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Original article

Metabolic characteristics of semitendinosus and gluteus medius muscles in bullfighting bulls at enzymatic level

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Abstract — Muscle metabolic characteristics can be determined by the ratios between key enzymes of the main energy-supplying pathways. It was shown that enzyme groups of constant proportions exit and represent no variable units within the enzyme activity. This study was aimed at comparing the enzymatic level of two bovine locomotor muscles. Biopsies were extracted from 24 male bullfighting bulls, aged between 4 and 5 years and belonging to four different Spanish farms. Four samples were taken from each animal: 2 from the semitendinosus muscle (ST) and 2 from the gluteus medius muscle (GM) at absolute depths of 30 and 50 mm. The activities of the enzymes CS (Krebs cycle oxidative potential), HAD (β-oxidation), HK (glucose phosphorylation), PHOS (glycogenolysis) and LDH (glycolytic capacity) were analysed. The following enzyme ratios were considered: PHOS/LDH; HK/CS; HAD/CS; LDH/CS and PHOS/HK. Some metabolic characteristics between both muscles and depths were found. No significant differences were detected in the HK/CS ratio in the ST and in the GM, whereas the LDH/CS ratio was higher in the ST at 30 mm and in the GM at 50 mm. No differences were detected between both depths in the GM muscle, for the HK/CS ratio and LDH/CS ratio. In contrast, in the ST muscle, the LDH/CS and HK/CS ratios were statistically higher at 30 than at 50 mm depth. The most evident differences were observed when the four farms were compared. This study showed that the main metabolic differences between the bovine ST and GM muscles were the relationships between the capacities to use extracellular energy sources, to oxidise Acetyl CoA in the Krebs cycle and to reduce pyruvate to lactate.

bovines / enzyme activities / metabolism / muscle

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Résumé — Caractéristiques métaboliques des muscles semitendineux et du muscle fessier moyen chez les taureaux de combat sur le plan enzymatique. Les caractéristiques métaboliques d'un muscle peuvent être analysées à partir des rapports entre les enzymes représentant les voies principales de resynthèse de l'énergie. L'objectif de ce travail a donc été d'effectuer une analyse comparative des muscles locomoteurs bovins sur le plan enzymatique. Des biopsies musculaires ont été realisées sur 24 taureaux de combat mâles, d'un âge compris entre 4 et 5 ans et appartenant à 4 élevages différents. Quatre échantillons ont été prelevés sur chaque animal : 2 au niveau du muscle semitendineux (ST) et 2 autres échantillons sur le fessier moyen (GM), à des profondeurs absolues de 30 et 50 mm. Les activités des enzymes CS (potentiel oxydant du cycle de Krebs), HAD (β-oxydation lipidique), HK (phosphorylation du glucose), PHOS (glycogénolyse) et LDH (capacité glycolique) ont été analysés. Puis, les rapports enzymatiques suivants ont été considérés : PHOS/LDH ; HK/CS ; HAD/CS ; LDH/CS et PHOS/HK. Certaines différences ont été observées entre les deux muscles et les différentes profondeurs. Ainsi, le rapport HK/CS ne présentait pas de différences entre le ST et le GM, tandis que le rapport LDH/CS était le plus élevé dans le ST à 30 mm et dans le GM à 50 mm. Aucune différence métabolique n'a été mise en évidence dans le muscle GM entre 30 et 50 mm pour le rapport HK/CS et le rapport LDH/CS. En revanche, dans le muscle ST, le rapport LDH/CS et le rapport HK/CS étaient significativement plus élevés à 30 mm qu'à 50 mm. Les différences les plus marquées sont apparues lors de la comparaison d'animaux de différents élevages. En conclusion, les principales différences métaboliques entre les 2 muscles ont été les rapports entre les capacités d'emploi des sources énergétiques extracellulaires, pour oxyder le Coenzyme A dans le cycle de Krebs et pour réduire le pyruvate en lactate.

bovins / activés enzymatiques / métabolisme / muscle

1. INTRODUCTION

Metabolic characteristics of muscle are linked to the main systems of energy-supplying metabolism, and are reflected most strikingly at the level of enzymatic organisation [1]. Previous research has shown that enzyme groups of constant proportions exist, and represent nonvariable units within the enzyme activity. Constant proportions have been observed in the metabolic systems of glycolysis, fatty acid β-oxidation, citric acid cycle and the respiratory chain. Although absolute enzyme activities can vary when different tissues, muscles or animal species are compared, the relative enzyme activities have been reported as constant patterns [1, 21].

