



## Short Communication

## Skeletal Muscle Fiber Composition of Untrained Mangalarga Marchador Fillies



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## ABSTRACT

Mangalarga Marchador (MM) is the most important and numerous equine breed in Brazil. So far, no studies have been carried out on the breed's skeletal muscle composition, which is important to develop specific physical conditioning programs. To characterize the skeletal muscle fiber composition of young MM females, *gluteus medius* muscle biopsies were obtained from 13 fillies between 2.5- and 3-year-old using a biopsy needle at 60-mm depth. Types I, IIA, and IIX fibers were determined by the metachromatic staining method of ATPase activity in myofibers using preincubation followed by incubation in alkaline medium. Relative frequency (%F), average cross-sectional area (CSA), and relative cross-sectional area (%CSA) of each muscle fiber type were determined. Considering %F, 29.5 ± 5.4% were type I, 40.3 ± 5.5% were type IIA, and 30.2 ± 5.9% were type IIX fibers. CSA of type I fibers was 2,633 ± 798 μm<sup>2</sup>, of type IIA was 3,407 ± 492 μm<sup>2</sup>, and of type IIX was 5,856 ± 1,237 μm<sup>2</sup>. %CSA was composed of 19.7 ± 4.9% of type I fibers, 35.4 ± 4.7% of type IIA, and 44.9 ± 7.4% of type IIX. The *gluteus medius* muscle of untrained MM fillies was predominantly composed of type IIA fibers, but the highest total relative area was occupied by type IIX fibers, suggesting moderate aptitude to the oxidative and glycolytic metabolisms.

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## 1. Introduction

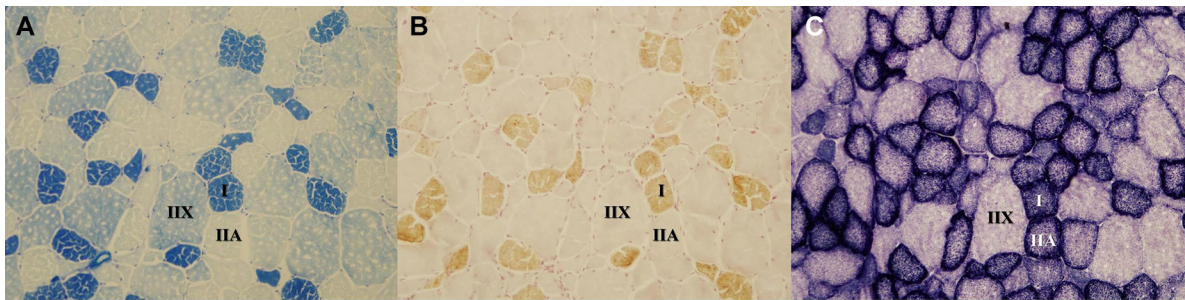
Mangalarga Marchador (MM) is the most important and numerous equine breed in Brazil. This breed's typical gait is the four-beat marcha, with alternate lateral and diagonal support interspersed by moments with triple support [1]. This movement dynamics allows the animal to always remain in contact with the ground during locomotion, which favors the stability of the animal's torso and provides

more comfort to the rider. The smooth gait of MM horses is favorable to activities such as cattle work, cavalcade, working equitation, and therapeutic riding. In April 2015, the Brazilian Mangalarga Marchador Breeder's Association (ABCCMM) counted 9,895 associated members, 598,457 registered horses, 66 regional breeders organizations in Brazil, and four regional organizations abroad (Germany, Italy, Argentina, and USA).

Horse breeds have key intrinsic characteristics regarding morphology, temper, physical aptitude, and physiology. They also have particular muscle types related to the aptitude to the types of exercises to which each breed is subjected [2]. The muscle fibers of some horse breeds raised in Brazil have been characterized: Quarter Horse and

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**Fig. 1.** Serial sections of *M. gluteus medius* of Mangalarga Marchador fillies. (A) mATPase histochemical staining: type I (oxidative, dark blue), type IIA (oxidative-glycolytic, light blue or no coloration), and type IIX (glycolytic, intermediate blue); (B) indirect immunohistochemical method: slow-twitch fibers or type I (golden color) and fast-twitch fibers or type II (lack of color); (C) NADH-TR: type I and type IIA (purple), and type IIX (light purple or no coloration;  $\times 200$ ). NADH-TR, nicotinamide adenine dinucleotide tetrazolium reductase.

