Examination of myosin heavy chain isoform expression in ovine skeletal muscles

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ABSTRACT: The contractile and associated metabolic characteristics of muscles are determined by their myosin heavy chain (MHC) isoform expression. In large mammals, the level of MHCIIB expression, which is associated with fast glycolytic-type muscle fibers, has not been fully characterized. In this study, quantitative reverse transcription-PCR and SDS-PAGE methodologies were developed for the analyses of adult ovine MHC isoform expression and used to characterize MHC expression in 3 skeletal muscles (LM, semitendinosus, and supraspinatus) from 66-d-old lambs. Three MHC isoforms (MHCI, MHCIIA, and MHCIIX) were detected at both the protein and messenger RNA levels in all 3 muscles, with greater proportions of type II than type I MHC. The expression of MHCIIB could not be detected at the protein level in any of the muscles and was detectable (in semitendinosus muscle) only at the messenger RNA level by using semiquantitative reverse transcription-PCR, indicating that MHCIIX is the predominant fast glycolytic fiber type in the sheep muscles studied. The methodologies developed are suitable for studying fiber type transformations at the molecular level, as well as allowing analyses of very small samples, including biopsies, when histochemical analysis may not be possible.

Key words: fiber type, myosin heavy chain, real-time polymerase chain reaction, sheep, skeletal muscle, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

INTRODUCTION

Skeletal muscles are dynamic in their fiber type composition and can change as an animal develops and ages (Picard et al., 2002) or in response to external factors, such as nutrition, growth promoters, or exercise (Lefaucheur and Gerrard, 2000). Muscle fibers are often classified based on their contractile and metabolic properties, and the different myosin heavy chain (MHC) isoforms form the basis of the contractile fiber type (Lefaucheur and Gerrard, 2000) because it is this component of the myosin molecule that has enzymatic activity, hydrolyzing ATP (Wagner and Giniger, 1981). Fibers are named according to the MHC isoforms expressed within them, and 4 adult isoforms (MHCI, MHCIIA, MHCIIX, and MHCIIB) are expressed in the skeletal muscle of many species, with each isoform originating from a different gene (Weiss and Leinwand, 1996). The conventional method for characterizing muscle fiber type is the histochemical measurement of myosin adenosine triphosphatase activity after preincubation at differing pH (Brooke and Kaiser, 1970). In sheep, 3 major fiber types (types I, IIA, and IIB) have been identified using this method (Peinado et al., 2004), with type I fibers being slow and types IIA and IIB being fast. With immunohistochemical methods, it has not been possible to distinguish between the MHCIIX and MHCIIB isoforms (Picard et al., 1999), and it is thought that fibers characterized histochemically as MHCIIB in sheep probably contain the MHCIIX isoform or both MHCIIX and MHCIIB (Picard et al., 1999; Greenwood et al., 2007), as observed in cattle (Picard et al., 1999). In sheep muscle, MHCIIB has been detected at the messenger RNA (mRNA) level (Vuocolo et al., 2007), but it is not clear whether the protein is expressed. Interestingly, MHCIIB mRNA can be detected in certain human muscle fibers (Horton et
al., 2001), but not the protein. The objective of the current study was to characterize the expression of adult MHC isoforms in sheep skeletal muscles by using PCR and SDS-PAGE methodologies.

**MATERIALS AND METHODS**

Sheep studies were approved by the University of Nottingham Local Ethical Review Committee and were carried out in accordance with the UK Home Office guidelines [Animals (Scientific Procedures) Act, 1986].

### Sample Collection and Preparation

Six Mule × Charolais wether lambs were weaned at 53 ± 2 d and slaughtered at 66 ± 2 d by electrical stunning and severing the carotid arteries. Newly weaned lambs were used because a change in nutrition had previously been shown to affect muscle fiber type composition (Greenwood et al., 2006); therefore, we expected fiber type transformations to be occurring, resulting in differences in protein and mRNA expression of the MHC isoforms. Samples of LM (from the 12th rib), and a complete transverse of the midsection of the supraspinatus (SS) and semitendinosus (STM) skeletal muscles were taken within 5 min of slaughter. Three cattle muscles, cutaneus trunci and the diaphragma from a 19-mo-old Charolais bull (Picard et al., 1999) and the longissimus thoracis from a 15-mo-old Blonde d’Aquitaine bull, were taken within 1 h of slaughter at the experimental INRA abattoir (Theix, France); each muscle had previously been shown to express MHCIIA and MHCIIX, MHCI and MHCIIA, and MHCI, MHCIIA, MHCIIX, and MHCIIB, respectively (Picard and Cassar-Malek, 2009). All samples were immediately frozen in liquid nitrogen, crushed, and stored at −80°C for protein or mRNA expression analysis.