The study of the relationships between the different muscular energy-supplying pathways can be made by comparing the activity ratios of key enzymes. According to Green et al. [10], these ratios may provide valuable information about the relationships between oxidative and glycolytic pathways, determining if there has been a metabolic transition in muscle fibers of types I, IIA and IIB or not. For example, the citric acid cycle/oxidative phosphorylation ratio has been documented as constant or at least comparable, whereas the glycolysis/citric acid cycle ratio is variable in different tissues and muscles, a variability that reveals metabolic differentiation [22].

In the present investigation, activity ratios of the enzymes representing the main metabolic systems in two bovine muscles have been compared. Citrate synthase (CS) and 3-OH-acyl CoA dehydrogenase (HAD) represented the citric acid cycle and the β-oxidation of fatty acids respectively. Other key enzymes of carbohydrate metabolism which act at the branch points of the Embden-Meyerhof pathways are: glycogen phosphorylase (PHOS) for glycogenolysis, hexokinase (HK) for glucose phosphorylation and lactate dehydrogenase (LDH) for glycogenolysis with lactate formation.

This study focused on the enzyme activity ratios in bullfighting bull semitendinosus and gluteus medius muscles at absolute depths of 30 and 50 mm, in order to metabolically differentiate both muscles.

2. MATERIALS AND METHODS

2.1. Animals

Twenty-four male bullfighting bulls (6 per farm), aged between 4 and 5 years were included in this study. They belonged to four Spanish farms with different genetic backgrounds (A, B, C and D). Mean live weights and carcass weights were 549 ± 28 kg and 308.3 ± 19.3 kg respectively.

2.2. Muscle biopsies

The muscle samples were obtained by needle biopsy as described by Lindholm and Piehl [17]. Four muscle samples were taken from each animal within the first 5 minutes after slaughtering at absolute depths of 30 mm (superficial sampling site) and 50 mm (deep sampling site) in the semitendinosus (ST) and gluteus medius (GM) muscles. The ST biopsies were extracted roughly 20 cm ventral to the tuber coxae. The GM biopsies were obtained roughly 15 cm caudoventral to the tuber coxae of the ilium in the line between this tuber and the tuber ischii. Earlier papers have shown the lack of differences between opposite muscles in horses [6] and the biopsies were therefore extracted indistinctly from both right and left muscles. In order to reduce sampling error, all the biopsies were withdrawn by the same investigator and special care was taken to standardise the location and depth of the samples, owing to the heterogeneous muscle fiber distribution. The exact depth was controlled by means of two cannulas that allowed to open the window of the biopsy needle at 30 and 50 mm.

Immediately after collection, biopsies were frozen in liquid nitrogen and stored at –80 °C until analysis.

2.3. Biopsies processing

Muscle biopsies were freeze-dried for 24 h, dissected free of blood, fat and connective tissue and weighed (mean weight was between 1.2 and 1.8 mg). After that, muscle was homogenised in ice-cooled potassium phosphate buffer at pH 7.3 in a glass homogeniser at a dilution of 1:400. The activities of the Citrate synthase (CS, Enzyme Commission, E.C. 4.1.3.7), 3-hydroxy-acyl-coenzyme A-dehydrogenase (HAD, E.C. 1.1.1.35), lactate dehydrogenase (LDH, E.C. 1.1.1.27), hexokinase (HK, E.C. 2.7.1.1.) and glycogen phosphorylase (PHOS, E.C. 2.4.1.1) were determined according to the methods described by Essén et al. [6] and Essén-Gustavsson and Lindholm [7]. The enzyme activities were measured by quantifying the changes in NADH and NADPH concentrations at 25 °C with fluorometric techniques. The activity of each enzyme was calculated in units of NADH or NADPH converted per min per g freeze-dried muscle [2].

