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Effects of gender and diet on back fat and loin area ultrasound measurements during the growth and final stage of fattening in Iberian pigs

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Abstract. Reared in extensive parcels, 50 castrated or spayed Iberian pigs were fattened with conventional or high-oleic-concentrate diets to evaluate the effect of diet and sex on the measurements of the loin area depth, back fat thickness and its layers during the last 6 months before slaughter at eight time points in two anatomical locations by serial scans. The scan was the factor that had the greatest influence, followed by sex and diet. Back fat thickness at 10th rib level was higher than at 14th rib level. The thickness of the outer, middle and inner layers progressively increased over the study period. Throughout the experimental period, the differences between two successive scans of the M. longissimus area did not differ significantly, obtaining the lowest value at the third scan and the highest at the fifth scan. The ultrasound back fat depth was affected by sex, being greater in females and in animals with a high-oleic diet. Positive significant correlations were observed for measurements assessed. The R^2 values for the regression equations to estimate M. longissimus area were lower than the values found for the prediction of fat measurements, and they differed between sex and diet. The relative back fat growth was higher than M. longissimus area, not permitting the establishment of a similar growth pattern for fat and muscle. The sex and diet was taken into account in the predictive models. The subcutaneous adipose layers in Iberian pigs grow at different rates during the last 6 months before slaughter; with the ultrasound serial scan, it is possible to show these changes. The change in diet and the sex affect the adipose tissue development, being more noticeable in the middle layer of back fat at 10th rib level and the inner layer at 14th rib level. However, the sex and the use of an enriched oleic acid diet do not affect to loin development. As the middle layer of back fat shows more growth, this layer could be the best to be included in predictive models. The middle layer of back fat could also be good to be included in predictive models. Back fat thickness at the eighth scan can be predicted with moderate accuracy from corresponding measurements taken 30 days earlier and with less accuracy as the interval between measurements increases.

1 Introduction

The autochthonous Iberian pig breed is traditionally bred in southwestern Spain and has a specific conformation and high adipogenetic potential, influenced by a diet based on grass and acorns. This pig breed is reared under different conditions, all of which are regulated by the Spanish Ministry of Agriculture (BOE, 2014) according to the features of the last fattening phase: intensive system, *Recebo*, *Cebo a campo* and *Montanera*.

In the last decades the pork industry has found features to improve meat quality. In this sense, the main trait of Iberian pig meat is its high content of intramuscular fat, which is desirable in obtaining dry-cured products (mainly ham, foreleg and loin) and important for flavour and to provide slow dehydration during the curing process (Fernandez et al., 2003). According to Lopez-Bote (1998), in the last phase of fattening (around 150 kg of body weight) the Iberian pig can reach 60 % carcass fat, 15 cm of back fat depth and 10–13 % intramuscular fat content. Factors such as diet or sex are the most investigated in Iberian pig production, with the purpose of improving intramuscular fat content (Ayuso et al., 2004).

Back fat layer thickness is an important parameter at all stages of pig production. It is used as a tool for the evaluation of dietary requirements in order to optimize growth and determine the price (McEvoy et al., 2007). Measurements of thickness of subcutaneous adipose tissue are useful for monitoring the production process in order to optimize growth and carcass composition. These measurements can be obtained using ultrasound techniques, the use of which has become widespread for the Iberian pig in recent decades either for descriptive studies or to predict carcass composition (Ayuso et al., 2013). Likewise, these measurements can be very useful in improving the response of genetic selection in economic traits (Moeller et al., 1998). It has been proved that back fat thickness, loin area and intramuscular fat content are good features to predict pig carcass characteristics (Ayuso et al., 2014).

The goal of this study was to evaluate, under outdoor intensive conditions, the effect of diet and sex on the measurements of the loin area depth, back fat thickness and its layers at eight time points in two anatomical locations by serial scans before and during the final fattening phase in Iberian pigs.

2 Material and methods

2.1 Animal management and experimental design

The experimental procedures used in this study were in compliance with the Spanish guidelines for the care and use of animals in research (BOE, 2007). The data were collected from 50 castrated or spayed Iberian pigs (25 males and 25 females). Pigs in the study were progeny from Iberian purebred sires and dams. The trial was conducted at Valdesequera Farm, a central Iberian swine test station (Badajoz, Spain). From 15 days of age to weaning (42–49 days old), piglets had free access to a commercial pre-starter feed. At weaning, all piglets were moved to an open-air fenced-in area where they had full access to feed and water. The pigs were reared in extensive parcels according to the Spanish legislation (BOE, 2007), without the presence of oaks to avoid the intake of acorns, and they received the same standard diet based on commercial concentrate feed. A gradual change from a starter diet (∼ 23 kg live weight) to subsequently receiving a growth diet (∼ 70 kg live weight) occurred. At the beginning of the fattening period (∼ 90 kg live weight), the males were castrated and the females were ovariectomized under anesthesia following the Spanish regulations. Then pigs of the same sex were sorted by weight into eight pens of 3–4 pigs each and were then randomly assigned to one of two dietary treatments: conventional concentrate feed (C) or concentrate diet containing a high level of oleic acid (HO). The pens had similar characteristics. The chemical composition of each feed is presented in Table 1. The diet was offered ad libitum.

Table 1. The chemical composition of dietary ingredients.

Analysed composition[a]	Concentrate[b]	Concentrate high oleic[b]
Crude protein %	16.10	15.00
Crude fat %	2.44	5.66
Crude fibre %	3.99	5.07
Ash %	5.80	5.10
Oleic %	0.54	3.30

[a] Expressed as percentage of dry matter. [b] By formulation.

