Muscle fiber type characterization and myosin heavy chain (MyHC) isoform expression in Mediterranean buffaloes

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ABSTRACT

This study aimed to evaluate myosin heavy chain (MyHC) isoform expression and muscle fiber types of *Longissimus dorsi* (LD) and *Semitendinosus* (ST) in Mediterranean buffaloes and possible fibers muscles modulation according to different slaughter weights. The presence of MyHC IIb isoforms was not found. Only three isoforms of MyHC (IIa, IIx/d and I) were observed and their percentages did not vary significantly among slaughter weights. The confirmation of the presence of hybrid muscles fibers (IIA/X) in LD and ST muscles necessitated classifying the fiber types into *fast* and *slow* according to their contractile activity, by m-ATPase assay. For both muscles, the muscle fiber frequency was higher for *fast* than for *slow* fibers in all weight groups. There was a difference ($P<0.05$) in the frequency of LD and ST muscle fiber types according to slaughter weights, which demonstrate that the slaughter weight influences the profile of muscle fibers from buffaloes.

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1. Introduction

The food demand for animal protein and healthy quality products has been increasing constantly. Thus, one more option is buffaloes (*Bubalus bubalis*) that have the capacity to supply the consumers' animal protein needs. In Brazil, buffaloes subjected to an intensive production system are usually slaughtered at live weights in the range of 450 to 540 kg. Production systems to increase muscle mass in a short time have shown that buffaloes are able to gain weight quickly and consequently provide high yield of carcass and retail cuts (Francisco et al., 2009; Jorge, Andrighetto, Millen, Calixto, & Vargas, 2006; Jorge et al., 1997; Vaz, Restle, Bondaini, & Pacheco, 2003).

The assessment of skeletal muscle fibers can be a tool used to follow the growth of animals, according on age and live weight, and the specificity muscles development. The profile of a muscle, in relation to the composition of muscle fibers, may be modified by factors such as gender, age, muscle function, type of enervation, genetic heritage, environmental conditions, as well as the production system. (Arnold & Meyer, 1988; Ashmore, 1974; Bee et al., 2007; Ozawa et al., 2000).

Despite speculations that the muscle fibers may respond to the production systems in different ways this relationship is not entirely clear (Maltin et al., 2001). According to Ashmore (1974), beef animals submitted to an intensive production system present a higher frequency of fast than slow contracting muscle fibers. In a study with

buffaloes, Solomon, West, and Carpenter (1985) observed the lack of fast glycolytic (FG) fibers in the *longissimus* muscle and the high frequency of slow oxidative (SO) fibers compared to fast oxidative glycolytic (FOG) in the profile of muscle fibers. Added to these speculations the scarcity of studies regarding the behavior of these fibers during the development in buffaloes, make it difficult to understand this species and possible improvements in the production system, whose purpose is to obtain meat-producing animals in less time.

Studies of several domestic species (Albrecht, Teuscher, Ender, & Wegner, 2006; Ashmore, Tompkins, & Doerr, 1972; Peinado et al., 2004; Pette & Staron, 2000) have shown how important the knowledge of muscle fibers characteristics and their possible modulations during growth is. The use of histochemical methods, like myofibrillar ATPase (m-ATPase), and electrophoresis technique allows a better classification of muscle fibers types (Bee, Solomon, Czerwinski, Long, & Pursel, 1999). The electrophoretic profile of myosin heavy chain (MyHC) reveals the myosin isoforms (MyHC I, MyHC IIa, MyHC IIx or MyHC IId and MyHC IIb) existing in the muscle, which could not be identified only with conventional analyses like m-ATPase (Bee et al., 1999; Picard & Cassar-Malek, 2009). However, there is a relationship between the fibers identified by ATPase and the myosin isoforms obtained by electrophoresis because a muscle fiber can specifically contain an isoform: a slow oxidative fiber (*slow twich oxidative* – SO, IA, βR or red) that contains MyHC I; a fast oxidative/glycolytic fiber (*fast twich oxidative glycolytic* – FOG, IIA, αR or red) that contains MyHC IIa; a fast glycolytic fiber (*fast twich glycolytic* – FG, IIB, αW or white) that contains MyHC IIb. These fibers are classified as pure fibers. But, a fiber can contain several MyHC isoforms

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simultaneously and be denominated hybrid fiber, in contrast to a pure fiber (Choi & Kim, 2009; Pette & Staron, 2000).

Considering the productive potential of the bubaline species (*Bubalus bubalis*) as meat producers and the lack of studies about the possible modulations of muscle fibers during the development of these animals, studies that help in the understanding of muscle development as well as in the characterization of muscle fiber type in this species are required.

