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Some aspects of meat production in pig autochthonous genetic types. III. Morphometric characteristics of fibre types and adipocytes[†]

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SUMMARY - The research was carried out on 12 pigs: 8 castrated males and 4 whole females belonging of autochthonous genetic types (AGT) Calabrese (n=4), Cinta casaldianni (n=2), Cinta senese (n=2) and Siciliano (n=4). On *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 and their percentage were determined. However the area of adipocytes from internal and external layer of adipose tissue from pig back fat, shoulder, dorsal and loin regions, was also determined. The results have showed that: (i) as regards muscle fibres, the SO is larger than FG and FOG ($P<0.05-0.001$); (ii) the size of FG fibre is significantly different among the 4 considered AGT, higher in Calabrese pig and lower in Cinta Casaldianni; (iii) *Longissimus dorsi* muscle is characterized by FG and SO fibres twice larger than PM (6109 vs 3184 and 6657 vs 3874; $P<0.001$); and (iv) there are no significant differences between sexes for size of three fibre types. As regards adipocytes: (i) among the AGTs, Calabrese pig has smaller cells ($P<0.001$) and Siciliano larger ($P<0.05-0.01$); (ii) the loin has bigger adipocytes, followed by the dorsal and shoulder regions ($P<0.05-0.001$); (iii) in comparison with the outer fat layer, the inner layer has more developed adipocytes (about 25%); and (iv) there are no significant differences between males and females.

Key words: Pig, fibre type, adipocytes, muscle.

RESUME - "Aspects de la production de viande de porcins autochtones. III. Caractéristiques morphométriques de la fibre musculaire et des adipocytes". La recherche a été faite sur 12 porcins, dont 8 mâles castrés et 4 femelles entières appartenant aux types génétiques autochtones (TGA) Calabrese (n=4), Cinta casaldianni (n=2), Cinta senese (n=2) et Siciliano (n=4). Les caractéristiques morphométriques des trois types de fibre musculaire (FG=fast glycolytic, FOG=fast oxidative glycolytic, SO=slow oxidative) ont été définies sur la base des échantillons musculaires [*Longissimus dorsi* (LD) et *Psoas major* (PM)] prélevés au moment de l'abattage. Les caractéristiques morphométriques des adipocytes ont été relevées sur des échantillons du substrat superficiel et profond du gras dorsal des parties suivantes : paleron, rein et croupe. Les résultats plus importants montrent que : (i) en ce qui concerne les caractéristiques morphométriques de la fibre musculaire, la fibre SO présente une surface de section transversale significativement supérieure aux autres deux types (FG et FOG ; $P<0,05-0,001$) ; (ii) les 4 TGA sont significativement différents entre eux en ce qui concerne la surface de la fibre FG qui donne la valeur maximum chez le Calabrese et la valeur minimum chez la Cinta casaldianni ; (iii) le muscle *Longissimus dorsi* est caractérisé par des fibres FG et SO de dimensions transversales doubles par rapport au PM (6109 vs 3184 et 6657 vs 3874 ; $P<0,001$) ; et (iv) il n'y a pas de différence significative entre les mâles castrés et les femelles entières pour les dimensions transversales des trois types de fibre. En ce qui concerne les adipocytes : (i) parmi les TGA étudiés, le Calabrese possède les cellules plus petites ($P<0,001$) et le Siciliano les plus grandes ($P<0,05-0,01$) ; (ii) la zone des reins présente des adipocytes plus grands, suivie du dorsal et du paleron ($P<0,05-0,001$) ; (iii) la partie profonde, par rapport à celle superficielle, présente des adipocytes plus développés (en moyenne de 25%) ; et (iv) on n'observe pas de différences significatives entre les sexes.

Mots-clés : Porcin, fibre musculaire, adipocytes, muscle.

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Introduction

The skeletal muscles consist of fibres that differ from each other in a number of aspects including contraction time, shortening speed and fatigue resistance. The proportions of the various fibre types may vary greatly in relation to the muscle function; also the cross sectional area of each fibre type may be determined by different factors such as genetic type, sex, age, feeding, etc. This study is a small part of a large research project on pig autochthonous genetic types and analysed the adipose tissue cellularity, size and distribution of fibre types of *Longissimus dorsi* (LD), one of the most studied muscles, and *Psoas maior* (PM) a representative muscle of animal leg.