2.2 Ultrasound measurements

The pigs were weighed and ultrasonically scanned throughout the fattening period. To obtain the ultrasound images, pigs were immobilized and restrained by the head in a squeeze chute and the image sites were determined by physical palpation to accurately ascertain the scanning sites. The animals were held manually, avoiding any abnormal situation that could stress the animal, and they were only scanned in a relaxed posture, permitting accurate measurements. A mix of Eco Gel and isopropyl alcohol was used as a sound conducting material to allow a better acoustic contact surface between the probe and the skin. An Aloka 500V real-time ultrasound machine (Aloka Holding Europe, Switzerland) equipped with a 12.5 cm, 3.5 MHz linear array transducer (Aloka Holding Europe, Switzerland) was used. The adjust gain settings were 90 for overall gain, −25 for near gain and 2.1 for far gain. The focus zones were 1 and 2 on the Aloka 500V for all images collected. Captured ultrasonic images were recorded and interpreted later with Biosoft® software (Biotronics Inc., Ames, IA, USA). Ultrasound images for measurement were taken along the dorsal midline at 10th and 14th rib projection with the transducer centred perpendicularly at the anatomical site. A cross-sectional image of the loin area and back fat thickness and its layers (outer, middle and inner) on the right side of the pig at the 10th intercostal space and just behind the last rib (14th rib) were obtained using an ultrasound stand-off guide (Superflab®) mounted on the linear probe and conforming to the curvature of the pig's back and ham. Images were digitalized and stored in a computer for later analysis with Biosoft® software (Biotronics Inc., Ames, IA, USA). Determination of the anatomical location of the 10th and 14th ribs was made based on presence of muscle systems viewed on the ultrasound image. Back fat thickness was measured at a midline of the distance along the loin muscle area from skin with a perpendicular line to skin. The loin area was measured to draw the outline around the loin muscle.

2.3 Statistical analyses

All the statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Since the study included repeated measurements (serial ultrasound scanning), the mixed procedure assuming non-equal covariances among serial samples was used to compare the eight serial ultrasound scanning measurements of the different traits (back fat thickness and loin area at the 10th and 14th rib levels). The model included sex, feed and scan as fixed effects and body weight as a covariate. Ultrasound scan was included as a repeated measurement in the repeated statement of the mixed procedures. Several interactions were also included in the model. The least significant difference test was used to compare least squares means. Correlations between the ultrasound measurements at 10th rib level and the same ultrasound measurements at 14th rib level were investigated in the fourth to seventh scans. Similarly, total back fat thickness and loin area regression coefficients relating the ultrasound measurements at the eighth scan and corresponding measurements at the previous scan were obtained too.

The allometric equation was used for the depiction of differential growth of muscle and fatty tissue. This model describes a part-to-whole relationship and has the following form:

$$\log Y = \log a + b \times \log X,$$

where is the intercept on the Y axis, b is the allometric growth coefficient (slope), X is the body weight, and Y is the total back fat thickness and its layers or loin area.

The allometric coefficients were t tested to determine if the allometric coefficient defined a fast, slow or equivalent pattern of growth. The t tests were computed as $t = (b - \mathrm{Bo})/\mathrm{SEb}$, which distributes as Student's t.

3 Results

Least square means (±standard errors) of the ultrasound measurements are listed in Table 2. As expected, the scan was the factor that had the greatest influence, followed by sex and diet.

Table 3 shows the least squares means (±standard errors) for the body weight, back fat thickness and M. longissimus area at the 10th and 14th rib levels. Table 3 also shows the thickness of individual back fat layers: outer, middle and inner. The live weight ranged from 65.6 ± 1.5 kg at the start to 161.6 ± 1.4 kg at the end of the experimental period. This represents an increase of 96.0 kg and an average daily gain of 458 g day^{-1} (360 and 720 g day^{-1} at the start and end of the trial, respectively). Increases in the ultrasound measurements with increased body weight were expected. BF10 and BF14 (see underneath Table 2 for an explanation of abbreviations) increased significantly ($p < 0.05$) during the studied period. In all scans, back fat thickness at 10th rib level was higher than that recorded at 14th rib level, although this relationship changed significantly ($p < 0.05$) as body weight increased (1.97 and 1.28 mm in the first and last scans, respectively) as a result of higher back fat growth at 14th rib level (152.8 vs. 1225.5 %). The thickness of the outer, middle and inner layers progressively increased over the period of study, representing 152.80, 115.8, 119.4, 135.3, 184.0 and 135.9 % of the initial value for BFO10, BFM10, BFI10, BFO14, BFM14 and BFI14, respectively. The greatest increase occurred at the 14th rib level. Regarding the back fat layers, the middle and outer layers showed the highest and lowest growth, respectively. In the data set, the outer, middle and inner layers of the fat represented 20.33, 55.28 and 24.73 % at 10th rib level and 22.97, 46.10 and 32.22 % at 14th rib level. As a proportion of the total back fat depth, a decrease appeared in BFO10 (from 57.05 to 53.94 %), BFO14 (from 23.43 to 20.76 %) and BFI14 (from 34.42 to 30.62 %), and an increase in BFM10 (from 52.88 to 56.68 %) and BFM14 (from 41.49 to 49.97 %), while BFI10 was relatively static over the time period (from 25.20 to 24.60 %).

The change in back fat thickness per unit of body weight was 0.456 and 0.308 mm kg^{-1} for BF10 and BF14, respectively, recording the highest values in the first scan (0.635 and 0.390 mm kg^{-1}, respectively). Similarly, to what was observed for back fat thickness, the M. longissimus area at 10th rib level was higher than at 14th rib level. The differences in the M. longissimus area between two successive scans are not significant ($p > 0.05$) throughout the experimental period, recording the lowest value (0.04 and 0.92 cm^2 for 10th and 14th levels, respectively) for the fourth scan and the highest for the first scan (1.41 cm^2). The M. longissimus area at both levels increased throughout the study period, representing 138.6 % for LA10 at the last scan and 123.6 % for LA14 at the first scan. The M. longissimus area per kilogram of body weight ratio decreased significantly as body weight increased: from 0.223 to 0.126 mm kg^{-1} for LA10 and from 0.209 to 0.105 mm kg^{-1} for LA14 for the first and last scans, respectively.

