The influence of the metacarpophalangeal joint angle on the transversal area and mean echogenicity of the superficial digital flexor tendon and suspensory ligament in gaited horses

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The objective of this study was to assess the influence of the metacarpophalangeal (MCP) joint angle on the transversal area (TA) and mean echogenicity (ME) of the superficial digital flexor tendon (SDFT) and suspensory ligament (SL) in gaited horses. Ultrasound images were obtained from 50 healthy adult horses of the Mangalarga Marchador (MM; n=25) and Campeiro (n=25) breeds. Static and dynamic angles of the MCP joint were measured from photographs and video recordings. Higher ME values were evinced for the SL only in the group with the smaller dynamic angles of the MCP joint in the MM horses, in addition to weak negative correlation between the dynamic angle and ME. Moreover, weak negative correlation was also observed between the static angle and TA of the lateral branch of the SL and between the static angle and the ME of the SDFT. However, the difference observed in the group of MM horses, as well as the weak correlation, was not considered sufficient to support the hypothesis that a smaller angle of the MCP joint (greater extension) is associated with larger TA and ME values for the structures. The results also suggest that the static and dynamic angles of the MCP joint do not influence the TA and ME values of the SDFT and SL in gaited horses.

Key words: *equine, fetlock, suspensory apparatus, ultrasound*

The equine suspensory apparatus is comprised of the suspensory ligament (SL), proximal sesamoid bones (PSB), and distal sesamoid ligaments, and its main function is to support the metacarpophalangeal (MCP) joint [13, 15]. When weight is placed on the limb, the suspensory apparatus and flexor tendons prevent excessive extension of the MCP joint, both in the static position and during locomotion [11].

The MCP joint extension angle influences the stress imposed over the superficial digital flexor tendon (SDFT) and SL directly [11]. Considering this biomechanical characteristic, greater extension of the MCP joint, which is associated with considerably more stress generated on the tendons and ligaments, may be a cause of structural modifications to these elements [10]. These modifications to the structure and function of tendons and ligaments affect the transversal area (TA) and echogenicity values directly. Both values are partially related to the number of collagen fibers, fat, muscle fibers, and nerves, which cause the reflection and dispersion of sound waves at their interfaces, affecting the acoustic impedance and the size of the ultrasound image [1].

The Mangalarga Marchador (MM) and Campeiro horses originate from Brazil and present the "marcha" as their main characteristic with great performance and comfort. MM horses are widely distributed throughout Brazil and are present in several countries [4]. The Campeiro horse is a locally adapted breed that has developed through natural selection in the southern region of Brazil and has its own characteristics related to adaptation to the environment [24].

The "marcha" is a four-beat gait in which the horses alternate between lateral, diagonal, and tripedal support without ever losing contact with the ground [16]. This specialized gait is developed through repetitive movements that have a high impact, especially on the forelimbs [5], possibly generating greater stress on the flexor tendons and ligaments.

The objective of the study was to assess the influence of the MCP joint angle on the TA and mean echogenicity (ME) values of the SDFT and SL of the forelimbs of gaited horses.

Materials and Methods

The study was approved by the Ethics Committee on Animal Use of Santa Catarina State University under protocol number 5868161216. The nature of the research was explained to the owners, and an informed consent form explaining the terms of the research was provided to them to read and sign. Therefore, animals were only included in the project after receiving the consent of their owners.

This study evaluated 25 MM horses and 25 Campeiro horses. The group of the MM horses included 16 females, six stallions, and three geldings with a mean age of 7.1 ± 3.3 years (2.5 to 17.0 years), mean wither height of 1.47 ± 0.03 m (1.40 m to 1.52 m), mean body weight of 384.00 ± 39.93 kg (327.00 kg to 474.00 kg), and mean body mass index (BMI) of 178.81 ± 17.18 kg/m^2 (155.22 kg/m^2 to 217.50 kg/m^2). The Campeiro horses included 18 females, six stallions, and one gelding with a mean age of 7.2 ± 3.6 years (3.0 to 18.0 years), mean wither height of 1.45 ± 0.02 m (1.39 m to 1.48 m), mean body weight of 394.60 ± 36.87 kg (321.00 kg to 459.00 kg), and mean BMI of 188.64 ± 18.52 kg/m^2 (156.69 kg/m^2 to 222.45 kg/m^2). The animals came from four farms located in the states of Paraná and Santa Catarina (southern region of Brazil).

