

Muscle fibre types, fat deposition and fatty acid profile of Casertana *versus* Large White pig

Salvatore Velotto^{1,*}, Claudia Vitale¹, Antonio Crasto¹

¹ University of Study of Naples Federico II, Department of Soil, Plant, Environmental and Animal Production Sciences - Faculty of Agriculture, Via Università 133, 80055 Portici (NA), Italy

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Used were 8 castrated males and 8 entire females of indigenous Casertana and Large White pigs, 4 animals of each breed. In *Longissimus dorsi* (LD) and *Psoas major* (PM) muscles the morphometric characteristics of FG (*fast glycolytic*), FOG (*fast oxidative glycolytic*) and SO (*slow oxidative*) fibre types was performed and their percentage determined. Determined was also the area of adipocytes from internal and external layer of adipose tissue from backfat, shoulder, dorsal and loin regions. The SO were larger than FG and FOG fibres ($P < 0.05-0.001$) and the size of FG fibres was significantly different among the two breeds considered, higher in Large White pigs and lower in Casertana. LD muscle was characterized by FG and SO fibres larger than PM ($P < 0.001$). Males had fibres larger than females in both muscles and breeds. The dorsal region had bigger adipocytes in both the pig breeds ($P < 0.05-0.01$). In comparison with the outer fat layer, the inner and middle layers showed more developed adipocytes. Differences in fatty acid composition were observed between breeds. Particularly, Casertana pigs showed higher level of essential fatty acids than did Large White. The results showed the particular characteristics of the Casertana breed as compared to pig lines intensively selected for growth efficiency.

KEY WORDS: adipocyte / fat / fatty acid / fibre type / meat / muscle / pig

The interest in indigenous pig breeds is related to meat products. Particularly among them, the Casertana pig is widely appreciated for the high quality of its niche products linked to the local gastronomic traditions [Barone *et al.* 2003, Gigante *et al.*

*Corresponding author: velotto@unina.it

2004]. The Casertana pig originates from the Campania region (Southern Italy). It is medium-small sized, the coat is bright black and mostly hairless; the standard type exhibits wattles. The importance of indigenous breeds is related to the conservation of genetic resources that has become a high priority goal to support future livestock improvement [Alfonso *et al.* 2005].

The Large White, also known as the English Large White, is a breed originating in Yorkshire. [http://en.wikipedia.org/wiki/Large_White_\(pig\)](http://en.wikipedia.org/wiki/Large_White_(pig)) – cite_note-Firefly-0 It is one of the most common pig breeds, widely used in pure- and crossbreeding in intensive pig farming around the world. The Large White is a long-bodied, large-sized animal with excellent ham and fine white bristle. The aim of this work was to compare the Casertana breed with Large White. The study focused attention on:

- size and distribution of fibre types of Longissimus dorsi (LD) and Psoas major (PM) muscles;
- adipose tissue cellularity;
- fatty acid profile.

Material and methods

Animals

Muscle samples were examined of Casertana (n=8) and Large White (n=8) pigs raised and fattened in a farm located in Campania, Italy. Four castrated males and four entire females of each breed were slaughtered at the age of one year and body weight of 150 ± 10 kg (Casertana) and 170 ± 10 kg (Large White). Animals were raised under semi-intensive conditions. The slaughter house had EEC mark with reference to rules 852/853/854/2004; 2076/2005; 1069/2009. Animals were treated according to the guidelines of the European Community on the treatment of experimental animals (Reg. EC 1/2005; directives 74/577/EEC; Law 439; 2 August 1978).

Muscle histochemistry

Examined were the *Psoas major (PM)* and *Longissimus dorsi (LD)* muscles. Samples were excised from the center of PM and the middle of LD at the level of the 8th thoracic vertebra. Immediately after excising the samples were frozen in liquid nitrogen (-196°C) and then stored at -80°C until histochemically analysed. Transverse serial sections ($8 \mu\text{m}$) were cut in a cryostat (LEICA CM 1100) at -20°C and transferred to glass cover slips. The sections were stained histochemically for myosin ATPase (to reveal muscular contraction) and succinic dehydrogenase (to reveal metabolism) on the same myofibres simultaneously [Solomon and Dunn 1988, Velotto *et al.* 2010]. The method used for the combined histochemical staining (acid myosin ATPase + SDH) consisted of several steps. Acid pre-incubation was performed at room temperature for 20 min, followed by two 1-min rinses of CaCl_2 in tris-hydroxymethyl-aminomethane buffer. Nitro-blue-tetrazolium (NBT) incubation was performed for SDH activity at 37°C for 20 min, followed by two rinses in distilled

