CHARACTERISATION OF PIEMONTESE CATTLE: HISTOCHEMICAL AND MORPHOMETRIC CHARACTERISTICS OF MUSCLE FIBRES

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Background

Muscular fibres could be classified according to structure, acting and metabolic characteristics as αW (white), ßR (red) and αR (intermediate) fibres (Ashmore and Doer, 1971). In each muscle all these three kinds of fibres can be observed, even if transformation from one to another type could occurred due to growth, exercise and so on. Moreover in each species fibres distribution and size seem be influenced by breed (Guenther, 1977; Bartlett *et al.*, 1979; Solomon *et al.*, 1981) so as by muscle type (Moody and Cassens, 1968; Beecher *et al.*, 1970; Hunt and Hendriks, 1977), and by muscular hypertrophy (Hendricks *et al.*, 1973; West, 1974). Muscular fibre classification is important not only in physiological studies, but also in determining the meat quality obtained from the skeletal muscles. In fact, the white fibres (αW) are larger than the red ones (both α and ß), and the red fibres metabolise and store more lipid than the white ones (Ashmore *et al.*, 1972), so important meat characteristics could be influenced also by the muscle fibre type (Ashmore, 1974, as recently investigated (Morita *et al.*, 2000; Ozawa *et al.*, 2000; Vestergaard *et al.*, 2000a, 2000b).

Objectives

Few data are available on characteristics of muscular fibres of Piemontese cattle, mostly on crossbred (Barone *et al.*, 1990; Tatum *et al.*, 1990) or morphological characteristics (Lazzaroni *et al.*, 1994), whereas the quality of meat obtained from such animals is well known so this breed is more and more used both as pure-bred and crossbred to obtain high quality meat, tender and lean. This trial aims to characterise the muscular fibres in double muscled pure-bred Piemontese young bulls, improving the knowledge on this breed and studying the effect of muscle on their cytochemical and morphological characteristics, as distribution and measures.

Methods

The trial has been carried out on *Triceps brachii* and *Semitendinosus* muscles of 6 hypertrophied young bulls of Piemontese breed, reared in the same environmental conditions and slaughtered at 16-18 months of age and 550-600 kg of live weight. The muscles were chosen for the similar and high hypertrophy ratio (Hanset and Ansay, 1972). Within 1 h from slaughter samples of about 2 cm³ have been picked up and frozen in liquid nitrogen. Consequently from each sample serial cross sections, 10 µm thick, have been obtained by a freezing microtome and stained following the adenosine-5-triphosphate method (Brooke and Kaiser, 1970) for microspopic examination. This method, changing the pH of the pre-incubation ground, allows differentiating the 3 types of muscular fibres. At alkaline incubation (pH = 9,4) the ßR fibres appear light whereas the αW dark. At acid incubation (pH = 4,3) the opposite result is obtained: the ßR fibres are dark and the αW fibres are light. To recognise the αR fibres the pre-incubation at pH 4,5 has to be used. With a comparison among different serial cross sections, pre-incubated at different pH, is possible to recognise the kind of the fibres. On prepared slides (4 for each muscle of each subject) morphological characteristics of each muscular fibre have been measured using a video image analysis system for optic microscope (METI, by Pertel, Torino, Italy: microscope mod. RME 5, objective A 10/0.25). On each sample serial sections pre-incubated at different pH have been compared. Afterwards each fibre has been measured, determining its area, perimeter, equivalent diameter, maximum and minimum axis. For each slide 4-5 fields have been observed, and on each of them 12-20 fibres have been measured. Data have been studied by ANOVA (Stat-Itcf, 1988) according to the model: $y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$ (where μ = general mean; α_i = muscle effect; β_j = fibre effect; $(\alpha\beta)_{ij}$ = muscle - fibre interaction; ε_{ijk} = subject or replication effect), and means compared by Newman-Keuls's test.

