The effect of breed-production systems on the myosin heavy chain 1, the biochemical characteristics and the colour variables of *Longissimus thoracis* from seven Spanish beef cattle breeds

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Abstract

The effect of breed-production system on the myosin heavy chain 1 (MHC-I), the biochemical characteristics and the colour variables of *longissimus thoracis* (LT) from seven beef breeds was studied: Asturiana de la Montaña (AM), Asturiana de los Valles (AV), Avileña-Negra Ibérica (A-NI), Bruna dels Pirineus (BP), Morucha (MO), Pirenaica (PI) and Retinta (RE) (Age at slaughter between 368 and 541 days; carcass weight between 249 and 334 kg). Significant differences between breed-production systems were found for all traits evaluated. LT from the MO, a rustic type breed, was the most oxidative (MHC-I, 39.3%; isocitrate dehydrogenase activity, 52 nmol min⁻¹ mg⁻¹; pigment content, 188.4 µg acid haematin g⁻¹) and showed a low *L** value (32.6) and high *a** and *C** values (24 and 27.2, respectively). In terms of meat colour (*L** and *a**) the canonical discriminant analysis separated the breeds into two groups, the AV, the PI and the A-NI (the lightest ones) from the AM and the MO breeds (the reddest and darkest) whereas the BP showed an intermediate position. The RE and the A-NI were distinguished from the others by their high intramuscular fat content. Meat colour was affected by the muscle biochemical traits in the breed-production systems studied. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Bovine breed; Myosin heavy chain I; Biochemical characteristics; Meat colour

1. Introduction

Beef meat consumption in Europe has been decreasing in recent years. Consumers are not only worried about meat quality, but also about its origin and how it

is produced. The use of rustic or local breeds as an alternative system of beef production has the advantage that these breeds are closely related to the environment and help to maintain biodiversity in agricultural production, especially in depressed areas.

Colour and tenderness are the two most important attributes to ensure consumer acceptance of beef (George, Tatum, Belk, & Smith, 1999; Issanchou, 1996). Both properties are influenced by the muscle biochemical and

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contractile characteristics together with external factors such as pre and post-slaughter treatments (Insausti, Beriain, Purroy, Albertí, Lizaso, & Hernández, 1999; MacDougall, 1982; Valin & Ouali, 1992) and the rearing-feeding regime (Vestergaard, Oksbjerg, & Henckel, 2000).

Histochemically classified type I fibres correspond to slow-twitch high-oxidative (SO or β -red) fibres and they express the myosin heavy chain 1 (MHC-I) isoform (Termin, Staron, & Pette, 1989). In most species, including cattle, only one slow MHC is observed in the adult animal and this isoform is a useful marker for type I fibres (Picard, Leger, & Robelin, 1994). To our knowledge, there are no studies available on MHC-I composition and metabolic properties [lactate dehydrogenase, LDH, and isocitrate dehydrogenase (ICDH), activities] of *longissimus thoracis* (LT) from the breeds reported here, although they have been characterized from the production point of view (Albertí, Sañudo, Santolaria, & Negueruela, 1995) and their sensorial qualities have been evaluated (Campo, Sañudo, Panea, Albertí, & Santolaria, 1999; Santolaria, Sañudo, Albertí, & Campo, 1997). These breeds have not suffered as much genetic pressure as other breeds (i.e. Charolais) and thus the biochemical characteristics of their muscles could be different.

Lately, meat quality in the EU has been assigned by trade marks associated with breeds in their own production systems. Therefore, the study of both the breed and the production system, as a means of establishing meat quality standards is of interest.

The aim of this paper was to ascertain the effect of the breed-production system on MHC-I percentage, the biochemical traits and the colour variables of LT from seven Spanish local beef cattle breeds. The relationships between production variables (age at slaughter and carcass weight), biochemical traits and meat colour measurements within and between breeds were also studied.

2. Materials and methods

2.1. Animals

This study was carried out on 478 yearling males of the following breeds: 70 animals from *Asturiana de la Montaña* (AM), 70 from *Asturiana de los Valles* (AV), 71 from *Avileña-Negra Ibérica* (A-NI), 75 from *Bruna dels Pirineus* (BP), 70 from *Morucha* (MO), 54 from *Pirenaica* (PI) and 68 from *Retinta* (RE).

The AV and the AM breeds are located in the north of Spain. The first is a double muscled breed, noted for its high muscle percentage (Franco, 1997). The AM breed is a small- to medium-sized rustic animal.