This study aimed to characterize the profile of myosin heavy chain isoforms and analyze the muscle fiber types of *Longissimus dorsi* (LD) and *Semitendinosus* (ST) in buffaloes finished in feedlots and slaughtered at different live weights. The hypothesis is that, in intensive finishing systems, there are different types of fibers in the ST and LD muscles of buffaloes and that the differences among the slaughter weights reflects variations in the profile of muscle fibers, confirming that the study of muscle fibers is a tool that can be used for understanding the growth of muscles in buffaloes.

2. Materials and methods

2.1. Animals and treatments

The experiment was carried out at the University Estadual Paulista – FMVZ – UNESP, Botucatu/SP, Brazil, and was done according to the ethical principles of animal experimentation (protocol No. 07/2008-CEEA) determined by the Ethical Committee of Animal Experimentation of UNESP. Muscle fiber analyses were done at the Department of Morphology, Biosciencies misspelled – UNESP – Botucatu/SP, Brazil.

Twenty-eight purebred uncastrated male Mediterranean buffaloes (*Bubalus bubalis*), offspring of the same bull, bred in native pasture and initial average age of 14 ± 1.66 mo and initial average live weight (LW) of 330 ± 9.62 kg, were bought from the Três Rios Farm in the city of Caraguatatuba, northern coast of the state of São Paulo, Brazil, to be used in this experiment. As soon as the animals were at the experiment location, were weighed after a 16-h solid fasting. Then they were kept in feedlots, randomly distributed in four collective stalls that had a total area of 300 m², in which 70 m² were covered with screen and provided with 10-m linear concrete bunk feeder and a 1500-liter Australian-type drinker with automatic float valve. The animals were randomly distributed in four groups of seven subjects each and they corresponded to the following established slaughter weight groups: 450, 480, 510 and 540 kg LW.

The adaptation period to the facilities and the feed was 28 days. During the whole experimental period, animals had *ad libitum* access to diets formulated according to the regulations of NRC (1996), level 2, so that there was an average daily gain of 1.3 kg LW. The concentrate:forage rate was approximately 70:30 in dry matter (DM) basis.

The animals were fed twice a day (equal meals) at 0800 h and 1500 h. The amount of feed was adjusted so that the leftovers were kept between 5 and 10% of the initial provision. The amount of provided ration as well as the amount of leftovers was weekly recorded so that the feed intake adjustment could be calculated. The composition of the diet used in this experiment is presented in Table 1.

2.2. Pre-slaughter management, slaughter and data collection

The animals were weighed every 28 days. When their weights were close to the pre-established weights for slaughter (450, 480, 510 and 540 kg LW), they were weighed at shortened intervals, every 14 days. Before slaughter, the animals were submitted to a 16 h feed-restriction fasting period, with free access to water. The slaughter was performed in a commercial slaughterhouse (Minerva, Barretos, SP, Brazil) within one regular working day.

Table 1
Percent composition of diet based on dry matter (DM).

Diet composition	DM (%)
Corn silage	7.8
Coast-cross hay (*Cynodon dactylon* L. Pers)	20.6
Cotton seed	8.2
Wet corn grain silage	46.0
Nucleus*	17.4
Estimated nutrients	
Total crude protein (%)	13.0
Total digestible nutrients (%)	73.2
Neutral detergent fiber (%)	23.6
Metabolizable energy (Mcal/kg)	2.7

*Nucleus composition: Ureia (6.25%), Dry Yeast (68.75%), Mineral Mixture (25%) and Rumensin® (3 ppm monensin).
Composition of mineral mixture per kilogram of product: 75 g P, 126 g Ca, 160 g Na, 240 g Cl, 20 g S, 15 mg Mg, 4.000 mg Zn, 1.800 mg Cu, 1.500 mg Fe, 1.400 mg Mn, 150 mg Co, 120 mg I,15 mg Se, 750 mg F (max), 50 mg flavors.

The carcasses were submitted to conventional cooling for 24 h at cooler temperature from 0 to 4 °C. After cooling, the left half-carcass of each animal was sectioned between the 12th and 13th ribs, and during the deboning procedures, samples from *Longissimus dorsi* muscle (LD) between the 12th and 13th ribs and from the *Semitendinosus* muscle (ST) were carefully taken from the same muscle region. Approximately 10 g of each muscle were collected. These samples were maintained at room temperature for 20 min, and then processed, which consisted the withdrawing 0.5 cm³ fragments, coating them in talc and immersing them in liquid nitrogen for 1 min; next, fragments were put into plastic microtubes with identification and, after that, they were transported in liquid nitrogen and stored in a freezer at −80 °C until the time of analysis by histological and electrophoresis techniques.