### Analysis of MHC Protein Expression

Muscle samples were homogenized in 10 vol of extraction buffer containing 0.5 M sodium chloride, 20 mM sodium pyrophosphate, 50 mM Tris, 1 mM EDTA, and 1 mM dithiothreitol, incubated on ice for 10 min, and then centrifuged at 2,500 × g for 10 min at 4°C. The supernatant was mixed with an equal volume of 87% (vol/vol) glycerol and stored at −20°C. Protein concentration was determined (Bradford, 1976) and samples were adjusted to equal concentrations, and then mixed with an equal volume of loading buffer [4% (wt/vol) SDS, 125 mM Tris, pH 6.8, 20% (vol/vol; 87%) glycerol, 10% (vol/vol) β-mercaptoethanol (β-Me), and 0.02% (wt/vol) pyronin Y], incubated at room temperature for 10 min, and then incubated at 70°C for 10 min. Four micrograms of protein was loaded per well and samples were run in duplicate on 0.75-mm-thick gels, using the Protean II system (Bio-Rad, Hemel Hempstead, UK); compositions of separating and stacking gels are shown in Table 1. The upper running buffer contained 100 mM Tris, 150 mM glycine, 0.1% (wt/vol) SDS, and 10 mM β-Me; the lower buffer was one-half this concentration without β-Me. Gels were run at a constant 70 V for 30 h at 4°C, and then fixed in 30% (vol/vol) ethanol and 5% (vol/vol) acetic acid, followed by staining in 25% (vol/vol) isopropanol, 10% (vol/vol) acetic acid, and 0.2% (wt/vol) Coomassie Blue R250. Gels were destained in a 30% ethanol (vol/vol) and 5% acetic acid (vol/vol) solution, as described previously (Picard et al., 1999). Gel images were captured and proportions of the different MHC bands were determined by densitometry (Image Quant, Amersham Biosciences/GE Healthcare Europe GmbH, Saclay, France).

### Table 1. Gel composition for 4× 0.75-mm gels

<table>
<thead>
<tr>
<th>Item</th>
<th>Separating gel</th>
<th>Stacking gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% acrylamide, mL</td>
<td>3.92</td>
<td>0.98</td>
</tr>
<tr>
<td>2% bisacrylamide, mL</td>
<td>1.57</td>
<td>0.39</td>
</tr>
<tr>
<td>2 M Tris-hydrochloride, pH 8.8, mL</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>1 M Tris-hydrochloride, pH 6.8, mL</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>1 M glycine, mL</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>100 mM EDTA, mL</td>
<td>6.9</td>
<td>3.45</td>
</tr>
<tr>
<td>87% glycerol, mL</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>10% SDS</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>N,N,N,N′,N′-tetramethylethylenediamine, µL</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Total volume, mL</td>
<td>17.4</td>
<td>6.43</td>
</tr>
</tbody>
</table>

1Adapted from Picard et al. (2007).

RNA Extraction and cDNA Synthesis

Total RNA was extracted using Trizol reagent according to the protocol of the manufacturer (Invitrogen, Paisley, UK). Total RNA was treated with deoxyribo-nuclease, and then first-strand cDNA was generated from 0.5 µg of total RNA by using random primers and Moloney murine leukemia virus reverse transcriptase in a 25-µL final volume as described by the manufacturer (Promega, Southampton, UK).