The BP — a meat type breed selected from the old Brown Swiss, similar to the American Braunvieh — and the PI are, respectively, located in the east and central-west

parts of the Pyrenees. They are medium to large as adults and could be considered as fast-growth breeds (Campo et al., 1999).

The A-NI and MO breeds are located in Western and Central Spain. The first is medium-sized and the second one is small- to medium-sized when adult. The RE breed is found in the west of Spain and is large when fully grown. All are rustic breeds (Sánchez Belda, 1981). Productive and carcass traits of all these breeds have been described by Piedrafito et al. (1999).

Since the experimental farms had limited space, the animals were evenly distributed over two consecutive years. The calves were reared in an extensive regime together with their mothers and started fattening at about 5–7 months old. They were fed a breed-specific diet fed ad libitum. The common characteristic is that both crude protein and energy levels are fairly high (crude protein between 13.8 and 16%; energy (UFC/kg), 0.9–1.04) and balanced for other components (Ca and P). The ingredients were always of vegetable origin. They included products and by-products both of cereals (wheat, barley and/or oats) and soybean. The diet was completed with straw or hay ad libitum.

The welfare of the animals was taken into account when handling them. The afternoon before slaughter, the young bulls were transported to the abattoir without mixing with animals from different batches. Pre-slaughter treatment may influence colour, so carcasses with a $\text{pH}_u > 6.0$ were not included in the study. Age at slaughter ranged from 12 to 18 months, depending on breed and animal. Carcass weight was measured immediately after slaughter.

2.2. Muscle sampling

Muscle samples for biochemical analyses were obtained at 24-h post-mortem. Samples for enzyme-linked immunosorbent assay (ELISA) and enzyme activity analyses were taken at the seventh-rib level, from the LT core. They were frozen in liquid nitrogen and stored at -80°C until analysis. Samples from all the animals over the 2 years were studied, except for PI breed in which only the samples from the first year were available; the samples from the second year were accidentally thawed during storage. Samples from LT were taken at the sixth-rib level for determination of haem pigment content and intramuscular fat (IMF). These samples were minced, vacuum-packed and stored at -20°C until analysis.

2.3. Colour measurements

Colour measurements were done at 8 days post-mortem using a Minolta CM 2002 Spectrophotometer in the CIELAB space (Lightness, L^* ; redness, a^* , yellowness, b^*) (CIE, 1976). Hue (H^*) and chroma (C^*) were

calculated from the a^* and b^* values according to Wysecki and Stiles (1967). A 3-cm thick chop located in the 7–8th ribs of the LT was cut and placed in a polystyrene tray, covered with plastic film permeable to O_2 and stored for 24 h at 4°C before measuring.

2.4. Muscle biochemical analyses

2.4.1. Contractile traits

The contractile traits of the muscle were determined by ELISA. The aim of this assay was to determine the percentage of slow 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, 1 mM EDTA and 1 mM DTT (Dithiothreitol), left on ice for 10 min and centrifuged for 10 min at 2500 g (4°C). The supernatant was then mixed with glycerol to a final concentration of 50% (v/v) and stored at –20°C until analysis. Sample protein concentration was determined less than a week before 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/ml 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 recognise the 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 standards were run in each microtiter-plate (Picard et al., 1994).

2.4.2. Metabolic traits

The metabolic traits of the muscle were determined by measuring the LDH activity according to Ansay (1974) and the ICDH activity according to Briand, Talmant, Briand, Monin, and Durand (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 6000 g for 15 min at 4°C. The supernatants were filtered through glass wool and stored in aliquots at –20°C for analysis within a week.

LDH activity was measured in 50 mM TEA, 5 mM EDTA, pH 7.5 buffer at 28°C, with 1.2 mM sodium pyruvate and 0.24 mM β-NADH in the reaction medium. For this assay the extracts had previously been

diluted 1/30 in homogenizing buffer and 50 µl were added in the reaction medium. Enzyme activities are expressed as µmol NADH min^{–1} mg of extracted protein^{–1}.

ICDH activity was measured in 38.9 mM Na₂HPO₄ 2H₂O, 0.5 mM manganese chloride, 0.5% Triton ×100, pH 7.3 buffer at 28°C, 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 are expressed as nmol NADPH min^{–1} mg of extracted protein^{–1}. The protein concentration of the extracts was determined following the method of Bradford (1976).

The concentration of haem pigment was determined according to Hornsey (1956). Results are given in µg of acid haematin g of muscle wet weight^{–1}. IMF percentages were determined according to the ISO 1443 method.