2.4. Enzyme activity ratios

The following enzyme activity relationships were investigated in both ST and GM muscles at 30 and 50 mm of depth: PHOS/LDH (glycogenolysis/glycolysis with lactate formation), HK/CS (glucose phosphorylation capacity/aerobic potential of the Krebs cycle), HAD/CS (fatty acid oxidation capacity/aerobic potential of the Krebs cycle), LDH/CS (glycolysis with pyruvate reduction/aerobic potential of the Krebs cycle) and PHOS/HK (glycogenolysis/glucose phosphorylation capacity).

2.5. Statistical analysis

Values are expressed as mean ± SD (otherwise indicated). An ANOVA/MANOVA

hierarchical model was performed for all the enzymatic relationships, considering the muscle and the farm as independent factors and considering the depth effect within the muscle effect.

3. RESULTS

Certain metabolic differences between the two bovine muscles studied have been found. No significant differences were detected in the HK/CS ratio between muscles. On the contrary, LDH/CS ratio was higher in the ST at 30 mm depth (mean value in ST muscle: 170.34; mean value in GM muscle: 140.70; F value: 9.450, $p < 0.001$) (Fig. 1A). This ratio was also higher in the GM at 50 mm depth (mean value in ST muscle: 116.70; mean value in GM muscle 149.70; F value: 10.12, $p < 0.001$) (Fig. 1B). In relation to the depth, any difference was found between 30 and

