FIBER CHARACTERIZATION OF MUSCLE *LONGISSIMUS THORACIS* FROM *BRUNA DELS PIRINEUS* CATTLE BREED

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Introduction

The *Bruna dels Pirineus* is a cattle breed for extensive meat production, mainly located in Pyrenean and Prepyrenean areas. The production system is semiextensive. Only few studies on the production parameters and carcass and on meat quality characteristics of the *Bruna* breed are still available (Font *et al*., 1997). On the other hand, the biochemical characteristics of the muscles have not yet been established.

The aim of this work was the fiber characterization of the muscle *Longissimus thoracis* (LT) from *Bruna dels Pirineus* cattle. The contractile and the metabolic traits of the muscle have been studied and related to carcass characteristics.

Material and Methods

Thirty-six *Bruna dels Pirineus* young bulls were randomly chosen at weaning (202 d and 250 kg of average age and weight, respectively). Animals were fed *ad libitum* a concentrated diet supplemented with hay. The diet was exclusively vegetal in origin. Animals were slaughtered in a local abattoir at an average weight of 551 kg, being 13 months the average age. Hot carcass weight, was measured. Carcass conformation and fatness were assessed using the EUROP classification: 1 = less fat or very poor conformation to 15 = very fat or very good conformation.

Muscle Sampling

Muscle samples for biochemical analyses were obtained 24-26 hours *post-mortem*. Samples for ELISA and enzyme activity analyses were taken at the 7th-rib level, from longissimus muscle core. They were frozen in liquid nitrogen and stored at -80ºC until use. For the determination of haem pigment content, samples from longissimus muscle were taken at the 6th-rib level. These samples were minced, vacuum-packed and stored at -20ºC until analysis.

Muscle Biochemical Analyses

Contractile traits

The contractile traits of the muscle were determined by enzyme-linked immunosorbent assay (ELISA). The objective of this assay was to determine the percentage of slow myosin heavy chain (MHC I) in the muscle with a specific MHC I monoclonal antibody (Picard *et al*., 1994).

Muscle sample extracts were obtained by homogenizing 200 mg of frozen muscle in 10 ml of buffer solution: 50 mM Tris, 0.5 M NaCl, 20 mM disodium pyrophosphate, 1mM EDTA and 1mM DTT (Dithiotheitol), left on ice for 10 min and centrifuged for 10 min at 2,500g (4ºC). The supernatant was then mixed with glycerol to a final concentration of 50% (v/v) and stored at -20ºC until analysis. Sample protein concentration was determined one week in advance as a maximum, prior to the ELISA assay (Bradford, 1976), using bovine serum albumin as a standard. For the assay, samples were diluted to a concentration of 2.4 μg of protein / μl and the microtiter-plate wells were filled with 50 μl each (triplicates).

A specific MHC I monoclonal antibody prepared from a human ventricle specimen was used (clone F36.5B9, 2C8, isotype Mouse IgG1, Biocytex Biotechnology) to recognize de MHC I myosin isoform in the muscle samples. A second antibody, (anti-mouse IgG Fab fragment from sheep, Boehringer Mannheim Biochemica) alkaline phosphatase conjugated was used. This provided the enzymatic reaction that enabled the detection in the variation of absorbance at 405 nm , by using 4-nitrophenylphosphate (Sigma) as substrate. The percentage of the MHC I in each sample was calculated by means of a standard curve prepared from two muscles having extreme characteristics: *Masseter* (100% MHC I) and *Cutaneus trunci* (0% MHC I). The standard curve was run in each microplate (Picard *et al*, 1994).

Metabolic traits

The metabolic traits of the muscle were determined by measuring the lactate dehydrogenase activity (LDH) according to Ansay (1974) and the isocitrate dehydrogenase activity (ICDH) according to Briand *et al*. (1981). Muscle extracts were obtained by homogenizing 200 mg of frozen muscle in 3 ml of 50 mM TEA, 140 mM sucrose, pH 7.5 buffer, and centrifuged at 6,000g for 15 min at 4ºC. The supernatants were filtered through glass wool and stored in aliquots at -20ºC for the analysis (within a week-time).

Lactate dehydrogenase activity was measured in 50 mM TEA, 5mM EDTA, pH 7.5 buffer at 28ºC, with 1.2 mM sodium pyruvate and 0.24 mM β-NADH in the reaction medium. For this assay, extracts were previously diluted 1/30 in homogenizing buffer and 50 μl were added into the reaction medium.