2.5. Statistical analysis

A least squares analysis was performed using the General Linear Model Procedure program of the SAS statistical package (SAS, 1988). The effects of year and breed were included in the model:

$$y_{ij} = \mu + \text{YEAR}_i + \text{BREED}_j + e_{ij}$$

In this model y_{ij} =dependent variables, μ =general mean, YEAR_i =main year effect ($i=1,2$), BREED_j =main breed effect ($j=1,\dots,7$) and e_{ij} =residual random term.

When F -tests were significant ($P<0.05$), differences among breeds were tested for significance using t -tests. Pearson correlations coefficients were generated between the variables studied. A canonical discriminant analysis (CANDISC procedure) was used to obtain the squared Mahalanobis distances in order to assess the proximity between breed-production system means based on biochemical and colour variables.

3. Results and discussion

3.1. Effect of the breed-production system on MHC-I, biochemical traits and colour

Least-square means of age at slaughter, carcass weight, MHC-I, biochemical traits and colour variables for the seven breed-production systems studied are presented in Table 1. Significant differences among breeds were found for all the traits studied ($P<0.001$). The RE breed (rustic type) showed the highest IMF percentage and the AV breed (double muscle condition) the lowest. The low level of IMF observed in AV is a characteristic of double muscle animals (Arthur, 1995) and it has been

widely reported not only in AV breed (Oliván et al., 1999) but also in other double muscle breeds such as Belgian Blue White (Clinquart, van Eenaeme, van Voooren, van Hoof, Hornick, & Istasse, 1994; Clinquart, Hornick, van Eenaeme, & Istasse, 1997) and Piemontese (Destefanis, Barge, & Brugiapaglia, 1996). IMF is an important trait which seems to influence the overall eating quality of beef meat (Dikeman, 1990).

The highest haem pigment content was found, as expected, in the breeds with the highest age at slaughter, AM and MO (Janicki, Kortz, & Rozyska, 1966). According to MacDougall (1982) and Moss, Millar, and Stevenson (1994), meat with higher pigment content has lower lightness (L^*) and higher redness (a^*). The results obtained for the AM and the MO breeds confirmed this finding since L^* showed the lowest value in these two breeds and a^* the highest. Also Insausti et al. (1999) reported a very high myoglobin content in the MO breed even with animals slaughtered at a younger age than in our study. AM and MO showed the lowest carcass weight, explained by their low daily gain: 1.03 and 1.12 kg day⁻¹ for AM and MO, respectively, compared with values higher than 1.40 kg/day for the other breeds in this study (Piedrafito et al., 1999).

Regarding the metabolic and contractile traits of the muscle fibres, the highest oxidative activity (ICDH) was found in the A-NI, the MO and the RE breeds, all of which are rustic types; MO, RE and also AM showed very high MHC-I percentages. The PI and the AV breeds showed high MHC-I percentages but the lowest ICDH activities and the highest LDH ICDH⁻¹ rates. These results could probably be explained by the percentages of fibres IIA (fast-twitch oxidative-glycolytic) and IIB (fast-twitch glycolytic) in the muscle, which were not examined in this study. Using the same analytical ELISA method as in this study, Jurie, Robelin,

Picard, Renand, and Geay (1995) reported a 27% MHC-I content for LT in Limousine cattle, 480 days old. This result is similar to those obtained from the A-NI and BP breeds in our study, although the average age at slaughter and carcass weight of both breeds were lower. Thus, it can be suggested that the LT from all the breeds studied had more oxidative characteristics than LT from the Limousin animals studied by Jurie and co-workers. Renand, Touraille, Geay, Berge, Lepetit, and Picard (1997) reported 25.3% of MHC-I in Charolais cattle. Maltin et al. (1998) reported lower percentages of MHC-I (21–25.3%) in *longissimus lumborum* from Aberdeen Angus×Charolais animals fed two different diets and slaughtered at different ages.

A low glycolytic activity (LDH) was found in the rustic breeds AM and RE, but not in the MO breed as would be expected due to its high oxidative characteristics (high MHC-I, ICDH and haem pigment values). Moreover, the RE breed showed the lowest LDH ICDH⁻¹ rate, whereas AM and MO had intermediate values for this variable.

A clear breed effect was found on lightness. The AV and the PI (meat breeds) were the ones with significantly higher L^* and H^* values as a result of their higher glycolytic capacity as judged by their LDH·ICDH⁻¹ rates (0.96 and 0.82, respectively). These two breeds also displayed the best conformation with a high lean percentage on the dissected sample rib (Piedrafito et al., 1999). In general, animals with increased muscularity have a paler colour and less IMF, as seen in double-muscled cattle (Fiems, de Campaneere, Bogaerts, Cottyn, & Boucqué, 1998), in which muscles show a higher glycolytic activity than those of normal cattle (Gagnière, Picard, Jurie, & Geay, 1997). A-NI, BP and RE showed intermediate lightness values. These results are similar to those obtained by Sañudo et al. (1999) in an experiment conducted on some of these breeds.