2.3. SDS-PAGE electrophoresis

For the analysis of myosin heavy chain, muscle samples were processed according to the methodology by Toniolo et al. (2007) and submitted to an electrophoresis run. MyHC isoform separation was carried out with some adaptations of the methodology by Mizunoya, Wakamatsu, Tatsumi, and Ikeuchi (2008). The samples were cut in a criostat (10 slices; 12 µm thick), collected in a tube and homogenized in 450 µl of Extracting Buffer (glycerol 10%, 2β-mercaptoethanol 5%, sodium duodecyl-sulfate 2.3%, Tris–HCl 0.757%, pH 6.8). Samples were denatured during 10 min at 60 °C and 4 drops of glycerol were added. Running buffer was composed of ultrapure glycine (28.8 g), trisma (6.068 g), sodium duodecyl-sulfate (2 g), 2β-mercaptoethanol (0.468 ml), distilled water (2 L). The targeted protein on each lane was the Myosin Heavy Chain, which is 200 kDa molecular weight. The separation gel was composed of 35% v/v glycerol (100%), 2.667% v/v acrylamide-bis (99:1), 0.995% v/v distilled water, 1.333% v/v 1.5 M Tris–HCl (pH 8.8), 1% v/v 1 M glycine, 0.4% v/v SDS (10%), 0.1% v/v ammonium per sulfate (10%), 0.005% v/v TEMED. The stacking gel consisted of 30% v/v glycerol (100%), 1.333% v/v acrylamide-bis (50:1), 3.362% v/v distilled water, 1.4% v/v 0.5 M Tris–HCl (pH 6.7), 0.4% v/v 100 mM EDTA (pH 7.0), 0.4% v/v SDS (10%), 0.1% v/v ammonium persulfate (10%), 0.005% v/v TEMED. Slabs 14 cm wide, 18 cm high and 0.75 mm thick were used. Each processed sample was loaded in each well (15 µl/well) and the electrophoresis run was performed at 4 °C for 26 h, with 180 V and 13 mA. The gels were stained with Brilliant Blue R (Sigma-Aldrich, Saint Louis, MO, USA) and photographed using Image Quant 300TL v.2002.01 (GE Health-Care, NJ, USA). The quantification of myosin chains was done by densitometry using Image Master VDS analysis software (v.3.0), with integrated optical density (IOD). Rat *Plantaris* muscle, obtained from an animal from at the university where this experiment was

performed, was used to classify myosin heavy chain isoforms. It was reserved one well in each gel, where was loaded 40 µl of sample processed.

2.4. Morphology and evaluation of muscle fiber types characteristics

The samples were taken from the freezer, transported in liquid nitrogen in appropriate containers and transferred to a Leica CM1900 cryostat microtome (Leica Biosystems, Nussloch, Germany) at −20 °C. For the cutting, the muscle samples were perpendicularly fixed in the metallic supports of the microtome with the help of *Jung Tissue Freezing Medium* resin (Leica Instruments GmbH, Germany), where several series of 10 µm thick cross-sections were obtained and fixed in previously identified slides. Muscle fiber orientation and sample integrity were verified in the first cross-section series through Hematoxilin-Eosin (HE) technique, as described by Lillie (1954).

A second series of cross-sections was used to demonstrate the oxidative metabolism of nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), using the modified technique by Dubowitz and Brooke (1973). A third series of cross-section was submitted to m-ATPase histochemical reaction (Lowe, 1997) with alkaline incubation; the pH was standardized for bubaline muscles in this study (pH = 9.83 and pH = 9.53, for LD and ST muscles, respectively). This technique was used to verify contractile activity and differentiate fiber muscle types, and consisted of the preparation of three initial solutions: Solution A – Glycine 0.1 M, NaCl 0.1 M; Solution B – solution A (Glycine/NaCl 0.1 M with $CaCl_2$ 0.75 M) and $CaCl_2$ 0.75 M (NaOH 0.1 M water added until pH value was between 9.6 and 9.8); Solution C (ATP) – ATP 0.01 M in Solution B, in which the pH was adjusted dropwise by adding NaOH 0.1 M (into more alkaline pH than the initial solution) or HCl 0.1 M (into less alkaline pH than the initial solution). The next steps consisted of the incubation of cross-section in the Solution C in a electric stove (37 °C) for the time determined in the standardization and according to each muscle (LD = 15 min; ST = 13 min); careful washing of slides with distilled water so that the cross-section did not loosen from the slide; application of $CoCl_2$ 2% on the cross-section for 5 min; rinsing of slides with distilled water; addition of ammonium sulfide solution (1:10) for 30 seconds.

