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Muscle fibre types in Iberian pigs: influence of crossbreeding with Duroc breed and rearing conditions

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Abstract — The effect of rearing conditions (outdoors on pasture vs indoors on concentrated feed) and crossbreeding (pure Iberian vs 50 % Iberian × Duroc-Jersey) on fibre type proportions and diameters of muscles *Biceps femoris* (BF), *Semimembranosus* (SM) and *Tibialis cranealis* (TC) was studied. The major fibre types identified were type I, IIA, and IIB. The proportion of oxidative fibres (type I and IIA) found was higher than the one described in the literature for selected pig breeds. Type IIB fibres showed the greatest diameters ($P < 0.05$). The muscle was the factor with a higher influence on fibre proportions, the TC showing the higher proportion of type I and IIA fibres ($P < 0.001$). This was most likely due to the anatomical location and physical function of the studied muscles in the live animal. Rearing the animals outdoors produced a slight increase in the percentage of type I fibres, although it was not significant in any of the studied muscles ($P > 0.05$). The crossbreed only influenced type IIA and IIB fibres diameter in the SM. (© Elsevier / Inra).

muscle fibre types / crossbreeding / rearing conditions / Iberian pigs

Résumé — Influence de la race et du mode d´élevage sur les types de fibres musculaires chez le porc Ibérique. L'effet des conditions d´élevage (extérieur au pâturage vs intérieur avec parcours et aliment concentré) et du type génétique (porcs Ibériques purs vs Ibériques × Duroc-Jersey) sur le type métabolique et contractile et le diamètre des myofibres a été étudié dans les muscles *Biceps femoris* (BF) *Semimembranosus* (SM) et *Tibialis cranealis* (TC). Les fibres étaient en majorité des

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types I, IIA et IIB. La proportion de fibres oxydatives (types I et IIA) était plus élevée que celles décrites dans la littérature pour des porcs de races améliorées. Les fibres IIB avaient les plus grands diamètres ($p < 0.05$). Le muscle était le facteur de variation le plus important pour la distribution des types de fibres, le TC étant le plus riche en fibres I et IIA ($p < 0,001$). Ceci était probablement lié à la localisation anatomique et à la fonction des muscles étudiés. L´élevage au pâturage a entrainé une augmentation du pourcentage de fibres de type I bien que cela ne soit pas significatif ($p > 0,05$). Le type génétique n'a affecté que le diamètre des fibres IIA y IIB dans le SM. (© Elsevier / Inra).

types de fibres musculaires / race / élevage / porcs Ibériques

1. INTRODUCTION

The Iberian pig is an autochthonous breed traditionally developed in the south-west of Spain. Contrary to selected breeds, pigs from the Iberian breed have not been subjected to any genetic selection to enhance meat production. They are adapted to the extreme conditions of the habitat where they are usually reared. However, due to the great increase in the sales of the derived dry-cured meat products from such breed in the present decade, the traditional production system has been modified considerably to increase production and profitability, the main strategies followed being indoor rearing and crossbreeding with the Duroc-Jersey breed. All these changes in the production conditions have brought out a decrease in the quality of the dry-cured meat products, which has been attributed to variations in the fatty acid composition [3, 4, 27] and muscle content of antioxidants [4]. However, the possible effects of the changes in rearing conditions or crossbreed on muscle fibre types and the subsequent influence on meat quality has not been studied yet in Iberian pig.

Muscle fibre composition appears to have a very important role in meat quality [12, 15]. In this sense, a higher lipid content in glycolytic rather than in oxidative muscles has been remarkable in several researches [11], although this relationship has not been observed in other works [2, 17]. Moreover, some authors have found a direct relationship between the content of different fibre types in the muscle and meat tenderness [8], flavour [22, 35], and colour [18]. Among all the factors that influence the proportions and characteristics of muscle fibres, physical activity is perhaps the most important [22]. Aerobic physical exercise is known to increase the oxidative capacity of skeletal muscles in selected breeds of pigs [25]. On the other hand, crossbreeding can also significantly affect the proportion of each muscle fibre type [35]. Therefore, the new trends in the management system of Iberian pigs, changing from rearing outdoors the pure Iberian breed to rearing indoors an Iberian x Duroc crossbreed, could influence the proportion of muscle fibres types, and hence, meat quality.