Material and methods

The research was carried out on 12 pigs: 8 castrated males and 4 whole females belonging to autochthonous genetic types (AGT) Calabrese (Cal, 2 castrated males and 2 whole females), Siciliano (Sic, 2 castrated males and 2 whole females), Cinta senese (CS, 2 castrated males) and Cinta Casaldianni (CC, 2 castrated males). Specimens were taken from *Longissimus dorsi* near the last vertebra, and *Psoas maior* muscles immediately (45' post mortem) frozen in liquid nitrogen and stored until they were sent to the laboratory. Simultaneous identification of the three fibre types (FG = fast glycolytic, FOG = fast oxidative glycolytic and SO = slow oxidative), was performed on cross sections (12 µm thick) obtained by cryostat set to -22°C, according to Matassino *et al.* (1993). For each fibre type, using an image analyser, the area, perimeter and diameter (minimum and maximum) were measured. Some specimens of adipose tissue from pig backfat, inner and outer layer at the first and last thoracic vertebra and last loin vertebra, were removed in order to detect the adipocyte size. Samples are 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 observed by image analyser. For each specimen not less than 100 adipocytes were observed.

The data were processed by variance analysis using two models, one for males of all 4 TGA (α) and another for Siciliano and Calabrese, males and females (β). For the histochemical parameters the models were: $y_{iklm} = \mu + \alpha_i + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\gamma\delta)_{kl} + (\alpha\gamma\delta)_{ikl} + \varepsilon_{iklm}$ and $y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\delta\gamma)_{kl} + (\alpha\beta\gamma)_{ijk} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + \varepsilon_{ijklm}$ 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: $y = \mu + \alpha_i + \gamma_k + \delta_l + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\alpha\gamma\delta)_{ikl} + \varepsilon_{iklm}$ and $y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + (\alpha\beta\gamma)_{ijk} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + \varepsilon_{ijklm}$, respectively. The significance between the mean values was evaluated using the Student's *t* test.

Results and discussion

The size (cross sectional area and diameters) of three fibre types varies in relation to the genetic type: Calabrese and Siciliano have FG fibre bigger than FOG ($P < 0.05$) and SO ($P < 0.001$); conversely both Cinta senese and Casaldianni have SO fibre larger than FOG ($P < 0.001$) and FG ($P < 0.001$). The occurrence of more developed SO fibres in LD and PM muscles may be related, especially in CS and CC autochthonous genetic types, to ancestrally degree (selection of domestic pigs), according to results of Essen Gustavsson (1993). This researcher found that there are differences in fibre area: in domestic pig breeds IIA and IIB fibre areas are larger than type I (SO), the opposite in wild pigs. Moreover, it is interesting to emphasize that, independent of genetic type, LD muscle shows for all three fibre types values area twice of that measured in PM (6109 vs 3184, 4678 vs 2288 and 6657 vs 3874 for FG, FOG and SO, respectively $P < 0.001$) (Table 1).

Looking at the histological picture of muscles (Fig. 1), LD seems lighter and more glycolytic than PM due to the higher percentage of FG (65% vs 29%), meanwhile PM shows higher oxidative capacity due to the higher percentage of SO red fibre (33% vs 15% LD, $P < 0.001$) that are grouped in clusters of 10-15 cells. The metabolic profile of muscles changes significantly in considered AGTs: 73% of FG fibres and 11% of SO type in LD of Siciliano, 62% of FG and 21% of SO in Calabrese pig; PM muscle has higher percentage of FOG fibres and less FG (20%) in CS and the same FOG and SO percentage in Sic (36 and 37%, respectively). Recently, Ruusunen and Puolanne (1997) evidenced a small difference among breeds in the fibres type composition and substantial variations within breed.