Testing Primer and Probe Specificity

The PCR was carried out on cDNA by using the primers shown in Figure 1. Reactions were performed...
in a 50-µL reaction volume of PCR Gold Buffer (Applied Biosystems, Warrington, UK), 1.5 mM magnesium chloride, 0.2 mM nucleotide mix, 0.25 µM forward and reverse primers, and 1.25 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems); these were incubated at 94°C for 10 min, 40 cycles of 94°C for 30 s, 60°C (MHCIIA) or 58°C (MHCI and MHCIIIX) for 30 s, and 72°C for 60 s, followed by 72°C for 5 min. Amplicons were run on a 1% (wt/vol) agarose gel and bands of the correct size (MHCI: 321 bp; MHCIIA: 282 bp; MHCIIIX: 332 bp) were cloned into the pGEM-T Easy vector, and the identity of inserts was confirmed by sequencing.

IX: 332 bp) were cloned into the pGEM-T Easy vector, corrected size (MHCI: 321 bp; MHCIIA: 282 bp; MHCIIIX: 332 bp) were cloned into the pGEM-T Easy vector, and the identity of inserts was confirmed by sequencing. To test for interference between templates in real-time PCR, vectors containing MHC isoform sequences were linearized by using SacI restriction endonuclease (New England Biolabs, Hitchin, UK), adjusted to 1 × 10⁻¹ ng/µL, and serially diluted before being mixed 1:1 with water (control) or 1 × 10⁻² ng/µL of DNA of each of the other clones. Real-time PCR primers and probes were designed using Primer Express software (version 1.5, Applied Biosystems) and are also shown in Figure 1. Real-time PCR reactions (in triplicate) were set up in 1× Universal Master Mix (Applied Biosystems) containing 0.3 µM of each forward and reverse primer and 0.2 µM of probe in a final volume of 12.5 µL in a 384-well plate; they were run on a Lightcycler 480 PCR machine (Roche, Burgess Hill, UK): 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s, 60°C for 1 min, and 72°C for 1 s (data acquisition). Crossing point values were calculated using the second derivative maximum method (Lightcycler 480 Software Version 1.2, Roche).

**Skeletal Muscle MHC mRNA Expression and Statistical Analysis**

First-strand cDNA generated from LM, STM, and SS muscles was diluted 1:4, and from this, a pool of cDNA was generated for each muscle and a dilution series made and used as a standard curve; individual samples were further diluted 1:16 for analyses. Real-time PCR reactions using the real-time PCR primers and probes described above were carried out in 1× GeneAmp Fast PCR Master Mix (Applied Biosystems) containing 0.3 µM of each forward and reverse primer and 0.2 µM of probe, in a final volume of 25 µL. Reactions were carried out in duplicate on a 96-well plate run on a 7500T fast PCR machine (Applied Biosystems): 95°C for 10 min, and then 40 cycles of 95°C for 1 s and 60°C for 20 s; fluorescence was detected in real time. Threshold cycle ($C_T$) values were calculated using SDS 2.2.2 software (Applied Biosystems).

Relative standard curves were generated for each MHC isoform primer and probe set to calculate PCR efficiency. The $C_T$ values (y-axis) were plotted against log₁₀ ng equivalent RNA (x-axis), and PCR reaction efficiencies (E) were calculated from the standard curve as $10^{(-1/slope)} - 1$ (Čikoš et al., 2007). An average $C_T$ value was obtained for each primer and probe set for each sample, and this was used to calculate the relative expression ratio (rER):

$$rER = \frac{(1 + E(\text{MHC target gene}))^{-C_T(\text{MHC control gene})}}{(1 + E(\text{MHC control gene}))^{-C_T(\text{MHC control gene})}}$$

Because of the nature of the analysis, any one of the primer and probe sets could be set as the MHC control gene, with the expression of each other MHC target gene being calculated relative to this. The rER of the MHC control gene was set at a constant value of 1. The sum of rER values for the different primer and probe sets was calculated and the relative contribution of each primer and probe set rER was determined as a percentage. Data were expressed as percentage of total adult MHC expression.

For method comparison of each isoform in different muscles, data were analyzed by 2-way ANOVA using Genstat (Lawes Agricultural Trust, Hertfordshire, UK).