The objective of the present experiment was to study the muscle fibre proportions and diameters in muscles *Biceps femoris, Semimembranosus*, and *Tibialis cranealis* of Iberian pigs, and to determine in which extent crossbreeding and rearing conditions are susceptible to affect them.

2. MATERIALS AND METHODS

2.1. Animals

This study was carried out with 20 Iberian (Ib) and 14 Iberian × Duroc 50 % (Ib × D) pigs weighting 90 ± 5 kg. Half the animals of each group were raised outdoors in a 30 Ha extension land during the fattening period, the exclusive feed source being acorn and pasture (OUT). The other half was raised indoors in a 6 Ha extension land and were fed on a concentrate feeding (IN). All the animals were slaughtered at about 140 ± 10 kg.

2.2. Sampling

Pigs were slaughtered by electrical stunning and exsanguination at a local abattoir, and sampling was carried out within the first following hour. Samples were taken from the outer areas of *B. femoris* (BF), *Semimembranosus* (SM), and *T. cranealis* (TC). The samples were blocks of about 0.5 cm height and width that were immediately frozen by dipping into isopentane (98 %, Panreac, Barcelona) and cooled with liquid nitrogen. Samples were stored at –80 °C until they were further sectioned.

2.3. Histochemistry

Serial cross-sections of 10 μm thick were cut using a microtome Leica CM1900 cooled at –20 °C. These muscle sections were then photographed and fibres were characterized as type I, IIA, IIB, oxidative IIB and IIC by myofibrillar ATP-ase activity staining after both acid and alkaline preincubation [5, 31], and by NADH-tetrazoliumreductase (NADH-TR) and Menadione-linked α-Glycerophosphate Dehydrogenase (M-GPDH) staining [5]. A computerised image analysis was performed using a semi-automated software system (VIDS IV of Analytical Measurement Systems. Cambridge. UK) adapted to a Flexscan 9065-S personal computer, to examine each section, which included about 250 fibres, in order to calculate the proportion (%) and cross-sectional diameter (μm) of each fibre type.

2.4. Statistics

The effect of muscle (BF, SM, or TC) was analysed by a one-way analysis of variance (ANOVA), whereas the effects and interaction of crossbreeding (pure Iberian or Iberian × Duroc 50 %) and rearing system (indoors or outdoors) within each muscle were analysed by a two-way ANOVA. In case the muscle effect or the interaction between crossbreeding and rearing system was significant, the Tukey's test was used at the 5 % level to make pairwise comparisons between sample means. All analyses were carried out using the GLM procedure [33].

3. RESULTS AND DISCUSSION

3.1. Histochemical composition

Main muscle fibres identified were type I, IIA, and IIB. Nevertheless, other fibres were identified as type IIC and type oxidative IIB. These muscle fibre types agree with previous studies in Iberian pigs [20, 21] and also in other pig breeds [10, 16].

As shown in *table I*, type I fibres exhibited an acid-stable myosin ATPase reaction, a high oxidative activity and a low glycolytic reaction. These fibres behave as slow twitch and fatigue-resistant fibres [19, 20, 28].

Table I. Intensity of staining reactions of fibre types identified in muscles *Biceps femoris, Semimembranosus* and *Tibialis cranealis*.

