

Changes in adipose cells of *Longissimus dorsi* muscle in Iberian pigs raised under extensive conditions

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ABSTRACT

Twenty-four Iberian castrated male pigs were used to characterize and evaluate the effect of the duration of "Montanera" in the adipocytes size and its relation with the backfat thickness and intramuscular fat. The animals were fed under extensive conditions during 30, 60 and 90 days in the "Dehesa" before slaughtered. Carcass weight, percentage of intramuscular fat, thickness of backfat and its three layers and adipocytes size of the intramuscular fat were obtained. The group which expended 90 days on fattening obtained the highest adipocytes, with an area higher by a 50% than those that only expended 30 days. The differences in diameter and perimeter adipocyte were not as marked as area. A significant positive correlation between the diameter, area and perimeter of adipocyte with the backfat thickness were found. The fat cells in Iberian pig hypertrophy during the "montanera stage", being this increase significant from month to month in this period of fattening. Also, this adipocyte increase is correlated with the animal weight. The correlation between adipocyte size and inner layer of backfat shows that the inner layer obtained in live pig by ultrasound techniques could be a good marker of fat infiltration in pigs fattening in "montanera" system.

Key words: extensive, adipocyte, backfat thickness, intramuscular fat.

INTRODUCTION

Traditionally, the Iberian pig is raised under extensive system being fed with natural resources, acorns and grass, during the final fattening phase, called "Montanera". This stage occurs from October to March and there is a high weight gain and fat accumulation at intramuscular and dorsal level. This type of feeding, along with genetic factors and processing conditions of the products

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is responsible for the high quality of meat and meat products of the Iberian pig.

The traditional food system in the management seems to be influencing the quality of Iberian pig products (Ventanas et al. 2005). Besides the agro-silvo-pastoral system in which the Iberian pig breeding has a positive image for consumers to be associated with increased animal welfare, environmental impact reduction and protection of traditional production system (Rey et al. 2006).

The fat thickness is one of the most important carcass traits of pigs. The fat deposition has come

to be one of the main objectives in improving pig production. Factors such as the size of the adipocyte (Etherton 1980, Hausman et al. 1983, Mersmann 1986) have been studied because it could explain the differences between lean and fatty pigs.

The development of adipose depots occur in three phases; the first one is characterized by a hyperplasia between 1 and 2 months old; the second one, between 2 and 5 months old, is based in both hyperplasia and hypertrophy; and in the last phase hypertrophy (Anderson et al. 1972, Henry 1977, Hood and Allen 1977).

Currently sufficient studies are made of the influence of type of feeding on carcass characteristics of Iberian pig, but there are few studies that examine as influences the duration of the Montanera in adipose tissue. Furthermore, while foreign breeds, mainly with a low-fat (Azain 2004, Domeneghini et al. 2006, Barnes et al. 2012) and high-fat (Hauser et al. 1997, Alfonso et al. 2005, Zhao et al. 2009, Nakajima et al. 2011) accumulation in their tissues, have been sufficiently studied as the fat of food is relationship with the adipocytes size of pigs. However, this fact has not been studied in Iberian pig. The difference in adipocyte size among breeds lean and fat, being higher in latter, is due to the genetic control of fat cells and affected by the backfat thickness (Nakajima et al. 2011), or a slow differentiation or lipid filling of adipose tissue cell in obese pigs, or to a later commitment to adipose differentiation (Hauser et al. 1997).

The feeding with food enriched in fat produces changes in performance as reducer intake, improved gain and feed efficiency and increases carcass fat (Azain 2004). This increase in carcass fat is correlated positively with the increase of adipocyte size (Steffen et al. 1978).

Therefore, the aim of this work is to characterize and evaluate the effect of the duration of "Montanera" in the adipocytes size in the Iberian pig and its relation with the backfat thickness and intramuscular fat.

MATERIALS AND METHODS

DATA COLLECTION

Twenty-four Iberian castrated male pigs of the Valdesequera line ("Valdesequera", Badajoz, Extremadura, Spain) progeny of four boars and twenty sows (two to three full siblings per litter) were used. Animals were randomly distributed into three groups of eight pigs each. The animals were fed under extensive conditions with acorn and grass during 30 (M_{30}), 60 (M_{60}), and 90 (M_{90}) days in the "Dehesa" before slaughtered. The animals were slaughtered with 463 ± 3 days of age, 484 ± 2 days of age and 504 ± 2 days of age for M_{30} , M_{60} and M_{90} groups, respectively. The initial body weight (BW) was 128.88 ± 5.46 kg, 129.25 ± 4.83 kg and 128.38 \pm 5.63 kg, for M_{30} , M_{60} and M_{90} , respectively. Pigs were slaughtered at the pre-planned age (at 30, 60 and 90 days of fattening) and the final BW was $157.56 \pm 6.40 \text{ kg for M}_{30}$, $190.38 \pm 5.01 \text{ kg for M}_{60}$ and 225.63 ± 16.12 kg for M_{00} .