In order to measure the frequency, the area and smallest diameter of muscle fibers, five areas were sampled in each cut, resulting in approximately 400 fibers per animal. A computerized image analyzer (Digital Image Analysis System QWin v3 for Windows / Leica, Wetzlar, Germany) with objective lens 20× (1 pixel = 0.4 µm) was used.

2.5. Statistical analysis

The experiment was a completely randomized design with four treatments (slaughter weights) and seven replications. Data was analyzed using the GLM (General Linear Model) of the Statistical Analysis System (SAS, 2001). The means were compared by Tukey's test and considered significant when $P < 0.05$.

3. Results

3.1. Electrophoresis

For all slaughter weight groups, electrophoresis of LD and ST muscles showed three bands of varying intensities that were characterized as MyHC isoforms when compared to the standard (rat *Plantaris* muscle). Weydert et al. (1983) explained that small rodents exhibit four MyHC isoforms (I, IIa, IIx/d, IIb).

Thus, in the muscles of the buffaloes of this experiment, we observed the presence of MyHC IIa, MyHC IIx/d and MyHC I isoforms, respectively (Fig. 1), and the lack of MyHC IIb isoform in LD and ST muscles. In Fig. 2a and c, the relative percentages of the three MyHC
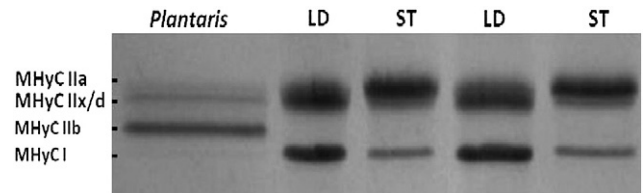

Fig. 1. Electrophoresis profile of myosin heavy chain isoforms (MyHC) in *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles of Mediterranean buffaloes. 8% SDS–PAGE. Rat *Plantaris* muscle was used as the parameter to classify myosin heavy chain isoforms.

isoforms found in both muscles are presented as well as the percentage (Fig. 2b, d) grouped in fast (MyHC IIa and MyHC IIx/d) and *slow* (MyHC I), for each group of slaughter weight. Data analysis shows that the frequency of the same isoform did not differ ($P > 0.05$) for LD and ST muscles among the slaughter weight groups. When grouping the isoforms, classifying them as either fast or slow, we observed that both muscles present a higher percentage of fast than slow isoforms (LD – 65, 61, 59 and 65%; ST – 89, 93, 92 and 92%, for the weight groups of 450, 480, 510 and 540 kg, respectively). However, the fast:slow ratio is higher for ST muscle than for LD (Fig. 2b, d), in visual assessment. The fast and slow percentages were not different among the slaughter weight groups.

3.2. Morphological analysis: HE, m-ATPase and NADH-TR

Hematoxylin-Eosine (HE) technique showed that muscle fibers presented normal morphology suitable for analysis. The cross-section presented fibers in mosaic shape, typical of animal tissue, with some apparently visible peripheral nuclei. Comparing the slaughter weight groups, there was no difference in the fiber sizes that were visually perceived for both muscles.

The analysis of alkaline m-ATPase in LD and ST muscles revealed three types of muscle fibers (Fig. 3) that allow classifying them according to the contractile activity as *fast* (strong and moderate intensity) and *slow* (weak color intensity). The NADH-TR reaction made the fiber metabolism evident and, through comparisons of serial cross-section, showed that some LD and ST muscle fibers that reacted strongly to the m-ATPase had both glycolytic and oxidative metabolism (Fig. 3). However, in both analyzed muscles, all fibers that showed low or moderate myofibrillar activity (weak and moderate color intensity, respectively) in the m-ATPase reaction, had high and moderate enzymatic activity, respectively, by the NADH-TR technique.

3.3. Morphology: frequency, area and diameter of muscle fibers

The fibers were classified according to their contractile activity into *fast* and *slow* in order to measure the parameters of frequency, area and diameter. Table 2 shows the results of LD and ST muscle measurements. The results for fiber type frequency in LD muscle showed that there was a difference ($P < 0.05$) among the slaughter weight groups. Animals with slaughter weights of 450 kg had the lowest frequency for slow fibers and highest frequency of fast fibers when compared to the fiber types frequencies of the 510 kg slaughter weight group. When data for this same parameter in the ST muscle were analyzed, a significant difference ($P < 0.05$) among the weight groups was also verified; however, the results showed that the 450 kg animals had the highest amount of slow fibers and the lowest amount of fast fibers compared to all other slaughter weight groups (Table 2). An increase frequency of fast fibers was maintained in the muscle fiber populations of both muscles regardless of slaughter weight.

