross-validation leave-one-out; RPD: Residual predictive deviation (s.d./RMSEP<sub>CV</sub>)

592



(d) GM

(e) BF

(f) SM







#### 601



## 603 genotype-sex.

Footnote: PL-F : Pietrain x (Landrace x Large White) gilts, LL-F: Landrace x Large White gilts,
DL-F: Duroc x (Landrace x Large White) gilts, PD-F: Pietrain x (Landrace x Duroc) gilts, PD-M:
Pietrain x (Landrace x Duroc) entire males, PD-C: Pietrain x (Landrace x Duroc) surgically
castrated males, PD-I: Pietrain x (Landrace x Duroc) immunocastrated males; TBW: live target
body weight