CARCASS TRAITS

The carcass measurements as carcass weight, percentage of intramuscular fat and backfat thickness and its three layers were taken at the slaughterhouse. The traits of backfat were taken at two levels, tenth and fifteenth rib. For this purpose from each carcass a half portion of loin containing four chops (spanning from the 11th to the 14th ribs) was extracted, chilled and used to measure the total backfat thickness and its layers at the two already mentioned rib locations. The intramuscular fat content was quantified according the method described by Folch et al. (1957).

ADIPOCYTE TRAITS

Adipocytes size (area, diameter and perimeter) of the intramuscular fat of *Longissimus dorsi muscle* were obtained. For this purpose a 1 cm³ centered portion of the *Longissimus dorsi* muscle tissue was selected and placed in liquid nitrogen. After that, the samples were embedded in OCT medium and stored at -80 °C until further cutting. The sections of 20 micron thick, collected on slides with intervals between each cut 300 microns were performed using a Leica CM1900 cryostat. The sections were fixed in a solution of 2.5% glutaraldehyde in PBS for 10 min, washed with PBS and then applied Oil Red O staining described by Lillie and Ashburn (1943). Using a digital camera coupled to a microscope Nikon Eclipse 90i photomicrographs were taken subsequently and analyzed by image analysis program (NIS-Elements RA). In each photograph the area, diameter and circumference of 1-12 adipocytes for each animal were obtained.

STATISTICAL ANALYSIS

The effect of the duration of fattening system called "Montanera" on the productive results, carcass traits and adipocytes size were studied by means of an analysis of covariance, using GLM procedure of Statistica 8.0 for windows. HSD Tukey's test was applied to compare the mean values. Mean values with standard deviation are reported. The correlations between carcass and adipocyte traits were investigated by Pearson's correlation analysis. Frequencies for adipocyte traits were obtained and represented graphically.

RESULTS AND DISCUSSION

Fat cells from adipose tissue of white pigs are closely studied by several groups of authors (Cagnazzo et al. 2006, Wimmers et al. 2007, Zheng and Mei 2009) that concluded that adipocyte size increases with live weight of animals. Other authors argue that the variation of the content of intramuscular and subcutaneous fat is closely related to changes in the size of adipocytes (Gondret and Lebret 2002, Alfonso et al. 2005, Barnes et al. 2012). In our study, the weight gain of Iberian pigs during the open range leads to increased adipocyte size

in addition to observe also the intramuscular fat content was consistent with increase of adipocytes size (Table I). The M₉₀ group obtained the highest adipocytes, with an area higher by a 50% than M_{30} group (26252.38 vs. 13693.49 μm^2). In the case of adipocyte diameter and perimeter the differences were not as marked as area, because it only increased by 26% between acorn-fed for three months and for a month (179.37 vs. 129.65 um and 647.57 vs. 476.13 um, respectively). Our results were higher than those obtained by Etherton and Allen (1979), Herpin et al. (1993), Smith et al. (1996), Hauser et al. (1997), Nünberg et al. (1998), Mourot (2001), Alfonso et al. (2005), Damon et al. (2006), Gardan et al. (2008), Zhao et al. (2009), Jiang et al. (2014), Szabó et al. (2010), Barnes et al. (2012), both in lean and obese breeds. Also, Nürnberget et al. (1998) affirmed that the rapid adipose tissue growth in pigs with 100-180 days of age is followed by a phase where adipocyte growth is minimal (180-220 days of age). In our case the pigs had more age (around one year) and there are no studies about growth of adipocyte in Iberian pigs, but if taken account studies in obese breeds as Meishan, shows that the growth in first months is more rapid than other months (Hauser et al. 1997).

In relation to the results obtained in commercial pig breeds, differences could be due to the weight of sacrifice (higher in our case) (Mourot 2001), as well as the genetics of the breed (Herpin et al. 1993, Hauser et al. 1997, Wood et al. 2004, Alfonso et al. 2005, Poulos and Hausman 2005).