The least squares means for the ultrasound measurements by sex are showed in Figs. 1 and 2. Sex did not significantly affect ($p > 0.05$) the body weight gain (95.7 and 96.2 kg in males and females, respectively) for the entire study period. All ultrasound measurements increased significantly as body weight increased, although at different rates. The back fat depth and rate of back fat growth were affected by sex ($p < 0.05$). The ultrasound back fat depth was greater in females, although the rates of back fat depth growth in the males were lower than those recorded in females (118.46 and 124.18 % for BF10 and 149.21 and 152.0 % for BF14 in males and females, respectively). The back fat thickness had higher growth in females (138.09 %) than the M. longissimus area (135.19 %); in males the opposite occurred (133.83 vs. 127.45 %, back fat thickness (BF) and M. longissimus area (LA), respectively). Also, it has been noted that the increase in back fat thickness was higher at 14th rib level, which caused the BF10 / BF14 ratio to de-

Table 2. Least squares means (±SE) for the data set of ultrasound traits studied by sex and feed and analysis of variance test.

	Sex		Feed		Effects						
	Male	Female	C	HO	Weight	Sex	Feed	Scan	Sex × feed	Feed × scan	Sex × scan
BF10 (mm)	45.19 ± 0.79	47.67 ± 0.86	44.72 ± 0.90	48.14 ± 0.84	< 0.0001	0.0177	0.0101	< 0.0001	0.2421	< 0.0001	0.2701
BFO10 (mm)	9.21 ± 0.20	9.67 ± 0.22	9.25 ± 0.25	9.64 ± 0.23	< 0.0001	0.0325	0.3369	< 0.0001	0.2346	0.0060	0.3713
BFM10 (mm)	24.08 ± 0.56	27.25 ± 0.61	24.75 ± 0.64	26.58 ± 0.60	< 0.0001	0.0045	0.0263	< 0.0001	0.0885	< 0.0001	0.0077
BFI10 (mm)	11.08 ± 0.25	11.88 ± 0.27	11.12 ± 0.30	11.84 ± 0.29	< 0.0001	0.5469	0.0542	0.0841	0.0533	0.0010	0.0148
LA10 (cm^2)	17.80 ± 0.21	18.06 ± 0.23	18.11 ± 0.24	17.76 ± 0.22	0.0067	0.2602	0.2209	< 0.0001	0.4333	0.0495	0.0001
BF14 (mm)	31.60 ± 0.72	32.36 ± 0.75	30.51 ± 0.92	33.44 ± 0.88	< 0.0001	0.0362	0.0608	< 0.0001	0.6659	< 0.0001	0.1668
BFO14 (mm)	7.25 ± 0.20	7.43 ± 0.21	7.22 ± 0.24	7.47 ± 0.22	< 0.0001	0.5736	0.4512	< 0.0001	0.0259	0.0670	0.2191
BFM14 (mm)	14.25 ± 0.43	15.23 ± 0.45	14.17 ± 0.55	15.31 ± 0.52	< 0.0001	0.0258	0.2150	< 0.0001	0.3102	0.0001	0.6585
BFI14 (mm)	10.23 ± 0.30	10.37 ± 0.32	9.63 ± 0.37	10.97 ± 0.35	< 0.0001	0.1164	0.0250	< 0.0001	0.1732	0.0263	0.4353
LA14 (cm^2)	15.55 ± 0.20	16.03 ± 0.22	15.75 ± 0.23	15.83 ± 0.22	< 0.0001	0.0251	0.9739	< 0.0001	0.1482	0.5185	0.6610

C: concentrated feed system; HO: high oleic system; BF10: ultrasonic back fat depth at 10th rib; BFO10: ultrasonic outer layer depth at 10th rib; BFM10: ultrasonic middle layer depth at 10th rib; BFI10: ultrasonic inner layer depth at 10th rib; LA10: M. longissimus area at 10th rib; BF14: ultrasonic back fat depth at 14th rib; BFO14: ultrasonic outer layer depth at 14th rib; BFM14: ultrasonic middle layer depth at 14th rib; BFI14: ultrasonic inner layer depth at 14th rib; LA14: M. longissimus area at 14th rib.

Table 3. Least square means (±SE) for body weight, ultrasonic back fat depth and loin area in pigs of the Iberian breed.

Scan	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
N	50	50	50	50	50	50	50	50
BW (kg)	65.59 ± 1.54	79.05 ± 1.58	88.27 ± 1.52	95.24 ± 1.52	110.78 ± 1.48	124.00 ± 1.43	139.91 ± 1.43	161.57 ± 1.43
BF10 (mm)	41.63 ± 1.73	41.98 ± 1.30	43.90 ± 1.00	46.24 ± 0.83	47.46 ± 0.79	48.34 ± 0.93	50.89 ± 1.36	50.98 ± 2.02
BFO10 (mm)	7.08 ± 0.43	7.95 ± 0.33	8.80 ± 0.27	9.34 ± 0.22	10.01 ± 0.21	10.73 ± 0.23	10.86 ± 0.36	10.77 ± 0.52
BFM10 (mm)	23.75 ± 1.09	23.66 ± 0.83	24.61 ± 0.63	26.17 ± 0.52	25.91 ± 0.56	26.23 ± 0.58	27.49 ± 0.86	27.50 ± 1.24
BFI10 (mm)	10.50 ± 0.54	10.79 ± 0.42	11.02 ± 0.32	11.55 ± 0.264	11.59 ± 0.22	11.77 ± 0.32	12.10 ± 0.44	12.54 ± 0.72
LA10 (cm^2)	14.66 ± 0.54	16.07 ± 0.42	16.89 ± 0.33	18.01 ± 0.28	18.55 ± 0.21	19.17 ± 0.28	19.77 ± 0.40	20.32 ± 0.63
BF14 (mm)	25.59 ± 1.38	26.85 ± 1.06	28.39 ± 0.89	31.60 ± 0.80	32.77 ± 0.86	34.81 ± 0.99	36.71 ± 1.28	39.10 ± 1.73
BFO14 (mm)	6.00 ± 0.39	6.33 ± 0.29	6.78 ± 0.23	7.51 ± 0.22	7.57 ± 0.20	8.14 ± 0.24	8.31 ± 0.32	8.12 ± 0.47
BFM14 (mm)	10.62 ± 0.79	11.18 ± 0.60	12.24 ± 0.48	14.13 ± 0.48	15.22 ± 0.53	16.67 ± 0.59	18.33 ± 0.77	19.54 ± 1.04
BFI14 (mm)	8.81 ± 0.69	9.43 ± 0.52	9.96 ± 0.41	10.63 ± 0.37	10.53 ± 0.32	10.31 ± 0.37	10.80 ± 0.57	11.97 ± 0.89
LA14 (cm^2)	13.69 ± 0.51	14.56 ± 0.37	15.48 ± 0.29	16.28 ± 0.24	16.32 ± 0.23	16.45 ± 0.24	16.63 ± 0.36	16.92 ± 0.55