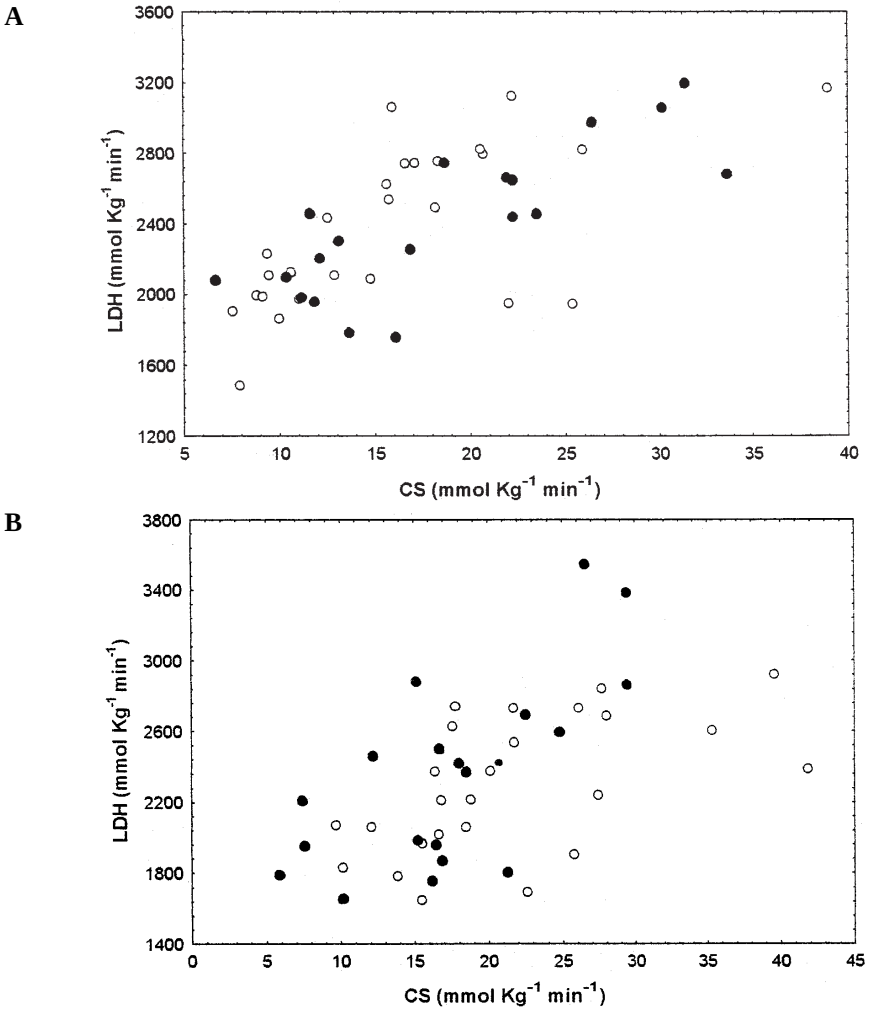

Figure 1. Significant enzymatic relationships in two bovine muscles at different depths (A and B: LDH/CS in the ST and GM muscles respectively). White circles: 30 mm depth; black circles: 50 mm depth.