Results and discussion

Number, percentage and morphometric characteristics (mean ± s.d.) of different type of fibre cells in muscle *Triceps brachii* and m. *Semitendinosus* of Piemontese young bulls are reported in table 1.
The αW fibres always show higher values (P<0.001) for the considered parameters (number, percentage, area, perimeter, equivalent diameter, maximum and minimum axis), than the αR and ßR fibres (according with Ashmore *et al.*, 1972), while no differences were found between the two studied muscles (in contrast with the previous assertion by Moody and Cassens, 1968; Beecher *et al.*, 1970; Hunt and Hendriks, 1977). However, considering the interaction between muscle and fibre type, significant differences (P<0.01) were found in the morphometric characteristics (area, perimeter, equivalent diameter, maximum and minimum axis), where the αW fibres of *Semitendinosus* muscle show always the highest values while the αR and ßR fibres of the same muscle show the lowest ones, and all fibre type of *Triceps brachii* muscle show intermediate measures. The high number of αW fibres found in this trial is in agreement with Tatum *et al.* (1990), and it seems characterise the Piemontese breed, even if the distribution of the other kind of fibres (αR and ßR) was different. Data on morphometric characteristics are comparable only with our previous data (Lazzaroni *et al.*, 1994) but not with other literature data for the different methods used to prepare and stain samples, that have a big influence on final fibres dimensions (Barone *et al.*, 1990).
As conclusion, in the two studied muscles there is an higher percentage of white fibres, probably due to their high hypertrophy ratio, and αW fibres have also bigger dimensions than the red ones (αR and ßR), similar in number and size. Moreover, the size differences found in αW fibres of *Semitendinosus* and *Triceps brachii* muscles suggest to study in depth the cytochemical and morphometric characteristics of Piemontese cattle muscles, studying also the correlation among them and meat quality parameters.

Pertinent literature

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Table 1. Number, percentage and morphometric characteristics of different histochemical type of fibre cells in two muscles of Piemontese young bulls (mean ± s.d.)

measurement	fibre type	*Triceps brachii* m.	*Semitendinosus* m.	mean
number (n)	αW	39.17±5.15	41.33±7.53	40.25±6.15 [A]
	αR	15.00±2.53	15.50±7.12	15.25±5.09 [B]
	βR	9.67±2.42	10.67±3.08	10.17±2.64 [C]
	mean	21.28±3.38	22.50±5.86	21.89
percentage (%)	αW	61.29±4.52	61.75±6.77	61.52±5.49 [A]
	αR	23.62±4.19	22.52±6.72	23.07±5.34 [B]
	βR	15.09±3.31	15.73±2.67	15.41±2.87 [C]
	mean	33.33±3.80	33.33±5.37	33.33
area (μm²)	αW	3062.87±365.20 [b]	4315.95±1110.82 [a]	3689.41±788.35 [A]
	αR	2689.25±405.41 [b]	2149.45±436.35 [b]	2419.35±401.56 [B]
	βR	2463.76±370.68 [b]	2160.72±473.86 [b]	2312.24±405.61 [B]
	mean	2738.63±357.74	2875.37±696.39	2807.00
perimeter (μm)	αW	243.13±14.21 [b]	281.48±34.69 [a]	262.31±25.27 [A]
	αR	218.11±15.01 [bc]	197.19±18.16 [c]	207.65±15.89 [B]
	βR	210.92±18.13 [c]	199.92±20.78 [c]	205.42±18.59 [B]
	mean	224.05±14.91	226.20±24.04	225.13
equivalent diameter (μm)	αW	61.91±3.90 [b]	73.02±9.44 [a]	67.46±6.89 [A]
	αR	58.08±4.46 [bc]	51.47±5.31 [c]	54.77±4.68 [B]
	βR	55.65±4.29 [bc]	51.29±5.11 [c]	53.47±4.50 [B]
	mean	58.55±3.96	58.59±6.50	58.57
maximum axis (μm)	αW	77.85±4.71 [b]	91.70±10.78 [a]	84.78±7.93 [A]
	αR	70.77±6.33 [bc]	63.95±6.45 [c]	67.36±6.10 [B]
	βR	67.46±6.22 [c]	64.89±5.63 [c]	66.17±5.66 [B]
	mean	72.03±5.45	73.51±7.47	72.77
minimum axis (μm)	αW	54.45±3.42 [b]	63.60±8.75 [a]	59.03±6.33 [A]
	αR	51.02±3.62 [bc]	45.06±5.20 [c]	48.04±4.27 [B]
	βR	50.06±2.61 [bc]	46.36±5.48 [bc]	48.21±4.09 [B]
	mean	51.85±7.91	51.67±6.27	51.76

Different superscripts, on the same column and for the same muscle, stand for significant differences: a, b, c: $P<0.01$, A, B, C: $P<0.001$.