Fibre types	Myosin ATP-ase activity pHs							NADH-TR	M-GPDH
	4.3	4.55	4.6	10.2	10.3	10.4	10.5		
I	++++	++++	++++	–	–	–	–	++++	–
IIA	–	–/+	–/+	++++	+++	++	+	++++/++	+/–
IIB	–	++	++	++++	+++	+++	+++	–/+	+++
IIBox	–	++	++	++++	+++	+++	+++	++	–/+
IIC	+/++	+++	+++	+++	+++	+++	+++	+++	–

– Low staining reaction; ++ moderate staining reaction; +++/++++ high staining reaction.

Type IIA fibres showed an acid-labile myosin ATPase reaction and stained high at alkaline pHs. They exhibited a high oxidative metabolism and a moderate glycolytic activity. These fibres behaved as fast twitch and their resistance to fatigue has not been clarified yet.

Type IIB fibres stained high at alkaline pHs and it was difficult to distinguish them from type IIA fibres in this staining. However, contrary to these ones, type IIB fibres showed a high glycolytic and a low oxidative activity. They behaved as fast twitch [6] and non resistant to fatigue.

Type oxidative IIB fibres (IIBox) present a similar histochemical behaviour to type IIB, except for the moderate oxidative activity shown by the former.

Type IIC fibres were eventually identified as alkaline-stable and slightly acid-stable showing a high oxidative and a low glycolytic metabolism.

Regarding the sizes of the different muscle fibre types, type IIB fibres showed the greatest cross-sectional diameter, which agrees with previous works [36].

3.2. Intermuscular variability

As *table II* shows, frequency and cross-sectional diameter of each fibre type, exhibited a high variability among the studied muscles.

TC showed a predominant oxidative metabolism, as it can be deduced by the high proportions of type I, IIA, and IIBox fibres (30, 19, and 13 % respectively), meanwhile type IIB fibres were 38 % of total fibres (*table II*). On the other hand, BF and SM showed a high percentage of type IIB fibres (48 and 56.2 % respectively). This is in agreement with what has been found in these muscles in most species studied, as cat and Guinea pigs [1], cattle [23], and also in other pig breeds [11].

Such a different fibre composition of the studied muscles can be attributed to their contrasting location and function at the hindlimb, since many authors have proved that fibres frequency remarkably vary among muscles according to their functional characteristics [1, 26]. TC, which is distally located at the hindlimb, exhibits an impor-

Table II. Means and standard errors of fibre proportions and diameters on *Biceps femoris* (BF), *Semimembranosus* (SM) and *Tibialis cranealis* (TC).

Proportions (%)	BF	SM	TC	P
Type I	$19.6 \pm 2.0b^2$	$18.5 \pm 0.9b^2$	$29.3 \pm 1.9a^2$	***
Type IIA	$14.0 \pm 1.2b^2$	$10.9 \pm 0.9b^3$	$19.3 \pm 1.9a^3$	***
Type IIB	$48.0 \pm 2.4a^1$	$56.2 \pm 1.4a^1$	$37.5 \pm 1.8b^1$	***
Type IIC	1.0 ± 1.0^3	2.6 ± 2.0^4	1.0 ± 0.4^5	ns
Type IIBox	$17.3 \pm 1.1a^2$	$14.0 \pm 1.2ab^{23}$	$12.8 \pm 1.4b^4$	*
(% I + % IIA) / % IIB	$0.9 \pm 0.1b$	$0.6 \pm 0.0b$	$1.4 \pm 0.2a$	***
Diameter (µm)				
Type I	$46.6 \pm 1.2a^2$	$54.4 \pm 1.4b^2$	$40.7 \pm 0.9c^2$	***
Type IIA	$40.6 \pm 1.4a^3$	$47.8 \pm 1.3b^3$	$38.6 \pm 1.0a^2$	***
Type IIB	58.6 ± 1.3^1	61.0 ± 1.4^1	45.6 ± 1.3^1	ns
Type IIC	41.4 ± 3.0^2	48.5 ± 4.5^{23}	32.9 ± 1.8^3	ns
Type IIBox	$45.6 \pm 1.0a^2$	$47.6 \pm 1.4a^3$	$39.9 \pm 1.0b^2$	***