The 45% of the adipocytes had an area situated in the range of 9000 to 15000 μm^2 in M_{30} group, of 15000 to 27000 μm^2 in M_{60} group and of 21000 to 33000 μm^2 in M_{90} group. In the case of perimeter in the range of 400 to 500 μm are the 41% of the adipocytes measured in the M_{30} group, of 600 to 800 μm in M_{60} group, and of 600 to 700 μm in M_{90} group. In the last case, the diameter, the 55% of adipocytes range from 100 to 140 μm in M_{30} group, from 100 to 160 μm in M_{60} group and from 160

TABLE I
Effects of duration of fattening period (D) on carcass ^{1.} and adipocyte traits.
Duration of fattoning ² .

	\mathbf{M}_{30}		\mathbf{M}_{60}		\mathbf{M}_{90}		P values	
	Mean	Std.	Mean	Std.	Mean	Std.		
Body weight at start, kg	128.9 ^b	5.5	129.2 ^b	4.8	128.4 ^b	5.6	0.95	
Body weight at slaughter, kg	157.6 ^a	6.4	190.4 ^b	5.0	225.6°	16.1	0.00	
Carcass weight, kg	119.6 ^b	4.6	163.0°	6.5	193.1 ^d	13.3	0.00	
IFAT, g/100g	2.74 ^a	1.25	4.72 ^b	1.41	6.22 ^b	2.45	\leq 0.01	
BF10, mm	7.26 ^a	0.75	$9.90^{\rm b}$	0.43	10.94 ^b	1.98	\leq 0.01	
OBF10, mm	1.57 ^a	0.23	2.00^{b}	0.25	1.87 ^b	0.34	\leq 0.01	
MBF10, mm	4.20^{a}	0.57	5.55 ^b	0.24	6.14 ^b	0.93	\leq 0.01	
IBF10, mm	1.48 ^a	0.26	2.35^{b}	0.24	2.92^{b}	0.95	\leq 0.01	
BF15, mm	5.60 ^a	1.04	7.63^{b}	0.84	9.56°	1.14	\leq 0.01	
OBF15, mm	1.25 ^a	0.29	1.59 ^b	0.27	1.62 ^b	0.20	\leq 0.05	
MBF15, mm	2.51 ^a	0.61	3.62^{b}	0.45	3.92^{b}	0.63	\leq 0.01	
IBF15, mm	1.84ª	0.42	2.42^{a}	0.63	4.01 ^b	0.77	\leq 0.01	
Area, μm²	13693.49 ^a	3085.12	20273.92^{ab}	5558.03	26252.38 ^b	8429.56	\leq 0.01	
Perimeter, µm	476.1a	57.3	582.8^{ab}	95.4	647.6^{b}	95.8	\leq 0.01	
Diameter, µm	129.6 ^a	15.0	157.6ab	22.7	179.4 ^b	26.9	\leq 0.01	

¹IFAT: Intramuscular fat; BF10: Backfat thickness at tenth rib level; OBF10: Backfat thickness of outer layer at tenth rib level; MBF10: Backfat thickness of inner layer at tenth rib level; BF15: Backfat thickness at fiftieth rib level; OBF15: Backfat thickness of outer layer at fiftieth rib level; MBF15: Backfat thickness of middle layer at fiftieth rib level; IBF15: Backfat thickness of inner layer at fiftieth rib level.

to 220 μ m in M₉₀ group (Fig. 1). The increase in adipocyte diameter is caused by the deposition of fat (Nürnberg and Wegner 1990).

Consistent with previous studies (Gondret and Lebret 2002, Alfonso et al. 2005, Velotto et al. 2012), a significant positive correlation between the diameter, area and perimeter of adipocyte with the backfat thickness were found (Table II), specifically with the inner layer taken at the 15th rib (0.42, 0.40 and 0.36, respectively). This correlation could be due to, and according with Fortin (1986), Geri et al. (1986), Barone et al. (2000) and Velotto et al. (2012), the adipocytes from inner layer are larger than the outer layer. Also, this inner layer of backfat (IBF15) has the most increase in size over "montanera" period of fattening, especially in the last section of fattening (Table I). In previous studies Ayuso et al. (2013) obtained a positive correlation between the inner layer thickness of backfat and intramuscular fat both in the carcass and in vivo by ultrasound methods (0.47 and 0.40, respectively). The greater adipocyte size is the major contributing factor in the greater increase on backfat tissue in obese pigs (Nakajima et al. 2011).