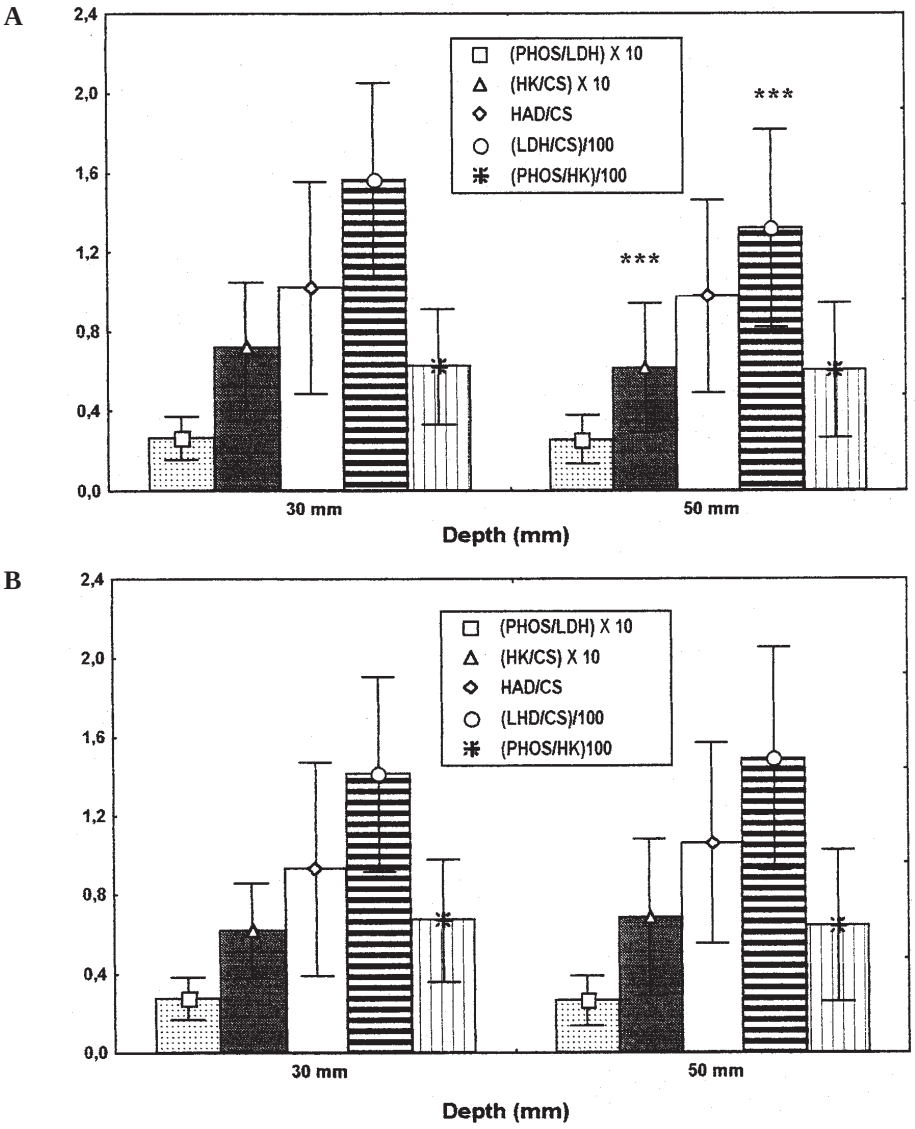

Figure 2. Influence of depth on the metabolic characteristics of two bovine muscles (A: ST muscle; B: GM muscle). Significant differences between depths at $p < 0.05$. (□ (PHOS/LDH) × 10; Δ (HK/CS) × 10; ◊ HAD/CS; ∗ (PHOS/HK) × 10.

50 mm in the GM muscle, for the HK/CS ratio and LDH/CS ratio (Fig. 2B). By contrast, the HK/CS ratio was higher at 30 mm of depth in the ST muscle (mean value at 30 mm: 0.082; mean value at 50 mm: 0.056; F value: 8.911, $p < 0.001$) (Fig. 2A). In the last, the LDH/CS ratio was higher at 30 mm of depth in the ST muscle (mean value at 30 mm: 170.3; mean value at 50 mm: 116.7; F value: 20.09, $p < 0.001$) (Fig. 2A).

The most evident differences were found between the farms. The maximum mean values were PHOS/LDH in farm B, HK/CS in farm C, HAD/CS in farm A, LDH/CS in farms A and C and PHOS/HK in farms A and B. The mean values for each farm are presented in Table I. Table II summarises the enzyme ratios in bovine GM at 50 mm of depth and in equine GM at 60 mm depth [2]. The results presented by Bass et al. [1] for total skeletal muscle for different animal species are included.

When the three factors, that is, muscles, depth and farm were considered together, no significant differences were detected.

4. DISCUSSION

These muscles (ST and GM) were chosen because they are the most frequently studied in horses, bulls and cattle and are considered to be propulsive muscles and very active during exercise [12, 18, 20, 24].

The results of the present research showed that the main metabolic differences between the bovine ST and GM muscles are the relationships between the capacities to use extracellular carbohydrates as fuel sources, to oxidise acetyl Coenzyme A in the Krebs cycle and to reduce pyruvate to lactate.

The metabolic characteristics of the ST muscle at 30 mm revealed the greater glucose phosphorylation capacity in relation to the oxidative potential. It might be thought that this result was associated with muscle composition. In fact, it has been reported in rabbit muscles that the slow-twitch I fibres, with a predominant oxidative metabolism, present higher HK activities [5]. However, this fiber type is also characterised by higher CS activities than the fast-twitch II fibres [26].

The difference in the HK/CS ratio seems to be accomplished by the higher CS activity in the ST muscle. In this way, the absolute CS activities were 15.65 and 19.65 $mmol \cdot kg^{-1} \cdot min^{-1}$ and the HK activities were 1.123 and 1.100 $mmol \cdot kg^{-1} \cdot min^{-1}$ in the ST and GM respectively. However, the PHOS/HK ratios of both muscles were statistically equal.

These data therefore showed that the bovine ST muscle has a higher aerobic capacity than GM, which was dependent on both lipid and carbohydrate metabolism, since no differences existed in the HAD/CS ratio. Earlier studies have shown that quantitative coordination of different metabolic systems appears to also exist in the case of fatty acid oxidation and the citric acid cycle. Although the coordination between HAD and CS is obvious, a constant ratio of these two enzymes is not necessarily expected, since the function of CS is not restricted to the catabolism of acetyl-coenzyme A derived from β-oxidation [1]

Table I. Mean values of the enzyme ratios in animals belonging to four different farms (data are presented as the mean of the ST and GM muscles at both 30 and 50 mm depth). Values in a same row with different superscript significantly differ.