The frequency of fast fibers decreased with slaughter weight in the LD but increased in the ST. The area of fast fibers increased with slaughter weight in the LD, but did not change in the ST. There was no change in the smallest diameter of the fast fibers with slaughter
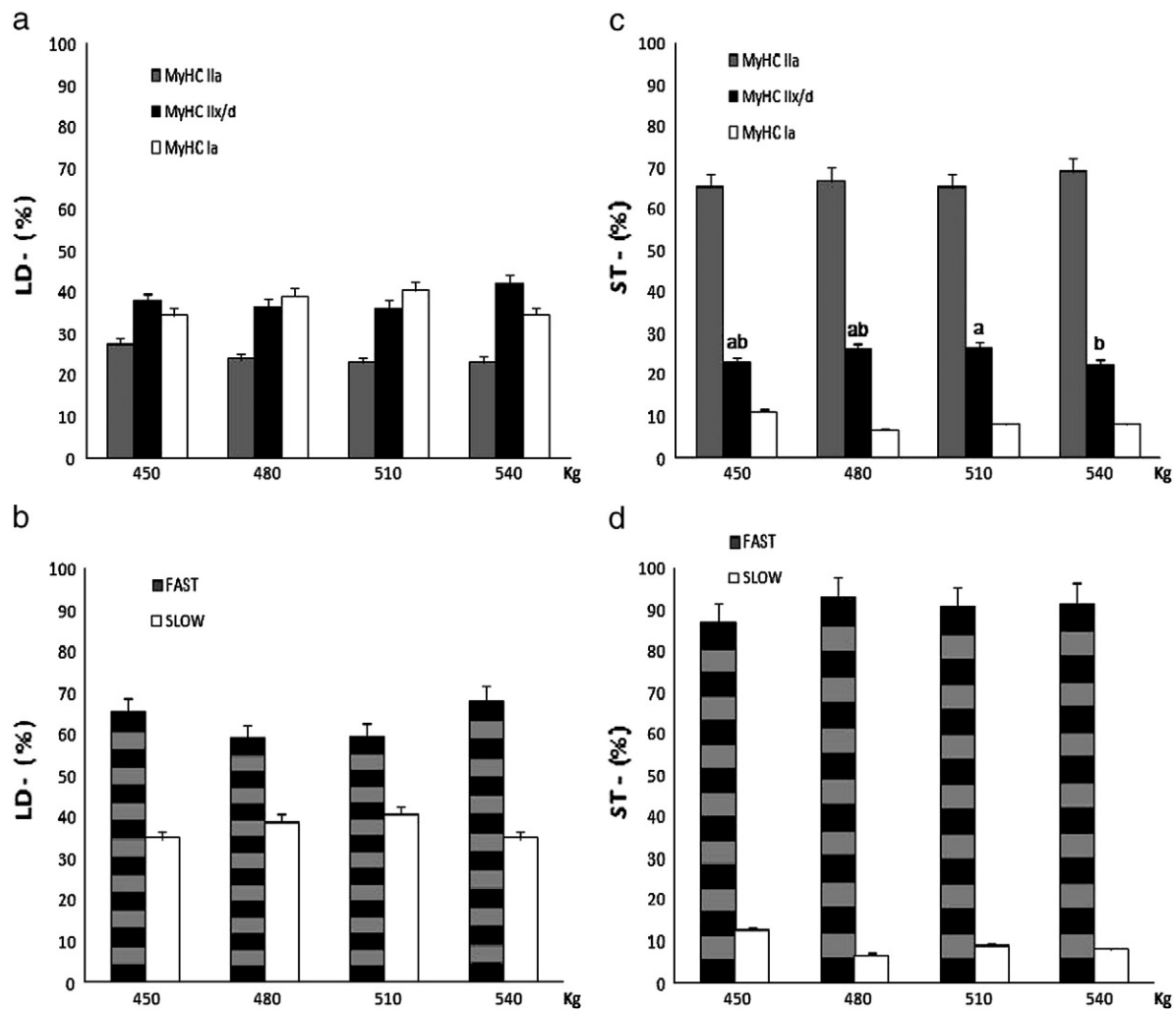
Fig. 2. Relative percentage (%) of MyCH isoforms present in *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles of Mediterranean buffaloes slaughtered at different weights (kg), quantified from the SDS-PAGE analysis. (a), (b) Relative percentage (%) of MyCH isoforms; (c), (d) Relative percentage (%) of MyCH isoforms, grouped according to the contractile activity (FAST, SLOW). [a,b] Means followed by the same letters in columns of the same color do not differ statistically by Tukey test (*P*<0.05).

weight. The frequency of slow fibers increased with slaughter weight in the LD but decreased with slaughter weight in the ST. The area of slow fibers increased with slaughter weight in the LD, but the ST showed no change due to weight increase. The diameter of slow muscle fibers decreased with slaughter weight in the LD and increased in the ST.