There were significant differences in the amount of intramuscular fat during the fattening phase on "montanera" system. These differences appear to be due to an adipocyte hypertrophy and the increased size of these cells are positively correlated with the weight gain, intramuscular fat and the inner layer thickness of backfat at the 15th rib level. The animals that spent 90 days on this system had higher deposits of intramuscular fat. The flavor, juiciness and tenderness of meat is variable and depends on fat content of meat (Fernandez et al. 1999, Brewer et al. 2001, Wood et al. 2004, Lonergan et al. 2007), due to this a specific development of intramuscular fat deposits is necessary (Hauser et al. 1997). Therefore, the animals that spend 90 days in fattening must present those carcasses with better organoleptic features. Zhao et al. (2009) suggested that fat pig breeds posses higher capacity to deposit

 $^{^{2}}M_{30}$ = Thirty days on fattening; M_{60} = Sixty days on fattening; M_{90} = Ninety days on fattening.

TABLE II									
Relationship	between	carcass	traits1.	and	adi	pocy	te	trait	s.

	IFAT	BF10	OBF10	MBF10	IBF10	BF15	OBF15	MBF15	IBF15
Area, μm	0.07	0.12	-0.01	0.21*	0.03	0.29*	0.08	0.05	0.40*
Perimeter, µm	0.14*	0.12	-0.00	0.21*	0.03	0.31*	0.10	0.10	0.36*
Diameter, µm	0.11	0.17*	-0.00	0.25*	0.08	0.33*	0.11	0.09	0.42*

¹·IFAT: Intramuscular fat; BF10: Backfat thickness at tenth rib level; OBF10: Backfat thickness of outer layer at tenth rib level; MBF10: Backfat thickness of middle layer at tenth rib level; IBF10: Backfat thickness of inner layer at tenth rib level; BF15: Backfat thickness at fiftieth rib level; OBF15: Backfat thickness of outer layer at fiftieth rib level; MBF15: Backfat thickness of middle layer at fiftieth rib level; IBF15: Backfat thickness of inner layer at fiftieth rib level. * $P \le 0.05$.

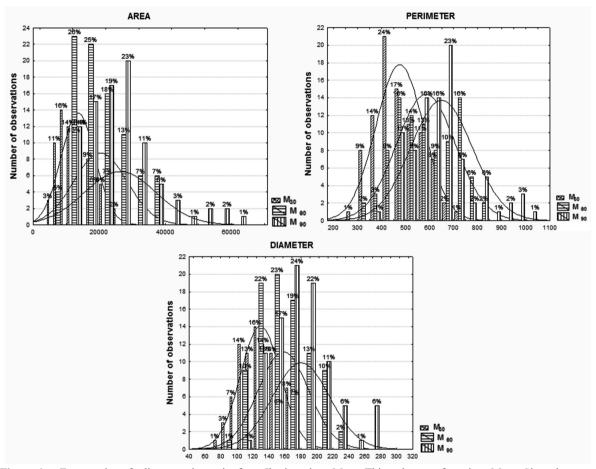


Figure 1 - Frequencies of adipocyte size traits from Iberian pigs. M_{30} = Thirty days on fattening; M_{60} = Sixty days on fattening; M_{90} = Ninety days on fattening.

triacylglycerol in muscle deposit than lean pigs because intramuscular fat content is higher in first breeds (5.58 vs. 3.90%). Also, higher intramuscular pigs appear to be because this kind of this breed has a high capacity of lypogenesis and fatty acid transport, lower potential of fat mobilization and fatty acid oxidation (Zhao et al. 2009). The increase in fat in pigs is associated with the increase of

intramuscular fat adipose tissue. This increase is attributable to the larger increase in intramuscular adipocyte size (Barnes et al. 2012).

CONCLUSIONS

The fat cells in Iberian pig hypertrophy during the "montanera stage", being this increase significant from month to month in this period of fattening.

Also, this adipocyte increase is correlated with the animal weight. Prolong the final fattening in "montanera stage" up to 90 days in Iberian pigs makes the marbling, and therefore the final quality of the meat is significantly higher than those animals with 60 or 30 days on "montanera" fattening and backfat is thicker. The correlation between adipocyte size and inner layer of backfat at 15th ribs shows that the inner layer obtained in live pig by ultrasound techniques could be a good marker of fat infiltration in pigs fattening in "montanera" system.

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