water. Myofibrillar acid ATPase staining was performed at 37°C at pH 9.45 for 50 min, followed by three 30 s rinses in CaCl₂ solution and incubation in CoCl₂ for 3 min. A standard ammonium sulphide staining of the acid ATPase procedure was performed and cover slips were applied using glycerol jelly. The basic myosin ATPase method was used for the first control procedure, consisting of sodium-cacodylate and sucrose incubation for 5 min, followed by two 1-min rinses in CaCl₂ in tris-hydroxymethyl-aminomethane buffer rinse solution. Sigma 221 and CaCl₂ solutions were used for 15 min (pH 10.3-10.5), followed by two 1-min rinses in CaCl₂ and tris-hydroxymethyl aminomethane buffer (MERCK & Co. USA). For acid ATPase the procedure was performed at 37°C at pH 9.4 for 50 min with three 30 s rinses in CaCl₂ solution and incubation for 3 min in CoCl₂, followed by ammonium sulphide staining. Cover slips were applied using glycerol jelly. The SDH method was used for the second control procedure, consisting of different phases: incubation in NBT at 37°C for 20 min, followed by two rinses in distilled water and formaldehyde immersion for 10 min. Cover slips were applied using glycerol jelly.

Fibre size

Fibre size was determined from the sections used to determine myofibres number. The area was measured using an image-analysing system LAS (LEICA Application Suite Interactive measurement). For each muscle not less than 250 fibres measured from eight random fields were determined. The mean fibre size was calculated.

Adipocytes

Some specimens of adipose tissue from pig backfat, inner and outer layer at the first and last thoracic vertebra and last loin vertebra, were collected in order to detect the adipocyte size. Samples were kept in Ringer's solution at room temperature, fixed in formaldehyde (37%) for 15 min, and then sectioned on cryostat (30 µm thick). The sections were floated in a drop of physiological saline buffer on glass slides and were examined by image-analysing system. For each specimen not less than 100 adipocytes were examined. The data were processed by variance analysis using two models: for males (α) and for females (β). **For the histochemical parametres the models were:**

$$y_{iklm} = \mu + \alpha_i + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\gamma\delta)_{kl} + (\alpha\gamma\delta)_{ikl} + \epsilon_{iklm}.$$

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\delta\gamma)_{kl} + (\alpha\beta\gamma)_{ijk} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + \epsilon_{ijklm},$$

where fibre type (γ), muscle (δ) and their interactions were considered as fixed factors.

As regards adipocytes, the two models, comprehensive also of region (γ) and sampling location (δ), were:

$$y = iklm \mu + \alpha_i + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\alpha\gamma\delta)_{ikl} + \epsilon_{iklm}$$

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + (\alpha\beta\gamma)_{ijk} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + \epsilon_{ijklm}.$$

The significance between the mean values was evaluated using the Student's *t* test.

Fatty acid composition

The left fifth and sixth chops taken at the meat-processing plant from each animal were used to analyse fatty acid composition, by gas chromatography. Fatty acid composition was determined after extraction (with chloroform:methanol, 2:1) and methylation (with boron trifluoride / benzene / methanol, 25:20:55) of adipose tissue of the muscle. The methyl esters were analysed on a chromatograph (HP-5890, HEWLETT-PACKARD Co., USA) equipped with a flame ionization detector and split injector (HP-7673, HEWLETT-PACKARD Co., USA). Separations were performed using a capillary column (100 m × 0.25 mm × 0.25µm) HPINNOWAX Crosslinked Polyethylene Glycol, HP Co., USA). The following conditions were applied: (a) carrier gas, helium at 1 mL per min; (b) oven temperature 150 to 210°C at 3°C per min, 210°C for 5 min, 210 to 250°C at 4°C per min, 25 min at 250°C; (c) injector temperature 225°C; (d) detector temperature 240°C. Methyl ester standards for fatty acids (SIGMA-ALDRICH Química, S.A., Spain) were used to identify the peaks. The results were expressed as relative percentage.