Table 1

Effect of breed-production system on age at slaughter, carcass weight, biochemical traits and colour measurements (Least squares means) of *longissimus thoracis*^a

	AM	AV	A-NI	BP	MO	PI	RE	RMSE
Age at slaughter (d)	541.0e	415.7c	363.3a	380.6b	438.9d	382.7b	417.7c	45.6
Carcass weight (kg)	249.9a	324.0d	279.4c	329.0de	259.9b	334.5e	286.3c	23.9
Intramuscular fat (g kg ⁻¹)	19.1b	9.9a	27.2c	24.3c	26.2c	19.4b	34.8d	10.0
Haem pigments (μg acid haematin g muscle ⁻¹)	186.2d	136.7a	136.0a	147.8b	188.4d	144.0ab	165.8c	29.4
Myosin heavy chain 1 (%)	35.5d	32.1cd	26.7a	27.9ab	39.3e	41.5e	31.1bc	11.8
Lactate dehydrogenase (μmol min ⁻¹ mg protein ⁻¹)	27.2a	31.5b	31.5b	32.2b	31.6b	30.4b	26.7a	4.3
Isocitrate dehydrogenase (nmol min ⁻¹ mg protein ⁻¹)	44.5b	38.6a	48.7bc	45.1b	52.0c	38.2a	53.3c	16.6
LDH ICDH ⁻¹ rate (μmol nmol ⁻¹)	0.71b	0.96c	0.77b	0.76b	0.72b	0.82bc	0.56a	0.38
L^*	32.2a	38.8d	37.7c	36.0b	32.6a	38.9d	35.5b	3.0
a^*	23.4b	20.9a	20.6a	21.3a	24.0b	21.1a	22.9b	2.5
b^*	13.2c	11.7ab	10.7a	11.2a	12.5bc	12.3bc	11.1a	2.9
Hue	28.9cd	29.1d	27.3abc	27.4bc	27.0ab	30.1d	25.8a	4.8
Chroma	27.0d	24.0ab	23.3a	24.2ab	27.2d	24.5bc	25.5c	3.2

^a Least squares means with a common superscript are not significantly different ($P > 0.05$). AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta; RMSE, root mean square error; LDH, lactate dehydrogenase; ICDH, isocitrate dehydrogenase.

3.2. Relationships between MHC-I, biochemical traits and colour within breed-production system

The significant correlations between a number of biochemical traits, carcass weight, age at slaughter and colour variables, within breeds are presented in Tables 2–4. Only variables with statistically significant correlations are shown. Several correlations between variables confirmed previous studies, indicating that the traits involved did not depend on the particular breed but were related to their common biological origin. Age at slaughter was correlated positively with haem pigments in the AM, the BP, the MO and the RE breeds. However, neither age at slaughter nor carcass weight had any correlation with IMF except for the AM breed (carcass weight vs. IMF, Table 2). In this study there were small variations in age at slaughter and carcass weight within each breed.

The positive correlations between MHC-I and haem pigment content obtained for most of the breeds (AM, AV, A-NI, PI, RE; Table 3) confirmed that the higher the percentage of slow myosin in a muscle, the greater the oxidative metabolism (Cassens & Cooper, 1971). On the other hand, the correlations between MHC-I and ICDH (Table 4) and between haem pigment and ICDH (Table 3) were positive in some of the breeds studied

(AM, A-NI, BP, RE, and AM, A-NI, BP, respectively). It is worth mentioning that a high correlation was obtained between ICDH and MHC-I (0.67) in the A-NI breed. Jurie et al. (1995) reported a correlation coefficient of 0.39 in the same muscle in Limousin animals.

With respect to the enzymatic activities, ICDH and LDH were negatively correlated in five of the seven breeds studied (Table 4) agreeing with Ansay (1974) and Talmant, Monin, Briand, Dadet, and Briand (1986), who observed negative correlations between glycolytic and oxidative activities in several beef muscles. However, the BP and the MO breeds showed no significant correlation between ICDH and LDH activities; in both breeds ICDH and LDH activities were high (Table 1).