Thoroughbred [3], Andalusian and Arabian horses [4,5], Crioulo [6], Mangalarga (of São Paulo state) [7], and Brasileiro de hipismo (Brazilian Sport Horse) [8]. Although the MM breed is responsible for the largest and most representative horse herd in Brazil, its muscle fibers have not been characterized yet. The present study aimed to characterize the composition of skeletal muscle fibers of MM fillies.

## 2. Materials and Methods

Thirteen MM fillies between 2.5- and 3-year-old with average body weight of  $330 \pm 30$  kg that had never undertaken a physical conditioning program were used. Biopsies of the *gluteus medius* muscle were obtained at 60-mm depth using a Bergström needle with 6-mm external diameter as methodology adapted from Lindholm and Piehl [9]. Samples were frozen in hexane, precooled in liquid nitrogen, and stored at  $-80^\circ\text{C}$  until analyzed. The experimental procedures were approved by the Ethics Commission on Animal Use (CEUA – UFMG) - protocol 237/2012.

Muscle samples were sectioned serially (12- $\mu\text{m}$  thickness) in a cryostat (Mícron gmbH, H1599 OM, 69,190, walldorf, Germany) at  $-20^\circ\text{C}$ . Histochemical analysis was used to identify or differentiate the types I, IIA, and IIX fibers and consisted of adapting the metachromatic staining method of ATPase activity in myofibers described by D'Angelis et al [10] for the preincubation in acid medium [11,12] at pH 4.45 to 4.55 for 5 to 6 minutes at  $18^\circ\text{C}$  to  $20^\circ\text{C}$ , followed by incubation in alkaline medium [13] at pH 10.50 to 10.55 for 25 minutes at  $37^\circ\text{C}$ . To verify the mATPase histochemical data, the indirect immunohistochemical method (peroxidase–antiperoxidase) [10] was used after incubation with monoclonal anti-slow myosin primary antibody (Clone NOQ7.5.4D; Sigma–Aldrich, Química do Brasil Ltda, São Paulo, SP, Brazil). The slow-twitch fibers (type I) and fast-twitch fibers (type II) were identified by the golden color of the diaminobenzidine precipitate formed in the antigen–antibody complex in the former and lack of color in the latter. The oxidative potential of the skeletal muscle fibers was assessed through nicotinamide adenine dinucleotide tetrazolium reductase [10,14]. Type I and type IIA fibers were stained purple, and type IIX were stained light purple or had no coloration. The muscle fibers

were identified only through the mATPase histochemical staining as follows: type I (oxidative, dark blue), type IIA (oxidative-glycolytic, light blue or lack of color), and type IIX (glycolytic, intermediate blue; Fig. 1).

Three images were captured from each slide using a photomicroscope (Camedia Olympus 95-98 ME). These images were transferred to the image analysis software Scion Image, and the artifact-free regions that contained between 30 and 80 muscle fibers were selected. The relative frequency of muscle fiber types for each sample (%F) and the average cross-sectional area (CSA) were determined. The relative CSA (%CSA) that a fiber type occupied in a muscle sample was calculated by dividing the product of the percentage and the mean CSA of the fiber type by the sum of these products for all muscle fiber types [15]. Data were submitted to descriptive analysis, and results are expressed as means  $\pm$  standard deviation.

## 3. Results

Results are presented in Table 1.

## 4. Discussion

According to the ABCCMM, MM horses are direct descendants of Alter breed horses from the “Coudelaria de Alter do Chão” situated in the Alentejo region in Portugal [16]. Horses of this stud farm are originated from Andalus breed, and the common ancestry may explain the similarity between the distribution of skeletal muscle fibers in MM fillies and Andalus specimens. As in the present study, Andalus stallions [4] showed a prevalence of type IIA fibers (42.8%) followed by type IIX (29.5%) and type I (27.7%).

**Table 1**

Relative frequency (%F), average cross-sectional area (CSA), and relative cross-sectional area (%CSA) of types I, IIA, and IIX muscle fibers of Mangalarga Marchador fillies.

Variables	Type I	Type IIA	Type IIX
F (%)	$29.5 \pm 5.4$	$40.3 \pm 5.5$	$30.2 \pm 5.9$
CSA ( $\mu\text{m}^2$ )	$2,633 \pm 798$	$3,407 \pm 492$	$5,856 \pm 1,237$
%CSA (%)	$19.7 \pm 4.9$	$35.4 \pm 4.7$	$44.9 \pm 7.4$

Abbreviation: SD, standard deviation.

Data are expressed as means  $\pm$  SD.