For both types of evaluated fibers in the LD muscle, the animals in the groups of 450 kg and 540 kg presented smaller and larger areas (*P*<0.05), respectively, when compared to other slaughter weights; this evolution in the area size was not significant for ST muscle (*P*>0.05).

There was no difference (*P*>0.05) for the parameter of fast fiber diameter in both muscles among the evaluated groups. Slow fibers had the largest diameter in the 540 kg slaughter weight group, presenting differences among the other weight groups (*P*<0.05).

4. Discussion

The aim of this study was to characterize two muscles of Mediterranean buffaloes through the analysis of the electrophoretic pattern of myosin heavy chains (MyHC), muscle fiber types (by ATPase assay) and their possible modulations according to the established slaughter weights. It was possible to demonstrate that Mediterranean buffaloes finished in feedlot and slaughtered in

different weights presented three isoforms of myosin heavy chain (MyHC I, MyHC IIa and MyCH IIx/d) whose relative percentages were different for each analyzed muscle. This difference in the isoform percentages could be related to the function of each muscle. Postural muscles that are constantly used, such LD, present a higher amount of MyHC I isoforms when compared to fast contracting muscles that are occasionally used, like ST (Ashmore, 1974; Bottinelli, Betto, Schiaffino & Reggiani, 1994).

Moreover, it was verified that buffaloes do not have MyHC IIb isoform. Studies (Chikuni, Tanabe, Muroya & Nakajima, 2001; Mascarello, Maccatrozzo, Patruno, Toniolo & Reggiani, 2004; Toniolo et al., 2004) reported that in big mammals, MyHC IIb isoform occurred in mRNA of specialized muscles like extraocular, masseter and retractor bulbi muscles; however, the presence of MyHC IIb isoform was found only in the skeletal muscle of swine, rats and marsupials. In a study on several species, Chikuni, Muroya and Nakajima (2004) also verified the lack of this isoform in buffaloes. According to Graziotti, Ríos and Rivero (2001), in all these species, the differential distribution of their fast MyHC defines only two fast muscle fiber types containing a single MyHC isoform (IIa and IIx) and an intermediate hybrid fiber population containing the two fast MHCs (IIax), and the differences among species in the expression of fast MyHC are probably due to functional and structural divergences for each MyHC isoform (Rome, Sosnicke & Goble, 1990).
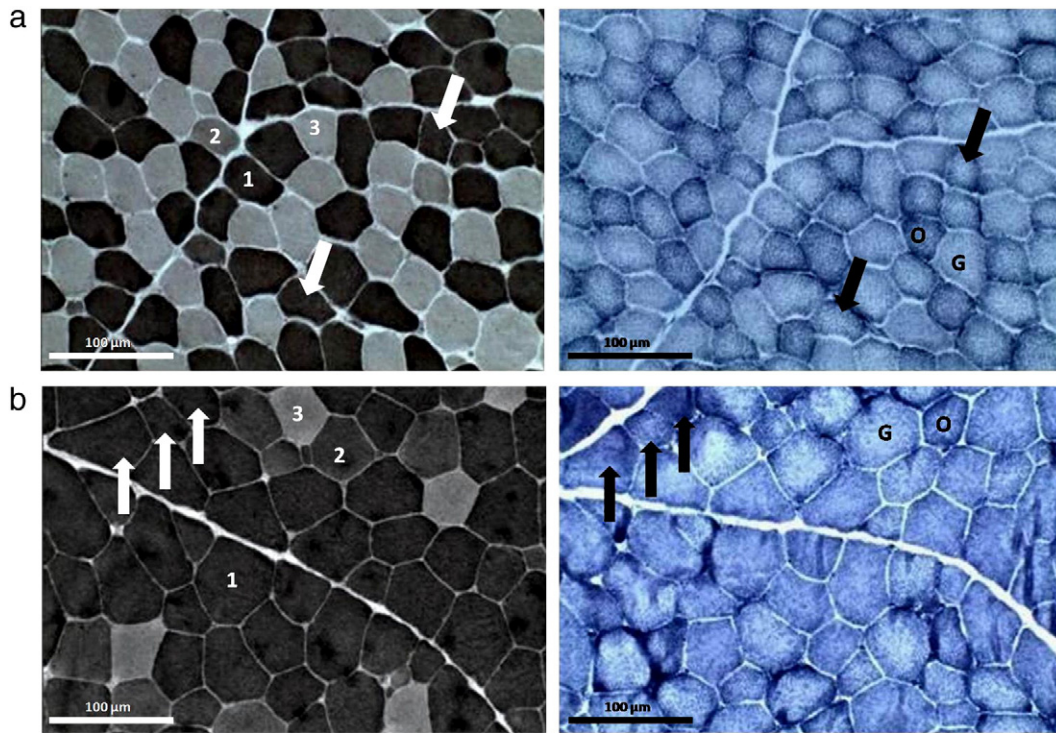
Fig. 3. Alkaline m-ATPase technique (left) and NADH-TR (right) performed in serial cuts of *Longissimus dorsi* (a) and *Semitendinosus* (b) muscles of Mediterranean buffaloes. The arrows point fibers that did not have the expected metabolic behavior according to m-ATPase. 1 and 2 = *fast* fibers, strong and moderate color intensity, respectively; 3 = *slow* fiber, weak color intensity; G = glycolytic metabolism; O = oxidative metabolism. 20× increase.