BF: *Biceps femoris*, SM: *Semimembranosus*, TC: *Tibialis cranealis*. Means within a row with different letters were different ($P < 0.05$). Means within a column with different superscripts were different ($P < 0.05$). ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

tant postural function (dorsiflexion of the ankle) and it bears the contractions of proximal muscles. Hence, the required characteristic is the resistance to fatigue, which explains the abundance of fibres with an oxidative metabolism. Other muscles that perform a constant, though not intense, physical activity, like the *Diaphragma* muscle, have also shown a predominant frequency of oxidative fibres [29]. On the other hand, BF and SM are proximally located at the hindlimb and operate as traction muscles: the SM extends the hip while the BF impulses the hindlimb when it is in contact with the ground [24]. Thus, active, fast and possibly severe muscle contractions are necessary to different degrees [13], and hence, type IIB fibres are the major ones in these muscles.

3.3. Breed and rearing conditions

The effects of breed and rearing conditions on the percentage of fibre types and cross-sectional diameters of muscle BF, SM, and TC are shown in *tables III, IV*, and *V*, respectively. Such effects were greatly variable among muscles, which support the opinion of many authors that the different function of the muscle determines to a large extent the fibre composition [7]. However, the kind of physical activity performed by a muscle depends, to a certain extent, on rearing conditions, and therefore muscles will be affected to different degrees.

In our work, breed did not appear to influence fibre size, except for SM, where significant differences in diameter between type IIA and IIB fibres were observed (Table IV). The scarce influence of breed on both size and proportion of fibres could be attributed to the high similarity between the studied breeds (Iberian and Iberian × Duroc 50 %), and to the fact that Duroc Jersey breed is not highly selected for muscularity. Pig breeds with a high muscularity would show a higher proportion of type IIB fibres [36] and larger fibre diameters. Several researchers have reported an effect of breed on fibre composition in wild and

Table III. Effects of genetic type and rearing conditions on proportions and diameters of muscle fibre types in the *Biceps femoris* muscle.

	Ib		Ib × D		Statistical significance		
Proportions (%)	OUT	IN	OUT	IN	Breed	Rearing	B × R
Type I	19.2 ± 3.9[2]	14.9 ± 2.3	27.5 ± 5.0	17.4 ± 3.3	ns	ns	ns
Type IIA	11.8 ± 1.7[2]	13.9 ± 1.9	13.4 ± 2.5	17.7 ± 4.3	ns	ns	ns
Type IIB	50.3 ± 4.5[1]	49.2 ± 3.3	45.5 ± 7.2	46.3 ± 4.4	ns	ns	ns
Type IIC	1.9 ± 0.5[3]	1.3 ± 0.8	0.3 ± 0.1	0.5 ± 0.3	ns	ns	ns
Type IIBox	16.9 ± 1.1b[2]	21.0 ± 2.6a	13.4 ± 1.7c	17.6 ± 2.1b	ns	*	ns
(% I + % IIA) / % IIB	0.8 ± 0.3	0.7 ± 0.1	1.00 ± 0.3	0.8 ± 0.2	ns	ns	ns
Diameter (μm)							
Type I	41.0 ± 2.1c[2]	50.3 ± 2.4a	46.5 ± 1.2b	49.0 ± 2.0b	ns	**	ns
Type IIA	35.6 ± 1.5[2]	40.6 ± 2.2	44.9 ± 4.9	42.4 ± 1.3	ns	ns	ns
Type IIB	54.5 ± 1.8[1]	61.6 ± 2.9	59.1 ± 2.6	59.6 ± 2.0	ns	ns	ns
Type IIC	38.2 ± 2.5[2]	44.0 ± 3.4	41.2 ± 14.9	48.6 ± 2.6	ns	ns	ns
Type IIBox	41.8 ± 1.7[2]	48.0 ± 1.7	46.3 ± 1.7	46.4 ± 2.1	ns	ns	ns

Ib: Iberian, D: Duroc, OUT: outdoor rearing system, IN: indoor rearing system. Means within a row with different letters were different ($P < 0.05$). Means within a column, either in proportions or diameters, with different superscripts were different ($P < 0.05$). **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

Table IV. Effects of genetic type and rearing conditions on proportions and diameters of muscle fibre types in the *Semimembranosus* muscle.