BW: body weight; BF10: ultrasonic back fat depth at 10th rib; BFO10: ultrasonic outer layer depth at 10th rib; BFM10: ultrasonic middle layer depth at 10th rib; BFI10: ultrasonic inner layer depth at 10th rib; LA10: M. longissimus area at 10th rib; BF14: ultrasonic back fat depth at 14th rib; BFO14: ultrasonic outer layer depth at 14th rib; BFM14: ultrasonic middle layer depth at 14th rib; BFI14: ultrasonic inner layer depth at 14th rib; LA14: M. longissimus area at 14th rib.

crease from 1.63 and 1.61 for males and females, respectively, in the first control to 1.29 and 1.31 in the last scan. For M. longissimus area no significant difference ($P > 0.05$) between locations was found, with a LA10 / LA14 ratio of 1.07 in the first scan for both sexes and 1.23 and 1.16 in the last scan for males and females, respectively. No significant differences were observed in the percentages of each back fat layer and total thickness between the sexes (54.15 and 56.16 % for BFO10, 20.20 and 20.48 % for BFM10, 25.23 and 24.17 % for BFI10, 23.68 and 22.28 % for BFO14, 45.27 and 46.84 % for BFM14, and 32.35 and 32.13 % for BFI14 in males and females, respectively). The back fat layer growth was different in both sex and anatomy levels (143.06 and 152.66 for BFO10, 137.63 and 130.36 for BFO14, 108.35 and 122.44 for BFM10, 178.88 and 181.76 for BFM14, 126.43 and 116.81 for BFI10, and 130.88 and 135.89 for BFI14 in males and females, respectively.)The back fat thickness / body weight ratio increased as body weight increased, with females showing a higher increase (0.378 vs. 0.405 $mm\,kg^{-1}$ for BF10 in males and 0.404 vs. 0.448 $mm\,kg^{-1}$ in females, 0.195 vs. 0.316 $mm\,kg^{-1}$ for BF14 in males and 0.206 vs. 0.350 $mm\,kg^{-1}$ in females). By contrast, the M. longissimus area / live weight ratio showed a significant ($p < 0.05$) decrease for LA10 in males and females (0.194 vs. 0.137 $cm^2\,kg^{-1}$ and 0.213 vs. 0.134 $cm^2\,kg^{-1}$, respectively) and LA14 in males (0.167 vs. 0.121 $cm^2\,kg^{-1}$) instead of an increase for LA14 in females (0.182 vs. 0.124 $cm^2\,kg^{-1}$). These results are in line with allometric coefficients that were obtained (Table 4).

The least squares means for the ultrasound measurements by diet are shown in Figs. 3 and 4. The growth was affected ($p < 0.05$) by feeding system. Total weight gain of pigs on a C ration was slightly lower (91.6 vs. 99.4 kg) than that recorded in pigs on a HO ration. The back fat thickness increased more (110.25 and 133.44 % for BF10, and 134.71 and 144.82 % for BF14 in C and HO groups, respectively) than the M. longissimus area (139.74 and 124.83 % for LA10, and 134.71 and 122.52 % for LA14 in C and HO groups, respectively). The back fat layers showed a higher increase at 14th rib level than 10th rib level except for the outer layer in the C group. The layer with the lowest growth was the medium layer at 10th rib level (106.22 and 125.14 % for C and HO, respectively) and the inner layer for C (116.09 %) and the outer layer for HO, both at 14th rib level.

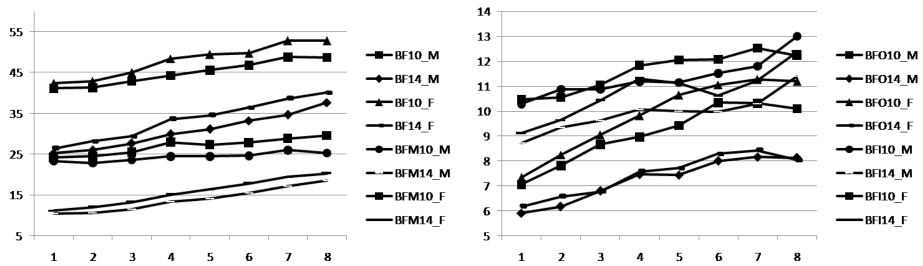

Figure 1. Changes in ultrasonic back fat depth and its layers as functions of scans in pigs of the Iberian breed.

Table 4. Allometric coefficients (R^2) for ultrasonic back fat depth and loin area in pigs of the Iberian breed.