In this study, the evidence that buffaloes do not have MyHC IIb isoform, by electrophoresis assay, generates doubts about the classification of the muscle fiber types through the m-ATPase method. Knowing that pure fibers are those that possess a single MyHC isoform, it can be stated that buffaloes do not have IIB-type fibers, and that, actually, fibers which react intensely in alkaline mATPase can represent hybrid fibers, possibly IIA/X fibers. Solomon et al. (1985), examining buffaloes *longissimus* muscle using m-ATPase and NADH-TR methods, observed the presence of two fiber types, FOG (IIA) and SO (IA), in which SO presented a higher frequency. In this study, three fiber types were evident using the same reactions as mentioned by these authors. Thus, it was possible to compare the contractile activity (m-ATPase) with oxidative metabolism of each fiber (NADH-TR) through serial cross-section performed in LD and ST muscles. This comparison showed that fibers that had the same contractile activity can reveal more intense or moderate metabolism, reinforcing the idea of the presence of a hybrid fiber. The conflicting findings presented between studies may be related to differences in sample size, age, methodology, and/or physical activity levels (Staron et al., 2000).

Data from the literature (Gil, López-Albors, Vázquez, Latorre, Ramírez-Zarzosa & Moreno, 2001; Gorza, 1990; Peinado et al., 2004) showed that a similar situation to the one described in this study occurred in other animal species (conidia, ovine, rodents, swine) where the authors described that FOG and FG fibers cannot be compared to IIA and IIB fibers, respectively, because some IIB fibers presented atypical oxidative activity that can correspond to the fiber type IIX, by histochemical assay.

Table 2
Values for frequency, area and diameter of *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles of Mediterranean buffaloes slaughtered at different weights, quantified from the ATPase assay.

Variable	Slaughter weight (kg)								CV[1] (%)	CV[2] (%)
	450		480		510		540			
	LD	ST	LD	ST	LD	ST	LD	ST		
Fast										
Frequency (%)	65.34[a]	84.13[B]	59.89[ab]	89.53[A]	53.52[b]	91.88[A]	56.79[b]	90.30[A]	6.90	2,54
Area (μm²)	499.97[c]	1065.81	748.43[b]	1252.29	567.30[bc]	1158.72	1100.89[a]	1354.90	14.86	27,07
Diameter (μm)	38.10	42.62	36.18	46.95	38.34	44.72	40.80	45.45	13.03	8,51
Slow										
Frequency (%)	34.64[b]	15.87[A]	40.11[ab]	10.47[B]	46.48[a]	8.12[B]	43.35[a]	9.70[B]	10.02	20,02
Area (μm²)	506.34[c]	923.73	781.32[b]	1048.61	724.09[b]	910.28	1168.77[a]	1301.70	13.39	25,73
Diameter (μm)	39.24[ab]	41.96[AB]	36.99[b]	42.69[AB]	35.51[b]	39.45[B]	43.79[a]	47.72[A]	9.34	9,36

[a,b,c] Means followed by the same letters on the same line for LD muscle do not differ statistically by Tukey test ($P<0.05$).
[A,B] Means followed by the same letters on the same line for ST muscle do not differ statistically by Tukey test ($P<0.05$).
[1] Variation coefficient for LD muscle.
[2] Variation coefficient for ST muscle.