As haem pigment contents were correlated positively to a^* and C^* in most breeds (AV, A-NI, BP (a^*), PI, RE; Table 3), a high myoglobin concentration was associated with redder meat (Cassens & Cooper, 1971).

Other correlations between these traits within each breed suggested that each breed-production system is a factor. Carcass weight was either related (positively or negatively) or not related to the metabolic and contractile characteristics according to the breed considered (Table 2). Carcass weight was associated positively with MHC-I in the AV (double muscled) and the PI breeds and negatively in the BP and RE. This trait was negatively correlated with haem pigment content in the A-NI rustic type breed, the only breed in which this correlation was negative.

Table 2

Correlations between age at slaughter and carcass weight with biochemical traits and colour in *longissimus thoracis* for the seven breed-production systems^a

	AM	AV	A-NI	BP	MO	PI	RE
<i>Age at slaughter with:</i>							
Carcass weight	–	–	–	–	0.30	0.38	–
Haem pigments	0.46	–	–	0.51	0.41	–	0.38
MHC I	0.51	–	–	–	–	–	–
ICDH	–	–	0.31	–	–	–	–
L^*	–0.44	–	–	–	–	–	–
a^*	0.42	–	–	–	–	–	–
b^*	0.48	–	–	–	–0.24	–	–0.27
Hue	0.39	–	–	–	–0.36	–	–0.36
Chroma	0.49	–	–	–	–	–	–
<i>Carcass weight with:</i>							
Intramuscular fat	0.35	–	–	–	–	–	–
Haem pigments	–	–	–0.24	0.23	–	0.34	–
MHC I	–	0.41	–	–0.23	–	0.40	–0.32
LDH	–	–	–	0.43	–	–	–
ICDH	–0.25	–	–	–	–	–	–
LDH:ICDH ^{–1} rate	0.48	0.27	–	–	–	–	–
a^*	–	–	–	–	–	0.29	–
b^*	–	–	–	–0.36	–	0.35	–
Hue	–0.26	–	–	–0.43	–	–	–
Chroma	–	–	–	–	–	0.37	–

^a Only variables with statistically significant correlations ($P < 0.05$) are shown. AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta; MHC I, myosin heavy chain isoform I; LDH, lactate dehydrogenase; ICDH, isocitrate dehydrogenase.

Table 3

Correlations between biochemical traits and colour in *longissimus thoracis* for the seven breed-production systems^a

	AM	AV	A-NI	BP	MO	PI	RE
<i>Intramuscular fat with:</i>							
Haem pigments	–	–	–	0.29	–	–	–
MHC I	–	–	–	–	0.30	–	–
ICDH	–	0.27	–	–	–	–	–
LDH:ICDH ^{–1} rate	0.30	–	–	–	–	–	–
L^*	0.43	–	–	–	–	–	–
a^*	–	0.29	–	–	–	0.31	–
Hue	–0.27	–	–	–	–	–	–
Chroma	–	0.26	–	–	–	0.28	–
<i>Haem pigments with:</i>							
MHC I	0.42	0.33	0.35	–	–	0.43	0.36
LDH	–0.28	–	–0.31	0.29	–	–0.41	–
ICDH	0.35	–	0.28	0.42	–	–	–
L^*	–0.33	–	–0.24	–0.28	–	–0.32	–0.54
a^*	–	0.49	0.49	0.43	–	0.40	0.37
b^*	–	–	–	–	–0.28	–	–
Hue	–	–0.33	–	–0.32	–0.40	–	–0.31
Chroma	–	0.42	0.38	–	–	0.34	0.24

^a Only variables with statistically significant correlations ($P < 0.05$) are shown. AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta; MHC I, myosin heavy chain isoform I; LDH, lactate dehydrogenase; ICDH, isocitrate dehydrogenase.

A positive correlation between IMF and MHC-I was found in the MO rustic type breed (Table 3). As presented in Table 1, this breed had high mean values of IMF, haem pigment content, MHC-I and ICDH. This agrees with Cameron (1993) who found that muscles with strong oxidative metabolism tend to show higher

IMF than muscles with a lower oxidative metabolism. This strong oxidative metabolism leads to a redder meat (higher a^* value) with low L^* value; both traits making meat have a darker appearance.