	Scans 1 to 8			Scans 1 to 4	Scans 5 to 8	
		Males	Females		C	HO
BF10	1.09 (0.88)	1.08 (0.86)	1.12 (0.89)	1.09 (0.67)	1.10 (0.65)	1.03 (0.79)
OBF10	0.99 (0.75)	1.03 (0.79)	0.98 (0.76)	1.13 (0.58)	0.70 (0.46)	0.61 (0.28)
MBF10	1.2 (0.81)	1.20 (0.80)	1.26 (0.87)	1.20 (0.56)	1.29 (0.59)	1.23 (0.79)
IBF10	1.10 (0.79)	1.08 (0.78)	1.14 (0.82)	1.17 (0.58)	1.00 (0.51)	1.08 (0.61)
LA10	0.52 (0.77)	0.56 (0.79)	0.48 (0.78)	0.53 (0.51)	0.43 (0.51)	0.40 (0.42)
BF14	1.56 (0.88)	1.54 (0.90)	1.61 (0.88)	1.74 (0.75)	1.37 (0.59)	1.45 (0.82)
OBF14	1.35 (0.79)	1.37 (0.84)	1.35 (0.75)	1.63 (0.63)	1.16 (0.58)	0.96 (0.45)
BF14	1.9 (0.85)	1.87 (0.87)	1.91 (0.87)	2.16 (0.72)	1.52 (0.50)	1.63 (0.76)
IBF14	1.46 (0.76)	1.42 (0.77)	1.52 (0.76)	1.84 (0.61)	1.39 (0.49)	1.34 (0.61)
LA14	0.58 (0.77)	0.61 (0.81)	0.56 (0.76)	0.68 (0.57)	0.55 (0.60)	0.43 (0.40)

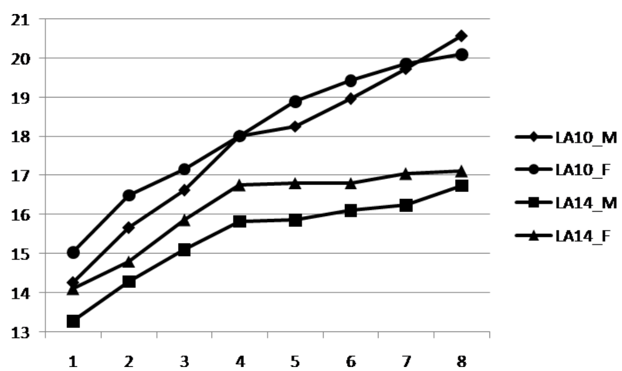

Figure 2. Changes in ultrasonic M. longissimus area as functions of scans in pigs of the Iberian breed.

These results are in line with the allometric coefficients obtained both in adipose tissue and muscle being higher in the back fat measurements. In relation to adipose tissue was the medium layer of back fat at 14th rib level with the highest growth rates and the outer back fat layer at 10th rib level with the lowest development speed. The M. longissimus area showed an allometric coefficient lower than 1 in all cases. Also, the allometric coefficients of ultrasound back fat depth measurements were slightly higher in females, while the allometric coefficients of M. longissimus area were higher in males. Although the differences were not statistically significant ($p > 0.05$). However, in relation to the diet, the C group presented a higher growth level.

Correlation coefficients between ultrasound measurements at the eighth scan with corresponding measurements at the previous scan (only those that showed statistically significant values) are shown in Table 5. Positive and in most cases significant correlations were observed for the measurements assessed, except for M. longissimus area at the 10th rib, which was not significantly ($p > 0.05$) correlated with the fourth, fifth and sixth scans and was positively significantly ($p > 0.05$) correlated with the seventh scan. These coefficients ranged from 0.18 to 0.89 ($p < 0.05$ when $r \geq 0.43$) and showed a tendency to increase this relationship as we approach the eighth scan. The correlations between M. longissimus area measurements were lower than for the other ultrasound measurements.

The R^2 values for the regression equations (Table 6) to estimate M. longissimus area were lower (0.15 to -0.01) than the values found for the prediction of fat measurements (0.62 to -0.02). As the time between scans increased, the regression coefficient decreased.

4 Discussion

There are no previous studies on serial scans of adipose and muscular tissue during growth and the final fattening phase in adipogenetic breeds such as the Iberian pig. However,

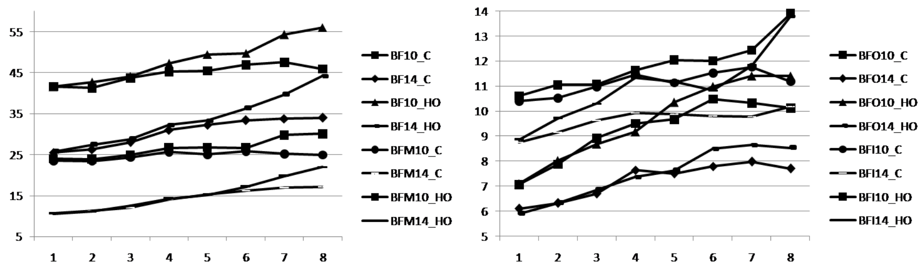

Figure 3. Changes in ultrasonic back fat depth and its layers as functions of feed in pigs of the Iberian breed.

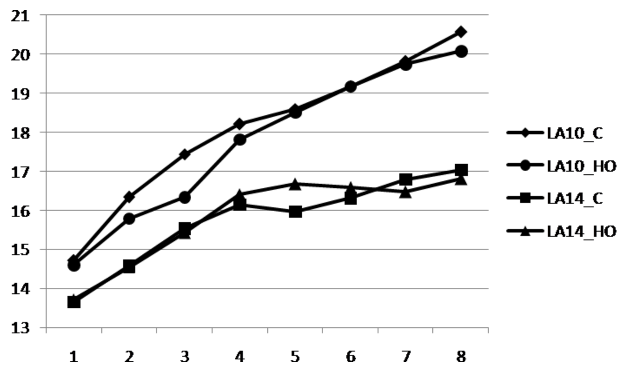

Figure 4. Changes in ultrasonic M. longissimus area as functions of feed in pigs of the Iberian breed.