**RESULTS AND DISCUSSION**

**MHC Protein Expression**

Polyacrylamide gel electrophoresis represents a direct approach for typing muscles based on the protein expression of the MHC isoforms, but the conditions of electrophoresis have to be optimized for different species. In sheep skeletal muscle, up to 4 adult MHC isoforms have been reported to be separated by gel electrophoresis (Maier et al., 1992; Sayd et al., 1998; Zhu et al., 2006), but interpretation of each MHC isoform band migration is inconsistent between studies. Because cattle and sheep are phylogenetically close, it is likely that their MHC isoforms will migrate similarly (Sayd et al., 1998). The separation of the 4 adult MHC isoforms in cattle skeletal muscle was first described in a study by Picard et al. (1999) and was confirmed recently in specific bulls (Picard and Cassar-Malek, 2009). Electrophoretic separation using an acrylamide gradient (Picard et al., 1999) was very difficult to reproduce, so the method used herein was a modification of the method used by Talmadge and Roy (1993), which provides better resolution and reproducibility than the previous method (Picard et al., 2007). The diaphragm and cutaneous trunci cattle muscles had previously been characterized as containing only MHCI and MHCIIA or MHCIIA and MHCIIIX, respectively (Picard et al., 1994). The longissimus thoracis sample used had 4 bands of MHC, characterized as MHCIIIB < MHCII A < MHCIIIA < MHCI, in order of migration (Picard and Cassar-Malek, 2009).

The sheep STM, SS, and LM muscles were chosen because we had previously shown (using histochemistry) that they varied in fiber type composition (Sazili
et al., 2005). When the migration of sheep MHC isoforms was compared with that of cattle, it appeared that 3 major isoforms were expressed, corresponding to MHCI, MHCIIA, and MHCIIX in cattle (Figure 2). No MHCIIB band was observed in any of the samples. It is possible that the MHCIIX and MHCIIB isoforms may have comigrated, as previously observed in pigs, where 4 isoforms were expressed but only 3 bands could be separated (Bee et al., 1999). Alternatively, MHCIIB protein may not be present at a detectable level in any of the muscles studied. It was suggested previously that, where 2 MHC isoforms are coexpressed, the expression of the minor isoform can be detected only when its staining is greater than 1% of the predominant band (Bottinelli et al., 1994); therefore, it could be possible that the minor isoform, MHCIIB, was expressed at a more reduced level than was detectable and therefore could not be observed using the gel electrophoresis method. At the protein level, the proportion of fast MHC isoforms (MHCIIA and MHCIIX combined) was greater than that of slow isoforms (MHCI) in both LM and STM, whereas in SS, the proportions were similar (Table 2). In LM and STM, MHCIIX was the most abundant isoform, whereas in the SS it was MHCI.

**MHC mRNA Expression**

Real-time PCR represents a quantitative method for the analysis of MHC isoform mRNA transcripts. It has greater sensitivity compared with the gel electrophoresis method and can generally detect as few as 50 to 500 copies of a transcript (Bustin, 2000). Only 3 partial-length cDNA sequences had been identified for sheep MHC isoforms, corresponding to types MHCI, MHCIIA, and MHCIIX. Therefore, the real-time PCR primers and probes were designed to detect both MHCIIX and MHCIIB combined.
Because of the great degree of homology among the MHC isoforms, it was necessary to test the specificity of primers and probes designed for real-time PCR. Sheep skeletal muscle total RNA was used to produce partial-length MHC isoform cDNA to act as positive controls (Figure 1). The MHC mRNA sequences are most variable in the 5′ and 3′ untranslated region, whereas the rest of the sequence is very similar. Therefore, to distinguish between MHC transcripts, PCR primers were designed within the 5′ untranslated region for MHCI and MHCIIX isoforms, but for MHCIIA, the primers were generic and would be expected to amplify all fast MHC isoforms (MHCIIA, MHCIIX, and MHCIIB; Figure 1). Specific PCR primers for sheep MHCIIB transcripts could not be designed because no sheep MHCIIB cDNA sequence was identified in the nucleic acid databases. The products from each primer set PCR reaction were cloned, and vectors containing partial-length MHCI, MHCIIA, and MHCIIX cDNA were identified by sequencing. Serial dilutions of each of the isolated MHC cDNA (MHCI, MHCIIA, or MHCIIX) were generated, to which fixed equal concentrations of the other 2 MHC cDNA were added separately. When real-time PCR was carried out using the primer and probe set corresponding to the serially diluted MHC cDNA isoform, no interference in amplification was observed (Figure 3).