FARMS	A	B	C	D
PHOS/LDH	0.021 ± 0.01^{a}	0.041 ± 0.01^{b}	0.022 ± 0.01^{a}	0.022 ± 0.01^{a}
HK/CS	0.044 ± 0.02^{a}	0.050 ± 0.02^{a}	0.109 ± 0.01^{b}	0.065 ± 0.03^{a}
HAD/CS	1.545 ± 0.56^{a}	0.861 ± 0.21^{b}	0.877 ± 0.15^{b}	0.867 ± 0.27^{b}
LDH/CS	179.4 ± 58.1^{a}	109.4 ± 28.8^{b}	171.9 ± 32.2^{a}	129.0 ± 53.2^{ab}
PHOS/HK	82.07 ± 27.3^{a}	91.72 ± 25.8^{a}	34.19 ± 13.7^{b}	44.06 ± 11.3^{b}

Table II. Summary of the muscle metabolic differentiation in some animal species, including bulls.

	Bullfighting bulls[1]	Untrained Andalusian horses[2]	Untrained man[3]	Rabbit (white skeletal muscle)[3]	Rabbit (red skeletal muscle)[3]	Chicken (white skeletal muscle)[3]	Chicken (red skeletal muscle)[3]
PHOS/LHD	0.027 ± 0.01						
HK/CS	0.071 ± 0.04	0.036	1.40	1.93	1.630	1.650	1.40
HAD/CS	1.063 ± 0.52	1.107	1.50	0.830	1.000	1.150	1.60
LDH/CS	149.7 ± 58	42.52	24.0	796.7	17.30	510.0	26.0
PHOS/HK	64.94 ± 39	22.27	15.0	119.7	2.70	81.0	3.10

[1] This experiment; [2] Blanco [2]; [3] Bass et al. [1].

The greater aerobic potential of the bovine ST muscle found in this study was contrary to that documented by Gottlieb et al. [9] in horses. After an increasing exercise test, lactate content has been significantly higher in the ST than in the GM, although the percentage of the different fiber types did not differ between the two muscles. Moreover, the CS activity has been 37% higher in the GM than in the ST. The differences between both studies may reflect the locomotor function of these muscles. It can be affirmed that muscles with a predominantly postural function show a higher percentage of oxidative I fibres. Inversely, muscles involved in locomotion show a higher percentage of type II fibres (IIA and IIB, with intermediate and low oxidative potential respectively) [4, 13, 14].

In spite of the inter-muscular differences with regard to the HK/CS ratio, this quotient was the least variable, with maximum and minimum values of 0.174 and 0.024. This smaller variability in comparison with the other enzyme ratios is important because it involves enzymes with extramitochondrial and intramitochondrial locations. However, it has been reported that although HK is essentially extramitochondrial, it is partly bound to the external mitochondrial membrane [25]. This is, probably, the main factor involved in the constancy of the HK/CS ratio.

The high relationship between the capacities to reduce and to oxidize pyruvate, producing lactate and acetyl-coenzyme A, respectively, seems somewhat controversial. It has been admitted that the greater the oxidative potential, the lower the glycolytic capacity. The only possible explanation is the heterogeneity of the muscle, which is composed of three principal types of fibres with different contractile and metabolic characteristics [3, 23].

The present research also presented evidences of muscle metabolic differences in animals belonging to different farms and thereby, subjected to different feeding and handling conditions with different genetic background (Tab. I). Moreover, some enzymatic activities, such as HK, could be affected by exercise. In absolute values, the glycolytic capacity was more developed in farm B. However, when it was expressed in relation to the Krebs cycle activity, higher glycolytic capacities were found in farms A and C. Therefore, the bulls of these two farms will tend towards pyruvate reduction by lactate dehydrogenase instead of pyruvate oxidation by pyruvate dehydrogenase or pyruvate deamination by pyruvate deaminase. It might, likewise, be thought that the greater amount of pyruvate produced by bovine muscles during contraction arises from the glycogen breakdown.