However, in the same study [4], Andaluz females had a virtually homogenous distribution among the three fiber types (34.8% IIX, 33.0% IIA, and 32.2% I). According to the authors, the difference found between the genders was attributed to the females being more inactive compared with the males in the study. The MM fillies in the present study, despite not performing physical conditioning activities, grazed in very large pens. Hence, their easy access to volunteer activity can justify the closer muscle composition to that of the active Andaluz stallions.

The MM and Mangalarga breeds had the same genetic origin, but different selection goals, which led to the creation of two different associations. In 1934, the ABCCRM was created in the state of São Paulo, and later in 1949, the Brazilian Mangalarga Marchador Breeder's Association (ABCCMM) was founded in the state of Minas Gerais. Roncati et al [7] observed that adult Mangalarga mares (5- to 12-year-old) with no athletic activity had approximately 48% type I muscle fibers, 28% type IIA, and 24% type IIX. A comparison between the results in the present study and those by Rivero et al [4] and Roncati et al [7] shows that, despite the genetic proximity with the Mangalarga of São Paulo breed, MM's muscle composition is closer to Andaluz's, which was the genetic basis of these two Brazilian breeds. This difference may be the result of including the Thoroughbred, Arabian, Anglo-Arabian, and American saddle horse breeds in Mangalarga's selection [17], which did not occur officially with MM.

Three myosin heavy chain (MyHC) isoforms (named types 1, 2A, and 2X) are functionally expressed in adult equine skeletal muscles [18]. A minute amount (<1%) of the fastest MyHC-2B isoform is also expressed, despite a MyHC-2B pseudogene present in the equine genome [19]. The latter suggests that an ancestral MyHC-2B gene has lost its function in horses during the course of their natural evolution. Type IIB fibers are not expressed on horses' muscles on a relevant amount neither on a functional nor a biological approach. Based on these facts, the present study uses the nomenclature of "IIX" to the fast-twitch glycolytic fiber type instead of "IIB," used in some scientific publications.

The proportion of the three muscle fiber types in a horse is related to the breed's aptitude to athletic activities. The Quarter Horse breed showed muscles composed by 6.8% of type I fibers, 48.3% of type IIA fibers, and 44.9% of type IIX fibers [3]. The predominance of fast-twitch fiber (types IIA and IIX) is crucial for the animals to perform high-speed and high-power exercises for a short period of time. On the other hand, resistance to fatigue and high aerobic energy production capacity are essential for long duration athletic activities; thus, the higher number of type I (slow-twitch oxidative) and type IIA (fast-twitch oxidative/glycolytic) fibers is important. Arabian adult females presented 42% of type I fibers, 30% of type IIA fibers, and 28% of type IIX fibers in the study of Rivero and Diz [20]. Later, Rivero et al [21] reported 51% of type I fibers, 46% of IIA fibers, and 3% of IIX fibers in Arabian horses trained for endurance race. In the present study, MM fillies had a larger number of type IIA fibers, which suggests an ability to perform long duration, moderate intensity exercises because this fiber type is faster twitching than type I fibers but has better resistance to fatigue than type IIX. The

characteristics of type IIA fibers favors MM's performance in its main functional activity, the marcha competition, in which the animal, ridden by its trainer, moves around an oval track in marcha gait for 20 to 70 minutes at a mean speed of 12 km h<sup>-1</sup> [22].

In the *gluteus medius* muscle of MM fillies, type IIX fibers take up a greater %CSA than type I and type IIA fibers. This result was not expected because the proportion of type IIA fibers was higher compared with the other two types. However, the greater CSA of type IIX fibers was responsible for the %CSA value obtained for this fiber type. According to Rivero and Diz [20], these results are not favorable to the performance in long duration, moderate intensity exercises. In these modalities, a greater %CSA of type IIA fibers compared with type IIX would be more appropriate because, despite type IIA fibers having slower twitching speed, they are more resistant to fatigue than type IIX fibers. Nevertheless, Rivero et al [5] assessed the effect of age on the muscle composition and morphometry of Andaluz and Arabian horses and reported that, as the animals got older, the proportion of type I fibers increased, the proportion of type IIX fibers decreased, and the %CSA of type IIA fibers increased. Hence, if these changes also occur in MM animals, there will be a larger %CSA of type IIA and type I fibers in adult animals compared with the fillies in the present study, which would be favorable to the performance in the breed's official marcha competitions.

## 5. Conclusions

The *gluteus medius* muscle of untrained MM fillies was predominantly composed of type IIA fibers, but the highest total relative area was occupied by type IIX fibers, suggesting moderate aptitude to oxidative and glycolytic metabolisms. Further studies are needed to describe the skeletal muscle composition of MM males and females at different ages.

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