We only included in the study animals that were registered in the genealogical registration book of the Brazilian Association of Mangalarga Marchador Horse Breeders and the Brazilian Association of the Campeiro Horse Breeders and presented "marcha" as their gait in its different forms. To minimize the physical activity effects on the tendons and ligaments, only horses that had not undergone physical training for at least six months were used [1]. Only clinically healthy animals with no history of injuries and no clinical alterations related to the digital flexor tendons and ligaments of the palmar/plantar region of the metacarpal/metatarsal or lameness of any nature were included in the ultrasound examinations.

The horses were subjected to clinical examinations specific to the locomotor system, as described by Baxter *et al*. [6], which included a history and static and dynamic inspections (straight line and in circles). Palpation of the limbs was conducted with a special focus on the palmar

structures of the metacarpal, initially with the limb resting on the ground and then with the limb suspended. Only animals that did not present any local alterations or lameness were used for the ultrasound evaluation.

The ultrasound examinations were performed with the horses in the standing position using a portable ultrasound device (A6 Vet, SonoScape Medical Corp., Shenzhen, China) equipped with a 5–12 MHz multifrequency linear transducer. To ensure better acoustic coupling, the palmar region of the metacarpal, as well as of the lateral and medial faces of the distal third, was shaved and then washed with soap and water. We applied 70% alcohol to the skin, ensuring that the entire area was covered with the aid of a compress and then used ultrasound gel between the skin and transducer during the examination. Horses that were untamed and those that did not allow the performance of any preparation step or the examination were sedated in advance by administering detomidine hydrochloride at a dose of 10 to 20 μg/kg intravenously.

Transverse ultrasound images were recorded in six zones of the palmar region of the metacarpal [1, 19]. The length of the metacarpal bones was measured in ten horses of each breed. The mean value of these measurements was then divided by six to determine the length of each zone in each breed. The measurement was carried out between the distal aspect of the accessory carpal bone and the proximal surface of the lateral PSB. Each zone designated for the examination was demarcated with white or blue chalk, according to the color of the coat, on the lateral face of the metacarpal to facilitate its localization during the examination. The transverse images were recorded at the central portion of each zone, and the evaluated structures consisted of the SDFT and SL. In the more distal zones, the SL branches cannot be visualized from the palmar face, and for this reason, they were evaluated from the lateral and medial palmar faces at three heights: 1) at the SL bifurcation level, 2) at the midpoint between the SL bifurcation and the ipsilateral PSB, and 3) at the insertion region on the PSB of each branch of the suspensory ligament of the fetlock.

All ultrasound images were obtained at a frequency of 8 to 12 MHz and a depth of 49 mm. The gain, focus, and time gain compensation (TGC) controls were standardized constantly for all evaluations. The examinations were carried out by a single individual (JS), and the images were recorded and stored on an external hard drive for later determinations of measurements.

The measured variables consisted of TA (mm^2) [8] and ME. The ME was determined by analyzing images according to a scale with 256 shades of gray, with zero being black and 255 being white [25]. Each variable was measured three times in each structure and each zone, with the mean values being used to calculate the overall means. The mean

TA and ME values for the SDFT, SL, LB-SL, and MB-SL were obtained from the mean values obtained in each zone. A single individual (JS) carried out all measurements using the ImageJ software [25].