Table 5. Correlation coefficients between ultrasound measurements at the eighth scan and previous scans.

Scan	Seventh	Sixth	Fifth	Fourth
BF10-8	0.81***	0.58*	0.54*	0.59*
BFO10-8	0.84***	0.68**	0.68**	0.55*
BFM10-8	0.80***	0.62**	0.62**	0.63**
BFI10-8	0.77***	0.55*	0.56*	0.49*
LA10-8	0.46*	0.24	0.22	0.18
BF14-8	0.82***	0.67**	0.57*	0.53*
BFO14-8	0.82***	0.66**	0.54*	0.45*
BFM14-8	0.89***	0.74***	0.69**	0.61**
BFI14-8	0.75***	0.60**	0.55*	0.46*
LA14-8	0.63**	0.55*	0.43*	0.45*

BF10-8: ultrasonic back fat depth at 10th rib in the eighth scan; BFO10-8: ultrasonic outer layer depth at 10th rib in the eighth scan; BFM10-8: ultrasonic middle layer depth at 10th rib in the eighth scan; BFI10-8: ultrasonic inner layer depth at 10th rib in the eighth scan; LA10-8: M. longissimus area at 10th rib in the eighth scan; BF14-8: ultrasonic back fat depth at 14th rib in the eighth scan; BFO14-8: ultrasonic outer layer depth at 14th rib in the eighth scan; BFM14-8: ultrasonic middle layer depth at 14th rib in the eighth scan; BFI14-8: ultrasonic inner layer depth at 14th rib in the eighth scan; LA14-8: M. longissimus area at 14th rib in the eighth scan. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Table 6. Coefficients (R^2) for the regression function relating the ultrasound measurements at the eighth scan to corresponding measurements at the previous scan.

Scan	First	Second	Third	Fourth	Fifth	Sixth	Seventh
BF10_8	−0.03	−0.02	0.03	0.18**	0.08*	0.24***	0.53***
BF14_8	−0.02	−0.03	0.04	0.02	−0.02	0.47***	0.62***
LA10_8	0.09*	0.06	0.08*	−0.01	0.07*	0.00	−0.01
LA14_8	0.15**	0.08	−0.01	0.06	0.10*	0.04	0.12**

BF10-8: ultrasonic back fat depth at 10th rib in the eighth scan; LA10-8: M. longissimus area at 10th rib in the eighth scan; BF14-8: ultrasonic back fat depth at 14th rib in the eighth scan; LA14-8: M. longissimus area at 14th rib in the eighth scan.
* $p < 0.05$. ** $P0.01$. *** $p < 0.001$.

there is consensus in the literature on the influence of breed on back fat thickness (Rybarczyk et al., 2011). Warriss et al. (1990) recorded average values of 11.0 and 23.0 mm in Pietrain and Large Black pigs, respectively. Also, Serrano et al. (2008) recorded values of 50, 51.1 and 80.1 mm for back fat thickness in Danish Duroc, Spanish Duroc and Retinto Iberian pig breeds, respectively. The means of back fat thickness at slaughter were similar to results in previous studies carried out on Iberian pigs (De Pedro, 1987; Dobao et al., 1987; Daza et al., 2005, 2006; Rey et al., 2006; Serrano et al., 2008; Ayuso et al., 2014). As expected, these values were higher than those reported for local or autochthonous breeds and improved breeds in previous studies (Minelli et al., 2013; Franco et al., 2014). The values were also higher than those reported for Celta pigs (38.0 ± 7.9 mm; a Spanish native breed adapted to the extensive production system) (Temperan et al., 2014). These results are not surprising since the Iberian is a local breed from the southwestern region of Spain with a distinct adipogenetic nature and shows a high subcutaneous adiposity, contributed to by their diet because as indicated by Cunningham et al. (1973), pigs fed with a low-protein diet were fatter than pigs fed with a high-protein diet. These differences could be considered as a consequence of selection for growth efficiency of lean meat or fat. The differences between breeds for back fat thickness remained when comparing the thickness / body weight ratio (mm kg^{-1}): 0.379 mm kg^{-1} in our study vs. 0.177 to 0.298 (Cunningham et al., 1973) or 0.271 mm kg^{-1} (Moeller et al., 1998). However, some local breeds have values close to those found in our study: 0.327 mm kg^{-1} in Nero Siciliano (Pugliese et al., 2003), 0.363 mm kg^{-1} in Cinta Senese (Franci et al., 2005), 0.308 mm kg^{-1} in Casertana, and 0.359 mm kg^{-1} in Mora (Fortina et al., 2005). This could be primarily due to the differences in slaughter weight, breed and feeding of the animals, factors that significantly influence the back fat thickness (Schinckel et al., 2002; Serrano et al., 2008).

The differences found between the back fat thickness at the 10th and 14th rib levels in our work demonstrate the lack of uniformity in the distribution of subcutaneous fat throughout the body of pigs, consistent with previous studies (Correa et al., 2006; Mas et al., 2010; Aro and Akinjokun, 2012). However, the back fat thickness at 10th rib level was higher in the first scans, but was higher at 14th rib level for the whole study period. In this regard, Fortin et al. (1980) indicated that fat over the back of the pork carcass was not evenly distributed when evaluating back fat thickness at various anatomical locations and positions. Comparison of the results of our work (Iberian pigs) with those obtained by Fortin (1986) in Yorkshire pigs clearly verifies the effect of breed (fattest vs. leanest) on back fat depth growth. In our work, we find a relative increase over live weight in back fat thickness during the control period studied (0.512 and 0.279 mm kg^{-1} at the first and last scans, respectively), whereas Yorkshire pigs descended from 20 to 120 kg live weight (0.51 and 0.282 mm kg^{-1}, respectively). McKay et al. (1984) also recorded declines in dorsal fat thickness / body weight ratio between 22.5 and 90.0 kg of live weight in Minnesotan (0.551 to 0.413 mm kg^{-1}), Pietrain (0.443 to 0.328 mm kg^{-1}) and Yorkshire pigs (0.412 to 0.308 mm kg^{-1}).