Results and discussion

The fibre type composition was delineated histochemically in pig muscle samples, identifying three fibre types: (1) FG, with high myosin ATPase activity and low oxidative activity; (2) FOG, with moderate myosin ATPase activity and intermediate oxidative activity and (3) SO, with low myosin ATPase and high oxidative activity. The results of the analysis of variance showed significant muscle × fibre and sex × fibre interaction ($P < 0.001$) for the morphometric characteristics evaluated. The differences between breeds for the LD muscle, with regard to fibre area were 21% for FG (6115 vs. 4833, $P < 0.001$), 19% for FOG (3568 vs. 2891, $P < 0.001$) and 16% for SO (7001 vs. 5845, $P < 0.001$). For the PM muscle, the interbreed differences with

Table 1. Means and variation coefficients (V%) for cross-section area (μm^2) of three types of fibres (FG, FOG and SO) of *Psoas major* (PM) and *Longissimus dorsi* (LD) muscles in pigs of two breeds

Muscle	FG fibres		FOG fibres		SO fibres	
	mean	V%	mean	V%	mean	V%
	Casertana breed					
PM	2946 ^A	32	1895 ^A	35	3416 ^A	38
LD	4883 ^B	32	2891 ^B	34	5845 ^B	37
	Large White breed					
PM	3552 ^A	34	2253 ^A	46	3958 ^A	44
LD	6115 ^B	40	3568 ^B	43	7001 ^B	45

^{AB} Within breeds and fibre types means bearing different superscript differ significantly at $P < 0.01$.

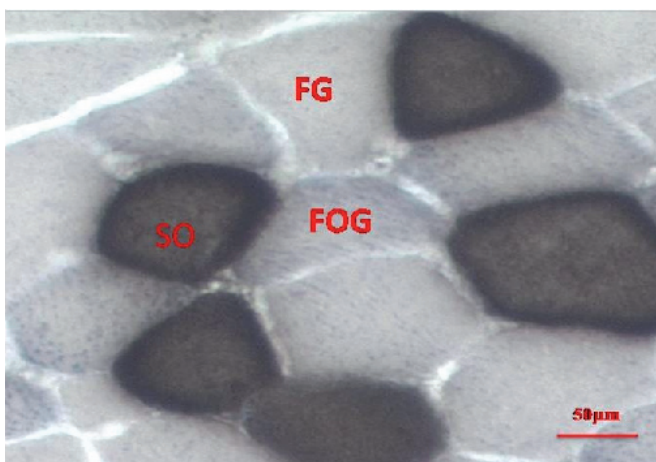


Fig. 1. *Longissimus dorsi* muscle (Casertana breed).

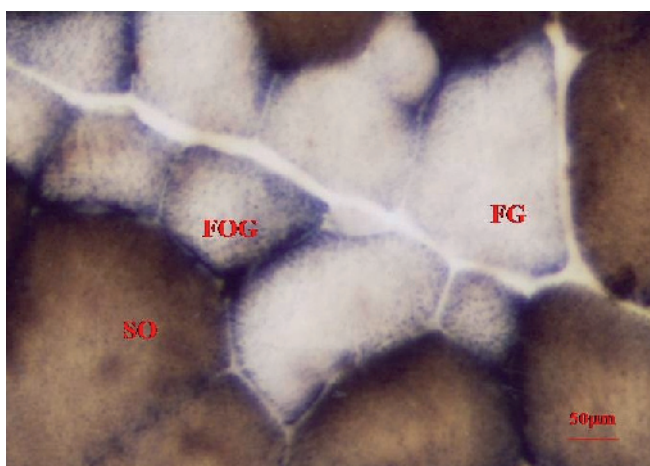


Fig. 2. Psoas major muscle (Large White breed).

regard to fibre area were 17% for FG (3552 vs. 2946, $P<0.001$), 15% for FOG (2253 vs. 1895, $P<0.001$) and 14% for SO (3958 vs. 3416, $P<0.001$). Besides, it is interesting to emphasize that irrespective of the pigs type, LD muscle showed for all three fibre types the area values larger than those found in PM (6115 vs. 3552, 3568 vs. 2253 and 7001 vs. 3958 for FG, FOG and SO, respectively, $P<0.001$ in Large White, whilst 4833 vs. 2946, 2891 vs. 1895 and 5845 vs. 3416 for FG, FOG and SO, respectively, $P<0.001$ in Casertana) – Table 1. Regarding the histological picture of muscles, LD seems lighter and more glycolytic than PM due to the higher percentage of FG both in Large White than Casertana pigs (62% vs. 27%; 58% vs. 30%). Meanwhile, PM