Different combinations of MHC isoforms may occur in the same fiber, but the predominant isoform is the main determinant of their characteristics, such as the speed of contraction and fatigue resistance (Graziotti et al., 2001). In addition, there is evidence that an incompatibility of the mATPase based and metabolic enzyme-based classification may exist (Bee et al., 1999). Presumably for this reason, data obtained by analysis of ATPase and electrophoresis were not compatible as the percentage of *slow* and *fast* muscle fibers and MyHC, respectively, according to the different slaughter weights. Thus, due to the great difficulty in differentiating pure fiber types such as IIA and IIX from hybrid fibers such as IIA/X using histochemical reaction intensity and considering that this method results in frequent misclassification, we preferred to classify and then measure the area and diameter of muscle fiber types only based on the contractile property, i.e., *fast* and *slow* fibers.

Therefore, it was possible to verify a higher frequency of fast than slow fibers in LD and ST muscles by using the MyHC isoforms as well as by analyzing the fibers using the m-ATPase method. This result may be related to the production system because animals developed for beef yield and conditioned to an intensive system, as the conditions of this study, are likely to cause fiber modulation (Ashmore, 1974). In this type of production system, the animals are inactive and, thus, their muscles are unlikely to change their contractile properties. The changes resulting from the growth for a given muscle can be evaluated by analyzing the frequency and area occupied by different types of fibers. Thus, it is expected that the muscle fibers increase with the age and live weight gain (Solomon, West & Hentges, 1986), since other factors such as disease and food restriction does not interfere with development.

Muscle fiber modulation with slaughter weight is evident when the frequencies of both muscle fiber types are analyzed by m-ATPase assay (Table 2). *Slow* fiber frequency of LD muscle increased until the 540 kg slaughter weight group when compared with data from the 450 kg slaughter weight group. Supposedly, animals raised in this system constantly use the postural muscles due to the need for balance when standing at the bunk feeder. As their body weight increases, the animals go to the bunk feeder less frequently and ruminate (lie down) for prolonged time because of their difficulty to keep standing. Therefore, *slow* fibers that are less recruited begin to change into *fast* fibers. Gotoh (2003) verified this same fiber transition in *Longissimus thoraci* muscle of Japan Black calves, with an increase of slow fibers and parallel depletion of fast fibers. Studies on animal behavior related to frequency of times going to the feeder could help elucidate such fiber modulation and this was not investigated in the present study.

Regarding ST muscle, a fast contracting muscle used as a bridge for contraction and relaxation of other muscles, its function is reduced in an intensive production system, thus, a muscle that is sporadically recruited increases its *fast* characteristics from the modulations of few existing *slow* fibers. Pette and Staron (2000) reported that in situations in which the muscles remain relaxed and overcharged, there is a decrease in *slow* fibers content and an increase of *fast* fibers. This can be verified by comparing the frequency data of fiber types in the first slaughter group (450 kg) compared to the other ones in this experiment (Table 2).

As muscle fibers develop and slaughter weight increases (Table 2), both muscles had increased area and diameter values in the last slaughter weight group (540 kg) as expected because muscle fiber hypertrophy, resulting in a muscle fibers increases size, caused weight gain. Moreover, the physiological maturity is also characterized by the increase of muscle fiber diameter (Hawkins, Moody & Kemp, 1985).

The *slow* fibers of LD muscle presented higher numerical values for area and diameter than the ones of *fast* fibers (Table 2) as reported by Peinado et al. (2004) in an ovine study; it was found that type I and IIB fibers were greater than IIA fibers. Also, the muscle fiber area did not vary statistically among the slaughter groups in the ST muscle.

5. Conclusion

It is possible to characterize and to predict the modulation process in the muscle fiber types in LD and ST muscles of Mediterranean buffaloes due to the animal development and according to the production system used. The presence or the absence of some fiber types is related to several factors such as a muscle type, live weight and production system. The SDS-PAGE technique used and the histochemical analysis contributed with valuable information for the fiber types and MyHCs isoforms characterization. LD and ST muscles of Mediterranean buffaloes do not have a MyHC-IIb isoform; only three isoforms of MyHC (I, IIa and IIx/d) were described; three muscle fiber types were also observed in the muscles, and they could be identified with alkaline m-ATPase and NADH-TR histochemical reactions. There was a higher frequency of *fast* fibers in all slaughter weight groups for both evaluated muscles. The results could support future studies related to bubaline species and their meat.

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