The MCP joint angle was evaluated with the horses in a static position (static angle) and during locomotion (dynamic angle). For these measurements, images of the left forelimb (LFL) were obtained from photographs (Sony Cyber-Shot 14.1 mega pixel, Sony, Tokyo, Japan) and videos (Sony HDR-CX220, Sony) of the animals resting and in motion, respectively. Photographs were used to measure the static angle and were taken from the left side of the animals at a height of 20 cm and a distance of 3 m, with the animals in a quadrupedal position with their limbs perpendicular to the ground. Videos were used to measure the dynamic angle and were filmed at a height of 20 cm and a distance of approximately 6 m while the horses wore a halter and performed their gaits. The videos were analyzed frame-by-frame using the Windows Media Player Classic^{TM} video player, with the images used for the measurements being frozen when the LFL was perpendicular to the ground, in bipedal support, in the phase of maximum weight support. Before the photographs and videos were taken, white or black stickers, according to the coat color, were attached to the surface of the limb to demarcate the locations for posterior measurements: 1) lateral face of the middle third of the metacarpal, 2) junction between the third metacarpal bone and the proximal phalanx, and 3) middle portion of the proximal phalanx [9]. A single individual (JS) measured the static and dynamic angles of the MCP joint using the ImageJ software. A line was traced over the markers, and the angle formed between the metacarpal axis and the pastern was assumed as the dorsal angle of the MCP joint (Fig. 1). The measurements were repeated three times, with the mean values being used to calculate the overall means. Based on whether the values were larger or smaller than the median, two groups were formed for each breed for both the static angle and dynamic angle of the MCP joint.

A descriptive analysis of the data was carried out by calculating the arithmetic means and standard deviations of the TA and ME and the medians of the static and dynamic angles of the MCP joint. Three measurements were taken for each structure in each zone, and the variation coefficient (VC) was calculated; measurements were repeated when the VC was over 5%. The normality of the data was evaluated with the Shapiro-Wilk test. Student's t-test or the Mann-Whitney U test were used according to the normality of the data to compare the variables between groups with larger and smaller static and dynamic angles of the MCP joint. Pearson's correlation coefficient was calculated for each variable in each structure. The analyses were carried out using the GraphPad Prism 7 software, and the significance level adopted was α=0.05.

Results

Using the values obtained for the static and dynamic angles of the MCP joint in the MM and Campeiro horses (Table 1), each breed was divided into larger and smaller angle groups based on the median values: group 1 ≤ median value and group 2 >median value (Table 2).

No significant differences (P>0.05) were observed in the TA and ME values of the different structures between the groups with the larger and smaller static angles of the MCP joint in the MM (Fig. 2), and Campeiro horses (Fig. 3). A significant difference (P=0.039) was evinced for the ME of the SL in the MM horses, with higher values being observed for the group with the smaller dynamic angles of the MCP


Fig. 1. Locations of attachment of the stickers for delimiting the lines traced for measuring the dorsal angle of the metacarpophalangeal joint (arrow). 1) Lateral face of the middle third of the metacarpal, 2) junction between the third metacarpal bone and the proximal phalanx, and 3) middle portion of the proximal phalanx.

Table 1. Mean, standard deviation, and minimum and maximum values of the static and dynamic angles of the metacarpophalangeal joint observed in the 25 Mangalarga Marchador (MM) horses and 25 Campeiro horses

Breed	Static angle (°)	Dynamic angle (°)
MM	148 ± 4 (141–158)	126 ± 7 (113–138)
Campeiro	152 ± 4 (145–162)	129 ± 4 (121–135)

Table 2.   Groups related to the static and dynamic angles of the metacarpophalangeal joint in the 25 Mangalarga Marchador (MM) horses and 25 Campeiro horses