Two methods are generally used to analyze real-time PCR data: the standard curve method and the ∆∆CT method. The method used herein to calculate MHC proportions was an adaptation of the C_T difference method of the gene expression used by Schefe et al. (2006), using an internal standard curve generated from sample cDNA to determine PCR efficiency (Čikoš et al., 2007). This method calculates the relative quantities of mRNA transcripts, allowing proportions of the different transcripts to be determined. It does not require a housekeeping gene because differences in the quantity of RNA in the PCR reaction and reverse transcription efficiency are taken into account because measurements for the different isoforms are carried out on the same cDNA sample.

The pig MHCIIX and MHCIIB sequences (GenBank accession numbers AB025262 and AB025261) are identical within the region to which sheep MHCIIX primers and probes were designed and are similar in other species; thus, if MHCIIB mRNA is expressed in sheep muscles, then the MHCIIX primers and probes are likely to detect it as well as MHCIIX. However, MHCIIB was detected previously in sheep muscle by using primers designed to the cattle MHCIIB mRNA sequence (Vuocolo et al., 2007). In that study, 4 adult MHC isoforms were detected in sheep LM skeletal muscle, with MHCIIX being the predominant MHC isoform expressed and MHCIIB being expressed in extremely small amounts (less than 1% of total adult MHC expression).

In the present study, the 3 sheep muscles used to characterize MHC isoform protein expression were also examined for MHC isoform mRNA expression by using real-time PCR (Table 2). The SS had the greatest proportion of MHCI mRNA, whereas the proportions in LM and STM were similar. The proportion of fast transcripts (MHCIIA and MHCIIX combined) was greater than the proportion of slow transcripts (MHCI) in all 3 muscles examined, and MHCIIX mRNA made up the greatest proportion of the MHC isoform transcripts. As described above, we predicted that the MHCIIX primers and probes would also detect MHCIIB mRNA. The analysis of MHC protein expression had failed to

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Myosin heavy chain (MHC) isoform protein expression. (A) Electrophoretic separation of MHC isoforms in (1) bovine longissimus thoracis muscle and (2) ovine supraspinatus (SS) muscle, indicating a lack of MHCIIB expression in ovine muscle. (B) Separation in SS (2), semitendinosus (3), and LM (4) ovine skeletal muscles and in bovine cutaneous trunci (1) and diaphragma (5) muscles, which contain only MHCIIA and MHCI IX or MHCI and MHCIIA, respectively.

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**Table 2.** Percentage of myosin heavy chain (MHC) expression as determined by real-time PCR [messenger RNA (mRNA)] and electrophoretic separation (protein)\(^1\)\(^2\)

<table>
<thead>
<tr>
<th>Item, %</th>
<th>LM mRNA</th>
<th>LM Protein</th>
<th>STM mRNA</th>
<th>STM Protein</th>
<th>SS mRNA</th>
<th>SS Protein</th>
<th>SED</th>
<th>Method</th>
<th>Muscle</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHCI</td>
<td>7.9</td>
<td>8.3</td>
<td>13.8</td>
<td>5.3</td>
<td>26.9</td>
<td>48.5</td>
<td>6.5</td>
<td>0.247</td>
<td>&lt;0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>MHCIIA</td>
<td>29.4</td>
<td>25.9</td>
<td>24.2</td>
<td>19.1</td>
<td>23.4</td>
<td>16.5</td>
<td>3.0</td>
<td>0.006</td>
<td>0.003</td>
<td>0.725</td>
</tr>
<tr>
<td>MHCIIX</td>
<td>62.7</td>
<td>65.8</td>
<td>61.9</td>
<td>75.6</td>
<td>49.7</td>
<td>35.0</td>
<td>6.3</td>
<td>0.852</td>
<td>&lt;0.001</td>
<td>0.014</td>
</tr>
</tbody>
</table>