	Ib		Ib × D		Statistical significance		
Proportions (%)	OUT	IN	OUT	IN	Breed	Rearing	B × R
Type I	19.1 ± 1.5^{1}	19.0 ± 1.7	18.0 ± 2.3	17.0 ± 2.0	ns	ns	ns
Type IIA	10.2 ± 1.5^{23}	9.2 ± 1.8	14.3 ± 1.7	10.8 ± 0.8	ns	ns	ns
Type IIB	56.8 ± 2.8^{12}	55.6 ± 2.0	52.9 ± 3.8	59.2 ± 3.8	ns	ns	ns
Type IIC	0.3 ± 0.2^{4}	1.2 ± 0.3	1.7 ± 0.8	0.2 ± 0.2	ns	ns	ns
Type IIBox	13.6 ± 1.6^{34}	16.1 ± 2.6	13.2 ± 4.1	13.1 ± 2.0	ns	ns	ns
(% I + % IIA) / % IIB	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	ns	ns	ns
Diameter (μm)							
Type I	52.1 ± 1.5^{12}	52.2 ± 2.1	57.3 ± 4.0	58.2 ± 5.1	ns	ns	ns
Type IIA	42.6 ± 2.2b^{12}	48.2 ± 1.9b	50.5 ± 2.5a	53.2 ± 1.7a	*	ns	ns
Type IIB	57.7 ± 2.3b^{1}	58.3 ± 1.8b	66.1 ± 2.6a	65.2 ± 4.5a	**	ns	ns
Type IIC	36.0 ± 2.5^{2}	48.2 ± 1.8	60.0 ± 7.6	39.9 ± 3.6	ns	ns	ns
Type IIBox	45.6 ± 2.0^{12}	47.4 ± 1.9	46.9 ± 2.2	52.5 ± 6.1	ns	ns	ns

Ib: Iberian, D: Duroc, OUT: outdoor rearing system, IN: indoor rearing system. Means within a row with different letters were different ($P < 0.05$). Means within a column, either in proportions or diameters, with different superscripts were different ($P < 0.05$). **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

Table V. Effects of genetic type and rearing conditions on proportions and diameters of muscle fibre types in the *Tibialis cranealis* muscle.

	Ib		Ib × D		Statistical significance		
Proportions (%)	OUT	IN	OUT	IN	Breed	Rearing	B × R
Type I	33.4 ± 3.9^{1}	28.6 ± 2.9	29.9 ± 2.4	22.4 ± 4.8	ns	ns	ns
Type IIA	21.1 ± 3.3^{3}	13.3 ± 3.3	22.8 ± 3.5	22.25 ± 4.3	ns	ns	ns
Type IIB	32.1 ± 3.2b^{2}	41.3 ± 2.1a	35.2 ± 2.8b	43.6 ± 5.2a	ns	*	ns
Type IIC	2.3 ± 0.9a^{4}	0.0 ± 0.0b	1.3 ± 0.6ab	0.1 ± 0.1b	ns	**	ns
Type IIBox	11.1 ± 2.8^{23}	16.8 ± 1.9	10.9 ± 2.0	11.7 ± 2.1	ns	ns	ns
(% I + % IIA) / % IIB	2.2 ± 0.5	1.1 ± 0.1	1.6 ± 0.2	1.2 ± 0.3	ns	*	ns
Diameter (μm)							
Type I	38.9 ± 1.2b^{1}	42.7 ± 1.2a	38.3 ± 1.6b	43.42 ± 3.8a	ns	*	ns
Type IIA	36.9 ± 1.2b^{1}	39.9 ± 2.0ab	35.8 ± 2.6b	42.62 ± 2.6a	ns	*	ns
Type IIB	44.0 ± 1.7^{1}	47.5 ± 1.9	42.9 ± 4.2	48.3 ± 3.3	ns	ns	ns
Type IIC	33.0 ± 2.7^{2}	45.1 ± 2.0	30.5 ± 2.3	32.5 ± 3.3	ns	ns	ns
Type IIBox	38.5 ± 1.3b^{1}	42.4 ± 1.8a	37.2 ± 2.7b	41.6 ± 1.2a	ns	*	ns