Table 1. Mean value (m) and variation coefficient (v.c.) of cross sectional area (μm^2) of muscle fibre types[†]

Autochthonous genetic type	Fibre type					
	FG		FOG		SO	
	m	v.c. (%)	m	v.c. (%)	m	v.c. (%)
<i>Longissimus dorsi</i>						
Calabrese	7477 ^A	40	5372	37	5851	31
Cinta casaldianni	4163 ^{AB}	34	3126 ^A	36	7910	31
Cinta senese	5371 ^{AC}	36	3180 ^B	38	6495	42
Siciliano	7409 ^{BC}	36	7680 ^{AB}	32	7581	35
All	6109	46	4678	43	6657	36
<i>Psoas maior</i>						
Calabrese	3682 ^A	51	2430 ^a	39	3492 ^A	36
Cinta Casaldianni	2562 ^{Aa}	48	2346	41	4787 ^{ABC}	28
Cinta Senese	3075	34	2015 ^a	41	3598 ^B	40
Siciliano	3418 ^a	45	2359	43	3620 ^C	42
All	3184	49	2288	42	3874	39

[†]Same letter in the column for each muscle means $P < 0.05$ if small and $P < 0.01$ if capital

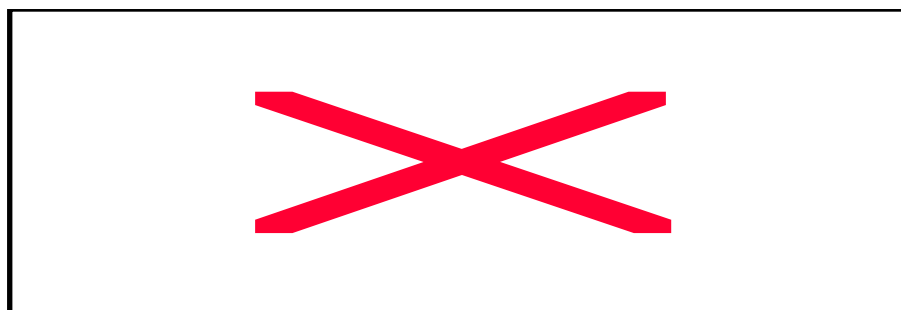


Fig. 1. Percentage of three fibre types of *Longissimus dorsi* and *Psoas maior* muscles in considered AGTs.

There are no differences between males and females regarding fibre type size; the only exception is represented by SO fibre which is significantly larger in *Longissimus dorsi* from castrated males ($P < 0.05$).

Regarding the adipocyte size of three considered regions (Table 2), the value rises from shoulder to dorsal region ($P < 0.05$) to loin ($P < 0.001$). No differences between Cinta Casaldianni and Siciliano were found for shoulder and dorsal region adipocytes as well as between shoulder and loin cells from Cinta senese pig. Moreover, according to results by Fortin (1986), Geri *et al.* (1986) and Zappa *et al.* (1992) on different breeds, adipocytes from inner layer are larger than the outer layer for any considered regions.

Table 2. Mean value (μm^2) and variation coefficient (%) of adipocytes area for region and location

Region	Autochthonous genetic type									
	Cal		CC		CiS		Sic		All	
	m	v.c. (%)	m	v.c. (%)	m	v.c. (%)	m	v.c. (%)	m	v.c. (%)
<i>Inner layer, I</i>										
Shoulder	8561	38	14287	31	13304	49	14975	38	12782	44
Dorsal region	11266	43	15270	38	15959	42	16824	38	14830	37
Loin	12511	30	17933	31	14739	42	17384	32	15870	46
<i>Outer layer, O</i>										
Shoulder	9272	41	11536	34	12076	42	11948	34	11208	39
Dorsal region	8460	38	10022	31	12485	69	10297	37	10079	57
Loin	10597	34	10159	41	11044	43	13123	39	11231	41
<i>I + O</i>										
Shoulder	8916	40	12911	34	12690	46	13462	38	11995	41
Dorsal region	9863	44	12263	41	14222	56	12689	45	12391	48
Loin	11554	34	14137	47	12892	58	15461	39	13556	43

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