showed higher oxidative capacity due to the higher percentage of SO fibres both in Large White than Casertana pigs (34% vs. 14% in LD and 32% vs. 16% in LD, $P<0.001$) – Fig. 1 and 2). Large White had fibres larger than Casertana in both muscles and such trend was noticed in both sexes – males had fibres larger than females in both breeds and particularly in Large White breed (Tab. 2). Looking at the adipocyte size of three regions considered (Tab. 3), the values varied according to the layer. Particularly the value increased from shoulder to dorsal region ($P<0.01$) in the deep and middle layers, while from loin to dorsal region – in the outer layer ($P<0.01$). The same trend was found in both breeds. Moreover, according to results by Fortin [1986], as well as Geri *et al.* [1986] and Zappa *et al.* [1992] on different breeds, adipocytes from inner layer were larger than those from the outer layer for any considered region. Finally, the adipose cells were greater in Casertana than in Large White pigs (Tab. 3).

Table 2. Means and variation coefficients (V%) for cross-section area (μm^2) of three types of fibres (FG, FOG and SO) of *Psoas major* (PM) and *Longissimus dorsi* (LD) muscles in pigs of two sexes

Sex	FG fibres		FOG fibres		SO fibres	
	mean	V%	mean	V%	mean	V%
Casertana breed						
Males	3265 ^a	24	2610 ^A	20	3756 ^A	21
Females	2759 ^b	25	1782 ^B	25	2744 ^B	22
Large White breed						
Males	4073	30	3255	25	4685	28
Females	3441	31	2223	31	3459	28

^{aA}...Within breeds and fibre types means bearing different superscript differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Table 3. Means and variation coefficients (V%) for adipocyte cross-section area (μm^2) as related to sampling region and sampling layer in pigs of two breeds

Region of sampling	Inner layer		Outer layer		Middle layer	
	mean	V%	mean	V%	mean	V%
Casertana breed						
Shoulder	13969 ^A	51	12679 ^a	44	13324 ^A	48
Dorsal	16756 ^B	44	13109 ^B	72	14933 ^b	59
Loin	15475 ^A	44	11599 ^B	45	13536 ^a	61
Large White breed						
Shoulder	12638	46	11472	40	12055	44
Dorsal	15361	40	11860	66	13510	53
Loin	14002	40	10488	41	12247	55

^{aA}...Within breeds and fibre types means bearing different superscript differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Table 4. Means and variation coefficients (V%) for fatty acids contents (per cent of a total) of *longissimus dorsi* adipose tissue in pigs of two breeds

Fatty acid	Casertana breed		Large White breed	
	mean	V%	mean	V%
C14:0	1.5	20	1.6	19
C16:0	25.7	10	26	8
C16:1	2.4	12	2.2	11
C17:0	0.4	0	0.4	25
C17:1	0.3	33	0.2	31
C18:0	14.5	5	15.5	4
C18:1	39.4	6	36.1	5
C18:2	8.2	11	10.9	10
C18:3+C20:0	0.7	14	0.6	13
C20:1	0.8	10	1.1	9

The profile of fatty acids is presented in Table 4. The most abundant were palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and stearic (C18:0). There was wide interindividual variation. Particularly Casertana pigs fat contained more essential, while that of Large Whites more saturated fatty acids.

Meat quality is a general term used to describe properties and perceptions of meat. In the case of porcine meat, these are primarily characteristics such as taste, tenderness and juiciness. In those terms it is important to compare pig breeds in order to evaluate their merits of value giving the possibility of choice to consumer. In the present study we compared two breeds – Casertana and Large White – analysing some important factors that may affect meat properties. As expected, all parameters evaluated showed differences between breeds. Muscle fibres, quantitatively the most important component of skeletal muscle, have long been thought to be important factors influencing meat quality. However, identifying a superior fibre type for meat production remains a tough issue [Lefaucheur 2002]. In many cases it seems that the ratio of slow twitch oxidative to fast twitch oxidative (type I to type II) muscle fibres affects meat tenderness and varies within individual animals as well as among breeds and crossbreds [Dinh Tran Nhat Thu 2006]. Most of adult mammalian skeletal muscles are composed of at least three fibre types: slow twitch oxidative (SO), fast twitch oxidative/glycolytic (FOG) and fast twitch glycolytic (FG). Muscle fibres number and size as well as percentage of individual fibre types may affect the *post-mortem* conversion from muscle to meat and subsequently alter meat quality. Recently, correlations were identified in pigs between muscle fibre characteristics and meat quality traits [Ryu and Kim 2005]. It is possible to identify light and dark muscles in the pig. Dark muscles contain predominantly type I and II A fibres, while light muscles are primarily of II B type. The present study particularly shows that LD muscle had a high percentage of FG fibre type in both breeds, while PM muscle – a high percentage of SO. Muscle with higher content of type II B is more prone to

