Study on the physiologic histochemical characteristics of lamb muscles

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INTRODUCTION

Histologic and histochemical studies contribute, with useful information, in understanding muscle growth and biological parameters influencing meat quality. Particularly when for these studies morphometric criteria are used. The most potentially useful parameter in this respect is histochemical muscle fiber typing and muscle fiber diameter measurements. In the following muscle fiber diameter measurements in relation to histochemical muscle fiber typing in lambs are presented.

MATERIALS AND METHODS

The study is concerned with 10 lambs of Serron race born in the Aristotelian University Farm. The animals were growing under identical management, housing, and feeding regimes. All animals were slaughtered under the same conditions in the University slaughterhouse at the age of 126.85±4.28 days. Their L.W. after a 24 hour fasting was 15.45±0.39, and percent yield was 50.11±0.48. Within 19.40±0.92 to 29.53±0.79 minutes from slaughtering, muscle tissue pieces weighting approximately 5g were taken from muscles Longisimmus dorsi, Biceps branchii, Semitendinosus and Semimembranosus. The samples were immediately snap frozen and preserved in -50^{0} C for a few days until all material was collected. Cryostat sections were prepared from all samples and stained for the demonstration of succinic dehydrogenase and ATPase activity. Muscle fiber diameters defined as the "maximum diameter across the lesser aspect of the muscle fiber" (Brook, 1970) were measured in about 100 muscle fibers of standardised magnification photographs. Other samples were taken at the same time, fixed in 10% formalin, embedded in paraffin wax and routinely stained in H&E. Again, in all preparations, muscle fiber diameters measured as it is described above. At the time of sampling pH measurements were also taking place using a portable pH meter.

Mean values for each muscle fiber type and muscle of each lamb were calculated for both of the enzymes histochemically demonstrated. Percentages for different muscle fiber types within each muscle of each lamb, were also calculated. Finally, mean values, standard deviations and standard errors were found for all measurements of each muscle for all animals examined. The same muscle diameter measurements and statistical procedures were applied in histologic sections stained in H&E. One way analysis of variance and the Duncan-Kramer test were used for comparing and evaluating statistically significant differences of muscle fiber diameter measurements and percentages of different muscle fiber histochemical types within and between muscles.

RESULTS

In table I mean values for muscle fiber diameters and percentage for different histochemical muscle fiber types demonstrated by the activity of ATPase are shown. In table II measurements for the same parameters after staining for demonstrating SDH activity are presented. Table III shows mean values for muscle fiber diameter measurements in histologic sections stained in H&E.

TABLE I

Mean values for muscle fiber diameter measurements (in μm) and percentage for muscle fiber histochemical types after staining for the demonstration of ATPase activity.

Muscles	Mean values			Muscle fiber type percentages		
	I	Intermediate	II	I	Intermediate	II
Longissimus dorsi	29,26	29,44	30,20	14,68	35,20	50,12
Biceps brachii	30,62	28,22	27,69	21,65	31,99	46,36
Semitendinosus	33,77	32,06	33,26	14.10	39,67	46,23
Semimembranosus	27,66	30,95	33,63	26,20	28,80	45,00

TABLE II

Mean values for muscle fiber diameter measurements (in μm) and percentage for muscle fiber histochemical types after staining for the demonstration of SDH activity.

Muscles	Mean values			Muscle fiber type percentages		
	I	Intermediate	II	I	Intermediate	II
Longissimus dorsi	25,81	27,99	29,37	25,64	41,54	27,98
Biceps brachii	30,92	31,58	28,98	29,99	43,05	26,96
Semitendinosus	27,69	31,61	32,23	33,17	27,58	39,25
Semimembranosus	33,87	37,25	37,08	23,03	38,40	38,56

TABLE III

Mean values for muscle fiber diameter measurements in histologic sections stained in H&E.

Muscles	Mean value
Longissimus dorsi	18,35
Biceps brachii	21,77
Semitendinosus	21,57
Semimembranosus	21,52

DISCUSSION

The demonstration of ATPase and SDH activity showed that all three histochemical muscle fiber types, that is red (I), intermediate and white (II), are present in lambs at the age of about four months. According to Beecher et al (1965, 1968), for a muscle to be characterised as red, a content of 40% red fibers is required. When this percentage is below 30% the muscle is characterised as white. Percentages for red fibers in tables I and II are in most cases below 30% or very close to it. All four muscles therefore, at least for the age and the lamb race studied could be reasonably safely characterised as white.

Muscle fiber diameter size is considered as one of the most important parameters in differentiating muscle fiber histochemical types (Gauthier, 1970). One way analysis of variance, published elsewhere (Rantsios et al, 1985; Tsagarakis et al, 1986) did not reveal any statistically significant differences for muscle fiber diameter measurements, for the means shown in tables I and II, either within or between muscles. Also, no statistically significant differences were detected between means for muscle fiber diameter measurements in histologic sections stained in H&E (table III). These means are statistically significantly lower than the ones produced from measurements in sections incubated for the demonstration of enzyme activity, due to tissue shrinkage when processed for paraffin embedding. Muscle fiber size homogeneity is therefore characterising lamb muscles, again, at least at the age and in the race studied.

Considering histochemical muscle fiber type percentages there are no statistically significant differences between muscles for any of the mean of muscle fiber types showing in table I and also table II. However, per-

centages for white muscle fiber means (tables I and II) are statistically significantly elevated for Longissimus dorsi ($p<0.001$), Biceps brachii ($p<0.001$) and Semitendinosus ($p<0.05$) when sections are stained for the demonstration of APTase activity. Relevant tables of one way analysis of variance are published elsewhere (Rantsios et al, 1985, Tsagarakis et al, 1986). Again therefore, all four muscles appear with a similar distribution in values of histochemical muscle fiber type percentages. Difference in histochemical profile of muscle tissue, depending on the activity of the enzymes used for identification of muscle fibers histochemical type have been already detected (Rantsios, 1981). In the present study individual fibers, in serial sections stained for different enzyme activity, were also found to be in different histochemical fiber types. These differences were large enough, so that statistically significant differences between histochemical muscle fiber types mean percentages were demonstrated, when classification was based on ATPase activity (Table I). Such differences were not detected when muscle fiber histochemical classification was based on SDH activity.

In conclusion, it could be stated that observations based on APTase and SDH activity, percentages of histochemical muscle fiber types, and measurements of muscle fiber diameter measurements, according to muscle fiber type and in toto, strongly suggest that a remarkable homogeneity in muscle histochemical profile exists in four month lamb Longissimus dorsi, Brachii biceps, Semitendinosus and Semimembranosus muscles. These muscles are of the white muscle type.

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