3.3. Relationship between the breed-production systems based on MHC-I, biochemical traits and colour variables

A canonical discriminant analysis of MHC-I, biochemical traits and colour variables in relation to breed is presented in Fig. 1 (a and b). Table 5 shows the squared Mahalanobis distances between breed-production system means. Although all breeds were significantly different from each other ($P < 0.01$), when considering these distances they can be grouped as follows: A-NI and BP, the closest breeds, with RE also close to them; AV and PI, two meat breeds with a high muscularity (Piedrafito et al., 1999) and finally the AM and MO rustic breeds. As expected, the largest distances were observed between the meat breeds and the rustic type breeds (AV vs. RE, AV vs. MO and PI vs. RE). Fig. 1 shows that the first four axes explained 97% of the variation. The first canonical axis discriminated according to meat colour (L^* , on the positive axis and a^* on the negative axis, together with haem pigment contents and C^*) the AV, PI and A-NI breeds on one side, and the AM, MO and RE (redder meat) on the other. The second axis discriminated according to IMF on the positive part and with MHC-I on the opposite. Also, the second axis allowed us to discriminate the breeds in two different groups, RE and A-NI on the positive side of the axis, and MO, AM, PI and AV on the negative side. The BP breed was placed near the origin in the first plot (Fig. 1a) and near the canonical coordinate explained by LDH in the second plot (Fig. 1b; third axis), together with the MO breed. Thus, according to the biochemical parameters and the colour variables studied, the BP breed was intermediate in relation to the others, as previously reported by Sañudo et al. (1999) for other characteristics. The fourth axis allowed us to discriminate the PI and the MO breeds

Table 4

Correlations between biochemical traits and colour measurements in *longissimus thoracis* for the seven breed-production systems^a

	AM	AV	A-NI	BP	MO	PI	RE
<i>MHC I with:</i>							
LDH	–	–	–	–	–	–	–0.50
ICDH	0.46	–	0.67	0.32	–	–	0.31
LDH:ICDH ⁻¹ rate	–0.38	–	–0.50	–0.33	–	–	–0.36
L^*	–0.27	–	–	–	–	–	–
a^*	–	–	–	–	–	0.42	–
<i>LDH with:</i>							
ICDH	–0.31	–0.44	–0.32	–	–	–0.58	–0.39
LDH:ICDH ⁻¹ rate	0.46	0.60	0.28	0.46	–	0.66	0.70
L^*	–	0.45	–	–	–	0.65	0.46
a^*	–	–0.27	–	–	–	–0.43	–
b^*	–	–	–	–0.40	–	–	–
Hue	–	–	–	–0.42	–	–	–
Chroma	–	–	–	–0.24	–	–	–
<i>ICDH with:</i>							
L^*	–	–0.39	–	–	–	–0.41	–0.28
a^*	–0.24	0.24	–	–	–	–	–
b^*	–	–	–	–0.26	–0.40	–	–
Hue	–	–	–	–	–0.44	–	–
Chroma	–	–	–	–0.24	–0.27	–	–
<i>LDH:ICDH⁻¹ rate with:</i>							
L^*	–	–	–	–	–	0.38	0.44
b^*	–	–	0.38	–	0.31	–	–
Hue	–	–	0.33	–	0.32	–	–
Chroma	–	–	0.27	–	–	–	–

^a Only variables with statistically significant correlations ($P < 0.05$) are shown. AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta; MHC I, myosin heavy chain isoform I; LDH, lactate dehydrogenase; ICDH, isocitrate dehydrogenase.

Table 5

Canonical discriminant analysis: squared Mahalanobis distances between breed-production system means^a

Breed-production system	AM	AV	A-NI	BP	MO	PI	RE
Asturiana de la Montaña	0						
Asturiana de los Valles	7.33**	0					
Avileña-Negra Ibérica	7.61**	4.49**	0				
Bruna dels Pirineus	4.72**	3.87**	0.86*	0			
Morucha	3.15**	8.83**	6.83**	3.87**	0		
Pirenaica	8.06**	2.64**	5.78**	5.10**	7.66**	0	
Retinta	5.46**	9.43**	3.05**	3.30**	5.63**	8.65**	0

^a AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta.

* $P < 0.01$.

** $P < 0.001$.

Fig. 1. Canonical discriminant analysis of the seven breed-production systems, according to biochemical parameters and colour measurements: (a) axis 1 and 2, (b) axis 3 and 4 of the total canonical structure. CAN_i (%), percentage of the total canonical structure explained by the axis; (■), breed centroids; AM, Asturiana de la Montaña; AV, Asturiana de los Valles; A-NI, Avileña-Negra Ibérica; BP, Bruna dels Pirineus; MO, Morucha; PI, Pirenaica; RE, Retinta. IMF, intramuscular fat; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MHC I, myosin heavy chain I; Rate, LDH ICDH⁻¹ rate; Haem, haem pigments.

from the other ones, showing the highest content of MHC-I, however the variance explained by this axis was only 8.9%.