The increase in back fat thickness recorded by Swantek et al. (2014) in Berkshire pigs (286.5 %) and Greer et al. (1987) in crossbreed pigs (250 %) was similar to that obtained in our study for BF10 (266.3 %) but much lower than the increase recorded for BF14 (409.3 %). The lowest increase recorded by Richmond and Berg (1971) in Duroc × Yorkshire, Hampshire × Yorkshire and Yorkshire × Yorkshire (143.4 %) pigs may in part be because the animals used belonged to a lean breed and also because a different weight range was considered (25.3 kg at baseline and 80.5 kg at the end), as was found in the work of Fortin (1980).

Subcutaneous fat tissue in pigs consists of two, and sometimes three, fat layers (Fortin, 1986). In the present study, the outer, middle and inner layers represented 21.65, 50.69 and 28.48 %, respectively, of the total back fat thickness. However, they differed from the results of Newcom et al. (2004), who recorded values of 50.2, 29.67 and 20.11 % for the outer, middle and inner layers, respectively, in Duroc pigs and the results of Alfonso et al. (2005) in Basque pigs (33.7, 40 and 26.27 %, respectively). Likewise, the importance of each layer differs between studies: middle > inner > outer vs. outer > middle > inner. Meanwhile, Pugliese et al. (2003), recorded only the outer and inner layers, providing percentages of 44.94 and 55.32 %, respectively, in Nero Siciliano pigs, confirming the differences between breeds in this work. The differences found by Dobao et al. (1987) could be mainly due to slaughter weight (130–140 kg vs. 162 kg in our study). The total back fat depth also influences the percentage of each layer (Moody and Zobrisky, 1966). These authors found that increasing the total back fat thickness decreased the middle layer and increased the outer layer, as occurred in our case at 10th rib level, while at 14th rib level the middle layer is the one that grows. The unequal rate of development of adipose layers is in agreement with Fortin (1986) and McEvoy et al. (2007).

Similarly to what was observed for back fat thickness, we check the lack of uniformity in the distribution of the different layers along the body of pigs, in line with Pugliese et al. (2003), who recorded percentages of 35.48, 44.82, and 54.51 % for the outer layer and 65.52, 55.18, and 45.26 % for the inner layer of back fat on the dorsal midline opposite from the first and last ribs, and over the M. Gluteus medius.

In contrast to the values reported for the back fat thickness, values of M. longissimus area recorded in our study (21.99 and 19.79 cm^2, or 0.136 and 0.122 $cm^2 kg^{-1}$ for LA10 and LA14, respectively) were lower than those recorded in lean breeds (Newcom et al., 2004; Stahl and Berg, 2003; Sullivan et al., 2007; Hsu et al., 2010; Mas et al., 2010; Rybarczyk et al., 2011), both in absolute values (30.3–56.7 cm^2) and relative to body weight (0.329–0.498 $cm^2 kg^{-1}$) or rate of deposition (0.088 $cm^2 kg^{-1}$ in our study vs. 0.304 $cm^2 kg^{-1}$ by Moeller et al. (1998). This difference may be explained in part by constant genetic improvement of this parameter.

Sex had a significant effect on back fat thickness, both overall and in each of the layers, except the inner layer of back fat, since the sexes differed significantly in distribution of back fat thickness. Females had thicker 10th and 14th rib level fat depth and bigger M. longissimus area than males. Kemster and Evans (1979) concluded that subcutaneous fat accumulated more ventrally in females and more dorsally in males. Sex differences in the present study are supported by previous reports for the effect of the sex (Serrano et al., 2008; Mas et al., 2010; Aro and Akinjokun, 2012; Minelli et al., 2013; Swantek et al., 2014; Suárez-Belloch et al., 2015). However, the differences in sex for back fat thickness were contrary to the observations of McKay et al. (1984). The higher adiposity of male carcasses resulted from the development of subcutaneous fat rather than internal fat tissue, and females having proportionally more fat depth over the shoulder and relatively less over the mid-back than males. Also, the higher fat depth in males is indicative of higher potential for fattening than females, in agreement with previous reports (Cisneros et al., 1996), which is likely related to the higher voluntary feed intake of males. Generally, females were leaner than males based on greater values for M. longissimus area, along with less back fat. However, in our study males were leaner than females and we did not find significant differences in M. longissimus area possibly because the animals were castrated. The effects of sex were probably reduced by castration of both males and females, as reported by Mayoral et al. (1999) for castrated male and female Iberian pigs.

Diet had significant effects on the back fat thickness, both overall and for each layer, while M. longissimus area was not affected by this factor, in agreement with Rey et al. (2006). Pigs fed a HO diet had significantly higher ($p < 0.05$) values for the total back fat thickness when compared with the group fed with the C diet. These results could be due in part to the growth rate, higher in the HO group, which suggests that the faster growth was a fatter growth, in agreement with Pugliese et al. (2003) and Millet et al. (2004). In contrast, Ayuso et al. (2014) indicated that *Montanera* animals showed similar back fat thickness compared to the high-oleic group, although the values were higher in the group fed a diet high in oleic; the M. longissimus area between those two groups was statistically different. Also, Martin et al. (2008), Guillevic et al. (2009) and Mas et al. (2010) working with crossbred pigs found no differences between swine fed elevated levels of monounsaturated fat and control animals for first-rib fat thickness and M. longissimus area.