Isocitrate dehydrogenase activity was measured in 38.9 mM $Na_2HPO_4.2H_2O$, 0.5 mM manganese chloride tetrahydrate, 0.5% Triton X100, pH 7.3 buffer at 28ºC, with 1.38 mM trisodium isocitrate and 0.36 mM β-NADP in the reaction medium. In this assay 200 μl of undiluted extract were added to the reaction medium. Enzyme activities were expressed as μmol / min per gram of wet weight (IU, International Units).

The concentration of haem pigment was determined according to Hornsey (1956). Results are given in μg of acid haematin per g of wet weight.

Results and Discussion

Table 1 shows the mean, the standard deviation and the minimum and maximum values obtained for the carcass characteristics and for LT muscle biochemical traits. Carcass conformation and fatness scores (11.3 and 6.9 respectively) indicated good characteristics of the *Bruna* cattle for meat production. The values obtained for MHC I (23.9 %) and LDH and ICDH activities (1203.0 and 1.7 μmol

/ min g wet weight, respectively) show *Longissimus thoracis* as a predominantly fast twitch glycolytic or white muscle type, as expected according to literature (Talmant *et al.* 1986).

The correlation coefficients between some of the carcass quality and biochemical characteristics studied are presented in Table 2. There were no significant correlations between fatness, conformation and biochemical traits (results are not shown). The percentage of MHC I was positively correlated with ICDH activity (+0.39, $p<0.05$) and negatively correlated with LDH activity (-0.43, $p<0.05$), while the correlation between the two enzymatic activities was also negative (-0.48, $p<0.01$). These results were already expected and are in accordance with the studies of Ansay (1974), who observed the negative relation between glycolitic and oxidative activities in beef muscles. Similar correlation coefficient values to those found in this study have been reported by Jurie *et al.* (1995) with Limousin breed. On the other hand, the haem pigment content was positively correlated with the ICDH activity (+0.33, $p<0.05$), since it is clearly related to muscle oxidative activity (Talmant *et al.*, 1986), and it was negatively correlated with the LDH activity (-0.42, $p<0.05$). We didn't find a significant correlation between pigment concentration and MHC I percentage. ICDH activity was also negatively correlated with carcass weight (-0.45, $P<0.01$), although no significant correlation with age was observed. This could be explained by the increase in fiber area observed when increasing carcass weight (Young and Bass, 1984) which would result in a lower capillarity ratio and, therefore, in a decrease in oxidative metabolism activity in heavier animals. Haem pigment concentration was positively correlated with age (+0.41, $p<0.05$) as it is well known that pigment content increases with age (Janicki *et al.*, 1966).

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Table 1
Carcass characteristics and biochemical traits of the *Longissimus thoracis* muscle of *Bruna dels Pirineus* breed

(n = 36)	*Mean*	*S.D.*	*Minimum*	*Maximum*
Carcass characteristics				
Age (days)	391.0	34.3	339.0	453.0
Live weight (kg)	551.0	25.5	483.0	602.0
Carcass weight (kg)	337.9	17.2	303.7	376.0
Fatness score	6.9	1.2	4	8
Conformation score	11.3	0.8	10	14
Biochemical traits				
LDH	1203.0	139.1	927.2	1473.7
ICDH	1.7	0.4	1.0	2.7
MHC I (%)	23.9	6.8	13.0	40.8
Haem pigment	158.3	18.3	118.3	201.0

Fatness / Conformation scores: EUROP classification: 1 = less fat / very poor conformation to 15 = very fat / very good conformation.
LDH: lactate dehydrogenase (µmol / min g wet weight).
ICDH: isocitrate dehydrogenase (µmol / min g wet weight).
MHC I: slow myosin heavy chain isoform.
Haem pigment (µg acid haematin / g wet weight).

Table 2
Carcass and biochemical traits correlation coefficients

	1	2	3	4	5	6
1. Age						
2. Live weight	-0.18					
3.Carcass weight	-0.09	0.90***				
4. LDH	-0.21	0.15	0.25			
5. ICDH	-0.12	-0.29	-0.45**	-0.48**		
6. MHC I	0.11	-0.12	-0.30	-0.43*	0.39*	
7. Haem pigment	0.41*	-0.24	-0.21	-0.42*	0.33*	0.13

Significance: *, $p\leq0.05$; **, $p\leq0.01$; ***, $p\leq0.001$.
LDH: lactate dehydrogenase.
ICDH: isocitrate dehydrogenase.
MHC I: slow myosin heavy chain isoform.