In conclusion, the breed-production systems studied affected the percentage of MHC-I and metabolic traits of LT. The MO, rustic type breed, was the most oxidative and as a consequence the meat was dark and red. In terms of meat colour (L^* , a^* and C^*) the canonical discriminant analysis separated the breeds into two groups, the AV, the PI and the A-NI (the lightest ones) from the AM and the MO breeds (the reddest and darkest ones) whereas the BP showed intermediate values. The RE and the A-NI were distinguished from the others by their high IMF content. Also, this study revealed the different biochemical composition of some of the breeds when compared with selected meat breeds. Meat colour was affected by the muscle biochemical traits and this information could help with the selection of animals to meet market demands.

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References

- Albertí, P., Sañudo, C., Santolaria, P., & Negueruela, I. (1995). Variación de la calidad de la carne, de las medidas de la canal y de los parámetros productivos de añejos de seis razas españolas. *Información Técnica Económica Agraria*, 16(II), 627–629.

- Ansary, M. (1974). Individualité musculaire chez le bovin: étude de l'équipement enzymatique de quelques muscles. *Ann. Biol. Anim. Bioch. Biophys.*, 14, 471–486.
- Arthur, P. F. (1995). Double muscling in cattle: a review. *Australian Journal of Agricultural Research*, 46, 1493–1515.
- Bradford, M. M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- Briand, M., Talmant, A., Briand, Y., Monin, G., & Durand, R. (1981). Metabolic types of muscle in sheep: I. Myosin ATPase, glycolytic and mitochondrial enzyme activities. *European Journal of Applied Physiology*, 46, 347–358.
- Cameron, N. D. (1993). Selection for meat quality objectives and criteria. *Pig News and Information*, 14, 161N–168N.
- Campo, M. M., Sañudo, C., Panea, B., Albertí, P., & Santolaria, P. (1999). Breed type and ageing time effects on sensory characteristics of beef strip loin steaks. *Meat Science*, 51, 383–390.
- Cassens, R. G., & Cooper, C. C. (1971). Red and white muscle. *Advances in Food Research*, 19, 1–74.
- Clinquart, A., Hornick, J. L., van Eenaeme, C., & Istasse, L. (1997). Relationship between meat quality attributes and animal performance or carcass composition in Belgian Blue double-muscle type bulls. In *Proceedings 43rd International Congress of Meat Science and Technology* (pp. 274–275). Auckland, New Zealand.
- Clinquart, A., van Eenaeme, C., van Vooren, T., van Hoof, J., Hornick, J. L., & Istasse, L. (1994). Meat quality in relation to breed (Belgian Blue vs Holstein) and conformation (double muscled vs dual purpose type). *Sciences des Aliments*, 14, 401–407.
- Commission Internationale de l'Eclairage (1976). Commission Internationale de l'Eclairage, 18th Session, 1975. *CIE Publication* 36.
- Destefanis, G., Barge, M. T., & Brugiapaglia, A. (1996). Meat quality in four muscles of hypertrophied Piemontese and Belgian Blue and White young bulls. In *Proceedings 42nd International Congress of Meat Science and Technology* (pp. 298–299). Lillehammer, Norway.
- Dikeman, M. E. (1990). Genetic effects on the quality of meat from cattle. In *Proceedings 4th World Congress Genetics Applied Livestock Production* (pp. 521–529). Edinburgh, United Kingdom.