A remarkable finding was the higher HAD/CS in farm A (1.545, 0.866, 0.877 and 0.869 in the four farms). In human and rat muscles a rise in the enzyme involved in fatty acid β-oxidation after a high-fat diet has been demonstrated. Our results can be related to the different diets supplied to the animals on the different farms. There is, however, a physiological advantage to the animal if the acetyl units are produced from fatty acids as opposed to carbohydrates. In fact, the CS enzyme, which controls the entry of acetyl-CoA into the citric acid cycle, produces more citrate from pyruvate than acetyl carnitine [11, 16]. Despite this potential for improved flux, the Krebs cycle is unable to differentiate between acetyl units produced from either fatty acids or carbohydrate sources. As a consequence, the fine tuning of the pathways to attain the most efficient production of acetyl-CoA must occur at the substrate level.

The low HK activity detected in animals of farm A was unexpected, (0.432 against 1.096, mean value of the three other farms). In spite of this fact, the HK/CS ratio was statistically equal between the farms. The reduced HK activity may be a limiting factor to use blood-borne glucose. However, these animals did not compensate this HK activity deficit with a higher citric acid cycle capacity, since CS activity in absolute values was intermediate in relation to the other

farms. One way to overcome this limitation appeared to be the use of the free fatty acids and the glycogen breakdown, as show by the HAD/CS and PHOS/HK ratios.

The muscles of ruminants have a lower capacity to use blood glucose, and hence hepatic glycogen to rephosphorylate ADP both at rest and during exercise. Three main factors can be mentioned concerning this restriction: hypoglycemia, low insulin activity and low HK activity. The three factors caused a low HK/CS quotient in our bulls, although this ratio was even lower in the horse. The muscular characteristics of the latter animal are peculiar, since they both have high oxidative and glycolytic potentials. For this reason, the low HK/CS ratio was related to a high CS activity, which was almost the double of that of the bulls. By contrast, the HK activity can be 4-fold higher in horses than in bulls. The limited capacity to phosphorylate extracellular glucose caused a high PHOS/HK ratio in bovine muscles, although it was higher in white muscles of rabbits and chickens. The white muscles show a predominant percentage of II fibres, mainly IIB fibres. This fibre type has a reduced capillary density, a low oxidative potential and a high anaerobic capacity. The muscles of the bulls included in the present research showed 39% slow-twitch fibres and 61% fast-twitch fibres [8, 19].

As mentioned before, the relationship between the capacities to oxidise both fatty acids and pyruvate to produce acetyl CoA is rather similar in the different animal species, and even when several muscles are compared. However, the ratio between aerobic and anaerobic capacities has a marked variability. The LDH/CS relationship in bovine muscles was intermediate between those of the horse (4.52), rabbit (white skeletal muscle 796.7; red skeletal muscle, 17.30) and chicken (white skeletal muscle, 510; red skeletal muscle, 26.0) [1, 2]. This result may be linked to muscle composition. If we suppose that the bovine ST and GM muscles were almost 100% of fast-twitch fibres, as happens in the white rabbit and chicken muscles, the LDH/CS ratio could be higher in the former species. Therefore, the different oxidative power, both between the kind of fibre and within a given fibre type seemed to be a determining factor. A previous report showed that when comparing the enzyme activities of the same fibre type from different animals of the same species, no characteristic enzyme activity could be attributed to a particular fibre type. The CS activity in type IIB fibres from one animal was greater than that in the type I fibres in another animal [26]. To the authors' knowledge, there is no report about the metabolic characteristics of individual muscle fibres in bulls in comparison with other species.

In summary, this research showed that, although a certain degree of metabolic differentiation exists between the bovine ST and GM muscles, their enzymatic profiles were rather similar. This finding would indicate the same locomotor function, although ST is a flexor muscle, whereas GM is an extensor muscle [12]. Some differences between farms have been detected. It was demonstrated that the I/II ratio is genetically determined, even though it can be modified by physical growth and training [15, 19].

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