\(^1\)Data are means (n = 6).
\(^2\)STM = semitendinosus muscle; SS = supraspinatus muscle; SED of the mean for the method × muscle interaction.
detect any MHCIIB. Therefore, to determine whether sheep MHCIIB mRNA could be detected in the muscles studied, semiquantitative reverse transcription-PCR was carried out using published primers (Vuocolo et al., 2007) previously shown to detect MHCIIB transcripts in sheep LM muscle, whereas the level of MHCIIX + MHCIIB combined was determined using the real-time PCR primers described in Figure 1. Expression of

![Graph A](image1)

**Figure 3.** Specificity of myosin heavy chain (MHC) isoform real-time PCR. Amplification of MHCI (A), MHCIIA (B), and MHCIIX (C) template cDNA using corresponding primer and probe sets. Each dilution was spiked with a fixed quantity of the other 2 templates to test for interference (the dashed line represents the predicted observation if an interference occurred). Cp = crossing point values.
MHCIIX + MHCIIB combined was consistently greater than MHCIIB alone, and, in several instances, the MHCIIB primers failed to generate a product (Figure 4); MHCIIB mRNA was barely detectable in the LM and SS muscles, but was detected in some of the ST muscles. Hence, MHCIIB expression was extremely low, suggesting that the predominant MHC isoform in fibers previously characterized as type MHCIIB (fast glycolytic) was actually MHCIIX.

The measures of percentages of MHCI protein and mRNA (Table 2) were similar in LM and ST muscles, but were quite different in SS muscle ($P = 0.009$, muscle $\times$ method interaction). Likewise, the percentages of MHCIIX protein and mRNA (Table 2) were similar in LM muscle, but were different in STM and SS muscles ($P = 0.014$, muscle $\times$ method interactions). Differences between muscles ($P = 0.003$) and methods ($P = 0.006$) were observed for percentage of MHCIIB, but no interaction. This was due to LM having a greater percentage of MHCIIB than the other 2 muscles, and the percentage of MHCIIB mRNA being slightly greater than protein. Although histochemical analyses of fiber type were not carried out here, previous studies in humans and cattle have shown good agreement between the electrophoretic separation method used herein and immunohistochemical determination of fiber type composition (Serrano et al., 2001; Picard et al., 2007). Differences between protein and mRNA measures of MHC isoforms have been observed by other groups and are suggested to occur when fibers are transforming (Andersen and Schiaffino, 1997), or they have been used as evidence that expression of MHC isoforms might be regulated at the translational level (Horton et al., 2001). We suggest that the data presented herein provide evidence for transcriptional regulation of the MHC genes because the 2 measures were similar in the LM. Thus, the difference in expression in the SS muscle is likely to represent a transition in MHC expression; this may be explained by the fact that the muscle samples were from newly weaned lambs, which have altered nutrition, a factor that has previously been shown to alter fiber type (Greenwood et al., 2006). Although we cannot rule out the effect of age, we suggest that the transitions are more likely to be an effect of weaning.

In conclusion, we have developed both PAGE and quantitative real-time PCR methodologies to detect sheep MHC isoforms that will be useful for investigating fiber type transformations at the molecular level, as well as for allowing fiber type determination on very small samples, such as biopsies, where histochemical analyses may not be possible. Although metabolic activity generally correlates with the contractile fiber type, a recent report indicates that the 2 components may be uncoupled (Park et al., 2009); therefore, combining the methodologies described in this paper with measures of metabolic enzyme activity would allow a more complete classification of muscle fiber type. In the current study, expression of the fastest isoform, MHCIIB, was observed only at the mRNA level in a subset of samples. This agrees with studies in other large mammals, including humans, in which MHCIIB mRNA transcripts have been detected, but not the protein (Horton et al., 2001). However, this is different from what has been reported for pigs (Lefaucheur et al., 2004) and rodents (Vadászová et al., 2006), in which both the mRNA and protein can be detected. Expression of the MHCIIB isoform at the protein level has been demonstrated recently in bovine skeletal muscle, but only in certain breeds of bulls (Picard and Cassar-Malek, 2009). Hence, there are some fundamental differences between species in terms of muscle biology, at least in terms of fiber type, which means caution is required when relating results from studies in one species to those in another.

**LITERATURE CITED**