- Fiems, L. O., de Campaneere, S., Bogaerts, D. F., Cottyn, B. G., & Boucqué, Ch.V. (1998). The influence of dietary energy and protein levels on performance, carcass and meat quality of Belgian White-Blue double-muscléd finishing bulls. *Animal Science*, 66, 319–327.
- Franco, J. (1997). *Características productivas de la calidad de la canal y calidad instrumental de la carne de 7 razas bovinas españolas*. Zaragoza, Spain: Máster, CIHEAM.
- Gagnière, H., Picard, B., Jurie, C., & Geay, Y. (1997). Comparative study of metabolic differentiation of foetal muscle in normal and double-muscléd cattle. *Meat Science*, 45, 145–152.
- George, M. H., Tatum, J. D., Belk, K. E., & Smith, G. C. (1999). An audit of retail beef loin steak tenderness conducted in eight U.S. cities. *Journal of Animal Science*, 77, 1735–1741.
- Hornsey, H. C. (1956). The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *Journal of the Science of Food and Agriculture*, 7, 534.
- Insausti, K., Beriain, M. J., Purroy, A., Albertí, P., Lizaso, L., & Hernández, B. (1999). Colour stability of beef from different Spanish native cattle breeds stored under vacuum and modified atmosphere. *Meat Science*, 53, 241–249.
- Issanchou, S. (1991). Consumer expectations and perceptions of meat and meat products quality. *Meat Science*, 43(S), S5–S19.
- Janicki, M. A., Kortz, J., & Rozyska, J. (1966). Meat quality in relation to age and live weight in pigs. *Technologia Misa*, 7, 73.
- Jurie, C., Robelin, J., Picard, B., Renand, G., & Geay, Y. (1995). Inter-animal variation in the biological characteristics of muscle tissue in male Limousin cattle. *Meat Science*, 39, 415–425.
- MacDougall, D. B. (1982). Changes in the colour and opacity of meat. *Food Chemistry*, 9(1/2), 75–88.
- Maltin, C. A., Sinclair, K. D., Warriss, P. D., Grant, C. M., Porter, A. D., Delday, M. I., & Warkup, C. C. (1998). The effects of age at slaughter, genotype and finishing system on the biochemical properties, muscle fibre type characteristics and eating quality of bull beef from suckled calves. *Animal Science*, 66, 341–348.
- Moss, B. W., Millar, S. J., & Stevenson, M. H. (1994). The use of reflectance measurements at selected wavelengths to predict the amount and proportion of myoglobin in a range of meat species. In *Proceedings 40th International Congress of Meat Science and Technology* (S-V.12), 1994, The Hague, Netherlands.
- Oliván, M., Osoro, K., García, M. J., Noval, G., Martínez, A., Villa, A., Diego, V., & García-Atance, P. (1999). Características de la carne de terneros añojos de las razas Asturiana de los Valles y Asturiana de la Montaña. *ITEA*, 20(I), 98–100.
- Picard, B., Leger, J., & Robelin, J. (1994). Quantitative determination of type I myosin heavy chain in bovine muscle with anti myosin monoclonal antibodies. *Meat Science*, 36, 333–343.
- Piedrafitá, J., Quintanilla, R., Sañudo, C., Olleta, J. L., Campo, M. M., Panea, B., Oliver, M. A., Serra, X., García-Cachán, M. D., Cruz-Sagredo, R., Osoro, K., Oliván, M. C., Espejo, M., & Izquierdo, M. (1999). Carcass variability in seven Spanish beef breeds. In *Proceedings of European Association for Animal Production*, August 1999, Zurich, Switzerland.
- Renand, G., Touraille, C., Geay, Y., Berge, P., Lepetit, J., & Picard, B. (1997). Variabilité des qualités organoleptiques de la viande bovine en relation avec les caractéristiques musculaires. *Renc. Rech. Ruminants*, 4, 311–314.
- Sánchez Belda, A. (1981). Catálogo de razas autóctonas españolas. II. Especie bovina. *M.A.P.A.*, Madrid, Spain.
- Santolaria, P., Sañudo, C., Albertí, P., & Campo, M. M. (1997). Estudio de la calidad organoléptica de la carne de añejo de siete razas españolas mediante una prueba de consumidores. *Información Técnica Económica Agraria*, 93A(2), 89–97.
- Sañudo, C., Albertí, P., Franco, J., Olleta, J. L., Campo, M. M., Panea, B., Lahoz, F., Jaime, J., Pardos, J. J., & Tena, R. (1999). Calidad instrumental de la carne de siete razas bovinas españolas. *Eurocarne*, 73, 37–42.
- SAS (1988). *SAS/STAT user's guide* (Release 6.03). Cary, NC: Statistical Analysis System, SAS Institute INC.
- Talmant, A., Monin, G., Briand, M., Dadet, M., & Briand, Y. (1986). Activities of metabolic and contractile enzymes in 18 bovine muscles. *Meat Science*, 18, 23–40.
- Termin, A., Staron, R. S., & Pette, D. (1989). Myosin heavy chain isoforms in histochemically defined fiber types of rat muscles. *Histochemistry*, 92, 453.
- Valin, C., & Ouali, A. (1992). Proteolytic muscle enzymes and post-mortem meat tenderization. In F. J. M. Smulders, F. Toldrá, J. Flores, & M. Prieto, *New technologies for meat and meat products* (pp. 163–178). Utrecht: ECCEAMST.
- Vestergaard, M., Oksbjerg, N., & Henckel, P. (2000). Influence of the feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. *Meat Science*, 54, 177–185.
- Wyszecki, G., & Stiles, W. S. (1967). *Color science*. New York: Wiley.