The outer, middle and inner back fat layers measured at two locations (10th and 14th rib level) followed the same trend as the back fat thickness and showed significant ($p < 0.05$) differences between feeding systems. Conversely, in the inner back fat layer more marked effects than those observed in the total back fat, outer and middle layers were detected. These results could indicate that the outer and middle layers tended to have a more constant fat deposition when compared with the inner layer. Wood et al. (1975) in previous research found that the rate at which cell diameter increased in size with increasing live weight was greater in the inner layer than in the other layers.

The allometric coefficients of back fat depth in relation to live weight allow one to better understand its evolution with growth. The isauxesis is condition of a linear measurement with respect to another of third degree, such as live weight, and is expressed by the allometric coefficient of 0.333 (Walstra, 1980) instead of unity. Lower or higher coefficients indicate slower or faster relative growth, respectively. The back fat development and its layers both in castrated Iberian male pigs and spayed Iberian female pigs showed differences through allometric coefficients. Each differential value could be interpreted as a different rate of maturation in tissue. Also, Wood et al. (1978) found different grades of back fat layer development and Mersmann (1982) evaluated the back fat development by ultrasound technique in different points of spin, concluding that the growth was not homogeneous.

The three back fat layers have distinct patterns of growth and should be considered as three separate tissues (Fortin, 1986). At birth, the outer layer is predominant. Then, at a given time during growth, the middle layer, due to a faster rate of development, becomes thicker. Finally, at a later stage, the inner layer begins to develop (Fortin, 1986). Fortin (1986) and Schinckel et al. (2002) indicated that, at light weights, the outer layer was usually more predominant, whereas, at heavier weights, the middle layer became predominant. In our study, the middle layer was predominant throughout the study period because the study began when the animals had a live weight of 64 kg.

Consistent with Fortin (1986) and Schinckel et al. (2002), in our study the allometric coefficients for the outer layer thickness of back fat ($b = 1.13$) were of lower magnitude than the middle layer thickness of back fat ($b = 1.20$). Contrary to the conclusions of Mersmann (1982) and according with Fortin (1986), the growth of the inner layer was intermediate between the outer and the middle. These results agree with those of McMeekan (1940), Sink and Miller (1962), and Sink et al. (1964), who reported that the inner (second) layer increased faster and had a greater thickness than the outer (first) layer as total back fat increased. Also, McEvoy et al. (2007) indicated that the change in thickness per unit change in body weight is greatest in the middle layer followed by the outer and inner layers.

The inner layer development is especially interesting because there are previous studies (Newcom et al., 2005; Ayuso et al., 2012) that relate thickness with a higher fat infiltration. We found remarkable differences in the allometric coefficient of the inner layer between the 10th and 14th rib levels. Thus, the level at which the measurement is recorded is important.

The M. longissimus area presented an allometric coefficient lower than 1, presenting a negative growth level of lean tissue as the fattening phase increased. This is in accordance with Virgili et al. (2003), who reported a decrease in lean yield of the loin from 143 to 183 kg live weight pigs and with Courchaine et al. (1996), who found that as age and body weight increased lean meat percentage decreased.

The Pearson correlation coefficients between ultrasound measurements at the fourth, fifth, sixth and seventh scans with the corresponding ultrasound measurements at the eighth scan ranged from 0.53 to 0.82 for back fat thickness and from 0.18 to 0.63 for M. longissimus area. These results were similar to those obtained by Tyra et al. (2001) in Polish Large White, Polish Landrace, Pietrain and Duroc breeds ($r = 0.567$–0.635 and $r = 0.552$ for back fat and loin area measurements, respectively) and higher than those recorded by Courchaine et al. (1999) in Yorkshire and crossbred pigs ($r = 0.639$ and $r = 0.453$, respectively), with initial and final average weights of 68.3 and 109.8 kg, respectively. As in the works cited, in our study the correlation coefficients between the traits of back fat thickness were greater than those obtained for the M. longissimus area. Robinson et al. (1987) recorded a correlation of 0.65 between ultrasound back fat at 17 and 20 weeks of age, and McLaren et al. (1989) reported moderate to high correlations of live animal ultrasound back fat measured throughout the nursery, grower and finisher stages to carcass back fat at slaughter. In line with the results of previous work on Iberian pigs (Ayuso et al., 2013), the highest correlation coefficients were recorded for the middle layer, followed by the outer and inner layers. This could be attributed, as indicated by these authors, to the lower accuracy in the measurement of the inner layer depth due to a wide variation in loin shape. As in the study of Kolstad et al. (1996), correlation coefficients decreased with increasing intervals between scans. Regression resulted as expected, thus corroborating the correlation results. Consistent with the results obtained by Courchaine et al. (1999) in Yorkshire barrows and Ayuso et al. (2012) in Iberian pigs, the regression coefficients for back fat measurements were higher than those obtained for M. longissimus area. However, the regression coefficients obtained from measurements taken between the seventh and eighth scans were higher than the regression coefficients between carcass and ultrasonic measurements for back fat layer variables and M. longissimus area at two anatomical locations obtained by the aforementioned authors in Iberian pigs.

5 Conclusions

The relative back fat growth was higher than M. longissimus area, not permitting the establishment of a similar growth pattern for fat and muscle. Also, the sex and diet must be taken into account in predictive models. The subcutaneous adipose layers in Iberian pigs grow at different rates during the last 6 months before slaughter, with the ultrasound serial scan it is possible to show these changes.

The changes in diet, in the fat development and the sex affect the adipose tissue development, being more noticeable in the middle layer of back fat at 10th rib level and the inner layer at 14th rib level. However, the use of an enriched oleic acid diet and the sex do not affect loin development.

As the middle layer of back fat shows more growth, this layer could be the best to be included in predictive models.

Back fat thickness at the eighth scan could be predicted with moderate accuracy from corresponding measurements taken 30 days earlier and with less accuracy as the interval between measurements increases. The M. longissimus area at the eighth scan can also be predicted, but with less accuracy.

Data availability. No data sets were used in this article.

Competing interests. The authors declare that they have no conflict of interest.

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