yield pale, soft and exudative (PSE) meat [Franck *et al.* 2007], while that with higher proportion of type I is more tender and presents more favourable quality [Sosnicki 1987]. Besides, it is possible to notice the presence of more developed SO fibres in LD and PM muscles that may be related, especially in indigenous Casertana, to selection of domestic pigs – Essen Gustavsson [1993]. Finally, LD muscle presented three fibre types more developed in both breeds than PM muscle. This is in accordance with Barone *et al.* [2000] who, working on LD muscle, identified a similar relation – all three fibre type areas were twice of those measured in PM muscle.

Differences were found between castrated males and entire females with regard to the size of all three fibre types. Castrated males showed larger fibres than did the females and this trend was noticed particularly in Large White pigs. This is in contrast with Barone *et al.* [2000] who found no differences between males and females regarding fibre type size. Velotto *et al.* [2010] found the same trend in Casertanas.

Casertana pigs retain the traits of slow growing and high fat-depositing compared to various genetic lines used in the pig industry. Compared with Large White, Casertana pig was far less competitive regarding growth performance, reaching a commercial slaughter weight at a considerably more advanced age and with a backfat thickness more than double [Salvatori *et al.* 2008]. The size of adipose cells was significantly greater in Casertana than in Large White in all the layers and regions considered (Tab. 3). The values increased from shoulder to loin region for deep and middle layers and from loin to shoulder region for outer layer. The mechanisms of accumulation and mobilization of fat reserves are particularly important in the middle and inner layers, explaining close to 90% of the difference in backfat depth between farrowing and weaning. Variation between sows at the same point of reproductive cycle, once corrected for parity, age, genotype and the technological group is wider in the middle layer, followed by the inner, and then in the outer layer [Alfonso *et al.* 2007]. Our results presented here are, in part, in accordance with those of Alfonso *et al.* [2007]. In fact, the size of adipocytes was greater in the deep and middle layers for both breeds.

Tenderness and flavour are important sensory traits that determine quality of meat. While tenderness is essentially affected by composition, texture of muscle, some biochemical processes taking place at slaughter, technology applied and carcass storing, the flavour, which is influenced by fat content, can be manipulated genetically by growth performance and dietary supplementation [Dinh Tran Nhat Thu 2006]. Yet fat and fatty acids, whether in adipose tissue or in muscle, contribute importantly to various aspects of meat quality and are crucial to the nutritive value of meat [Wood *et al.* 2008]. The same authors [2008] had shown that fatty acid composition of adipose tissue and muscle in pigs, sheep and cattle is related to the amount of fat in the carcass and in muscle. In the present study when comparing the two breeds we found that Casertana carcasses contained more fat tissue and presented higher content of essential fatty acids than Large White pig carcasses. In the latter more saturated and less monounsaturated and polyunsaturated fatty acids were found. Studies conducted at Bristol in the 1970s

and 1980s [Wood *et al.* 2004] showed that 18:0 and 18:2 are particularly important contributors to fat tissue firmness. As fatty acid composition was changed for reasons of diet, genetics, sex or fatness, these two showed the highest correlations with fat firmness. The 18:0 to 18:2 ratio was found to provide the best prediction of firmness. The role of 18:0 is particularly interesting as it varies across a smaller range than 18:2 acid [Wood *et al.* 2004]. In the present study, among the two considered breeds, the contents of these two fatty acids were higher in Large White pig.

In summary, the work reported here shows the particular characteristics of the Casertana as compared to the Large White breed. The former presents higher adipose tissue development with a larger area of adipose cells and higher content of essential fatty acids than the Large White. Moreover, the Casertana shows smaller fibres than the Large White, considered a state less prone to degradation. It is noteworthy that this study provides useful information to allow the design of future experiments aimed at answering a specific hypothesis on the importance of indigenous breeds.

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