Ib: Iberian, D: Duroc, OUT: outdoor rearing system, IN: indoor rearing system. Means within a row with different letters were different ($P < 0.05$). Means within a column, either in proportions or diameters, with different superscripts were different ($P < 0.05$). **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

domestic pigs [37] or in other species as bovine [30].

As long as the effect of rearing conditions is concerned, and with regards to fibre size, it seems to be more evident than crossbreeding, though it was heterogeneous too. Concerning the BF (*table III*), the largest cross-sectional diameter of type I fibres was remarkable in pigs fattened indoors and TC from this group of pigs also had a higher diameter in type I, IIA, and IIBox fibres (*table V*). This could be due to the high protein content in standard concentrates, whose effect has already been verified by Staun [34] in other pig breeds. This author also observed that a diet with a high fat content brought out a decrease in fibre size, which could explain the shorter diameter of fibres in pigs reared outdoors and fed on acorn, since fat content in such feed source is higher than in concentrates, as has been already shown [3, 27].

Concerning the proportions of muscle fibre types, it is remarkable that in all the studied muscles, animals reared outdoors tended to have a higher percentage of type I fibres than those reared indoors, although it was not significant. Animals reared outdoors also showed the higher rate between oxidative fibres (type I plus IIA) and glycolytic ones (IIB) in all the muscle studied (*tables III, IV* and *V*), but only to a significant extent in the TC. In our work, the variable rearing conditions involved both feeding and physical activity, though the latter was probably the one with the highest effect on muscle fibre proportions. Free-ranged pigs had to walk all around the area where they were kept to achieve the acorns, meanwhile pigs reared indoors did not have to perform any physical activity to take the feeding. However, both the short time the pigs were fattened (45 days) and the not very large area where they were reared outdoors, could explain the small differences between both rearing systems, although the trend points out a positive effect of rearing the pig outdoors on the proportion of oxidative muscle fibres.

There are different results regarding the effect of the exercise on the muscle fibre composition in the scientific literature, depending on the type, duration and frequency of the physical activity to which the animals are submitted. However, it is generally accepted that endurance exercise increase the oxidative capability of the muscle [25, 35], the harder and the longer the training, the clearer the effect on muscle fibres [9]. This agrees with the results found in the present study, in which the moderate exercise performed by the pigs reared outdoors slightly increased the proportion of oxidative fibres. Moreover, our findings reflect a real situation, where the pigs are not submitted to an extra exercise, but reared in the usual conditions used in the Iberian pig management. The higher percentage of type IIC fibres in the TC is remarkable in pigs raised outdoors. This could be related to the physical activity performed by these animals during the fattening phase, since some authors have verified an increase in percentage of type IIC fibres due to a higher muscular activity in different species, such as the rat [32] or man [14].

4. CONCLUSIONS

The different location and function of muscles have a greater influence on muscle fibres proportions of Iberian pigs than the rearing system during fattening or crossbreeding with the Duroc breed. Nevertheless, rearing the animals outdoors increases the proportion of oxidative fibres, and hence, it could have consequences on meat quality.

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