Breed	Static angle		Dynamic angle	
	Group 1	Group 2	Group 1	Group 2
MM	≤ 148° (n=14)	>148° (n=11)	≤ 125° (n=13)	>125° (n=12)
Campeiro	≤ 152° (n=14)	>152° (n=11)	≤ 130° (n=15)	>130° (n=10)
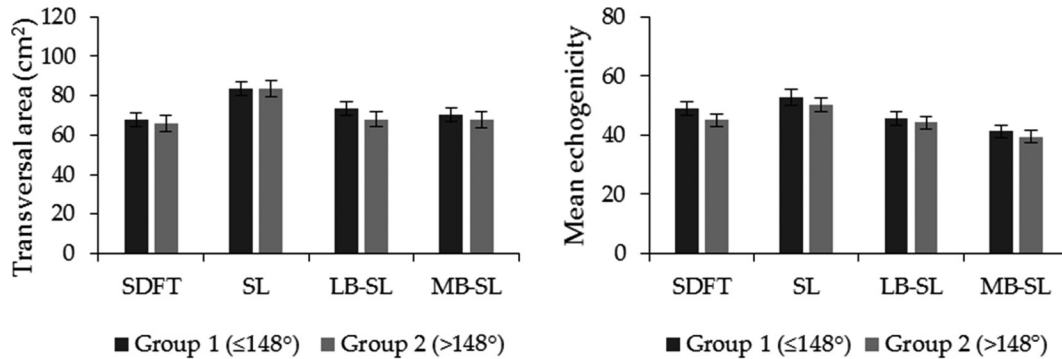
Fig. 2.   Comparison of the transversal area (cm^2) and mean echogenicity of the superficial digital flexor tendon and suspensory ligament between groups of MM horses according to the static angle of the metacarpophalangeal joint: group 1, 148° or smaller (n=14), and group 2, greater than 148° (n=11). SDFT, superficial digital flexor tendon; SL, suspensory ligament; LB-SL, lateral branch of the suspensory ligament; MB-SL, medial branch of the suspensory ligament.
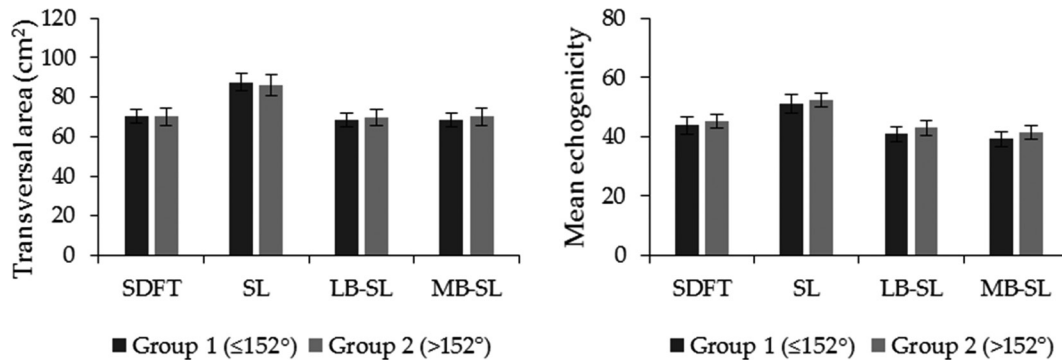
Fig. 3.   Comparison of the transversal area (cm^2) and mean echogenicity of the superficial digital flexor tendon and suspensory ligament between groups of Campeiro horses according to the static angle of the metacarpophalangeal joint: group 1, 152° or smaller (n=14), and group 2, greater than 152° (n=11). SDFT, superficial digital flexor tendon; SL, suspensory ligament; LB-SL, lateral branch of the suspensory ligament; MB-SL, medial branch of the suspensory ligament.

joint (Fig. 4). The other structures did not show significant differences (P>0.05) related to the TA and ME between the groups with the larger and smaller dynamic angles of the MCP joint in the MM (Fig. 4), and Campeiro horses (Fig. 5).

Weak negative correlation was evinced between the static angle of the MCP joint and the TA of the lateral branches of the suspensory ligament (LB-SL) and between the static angle and the ME of the SDFT. Regarding the dynamic angle, weak correlation was observed with the ME of the SL. The other variables and structures did not show correlations with the static or dynamic angles of the MCP joint (Table 3).
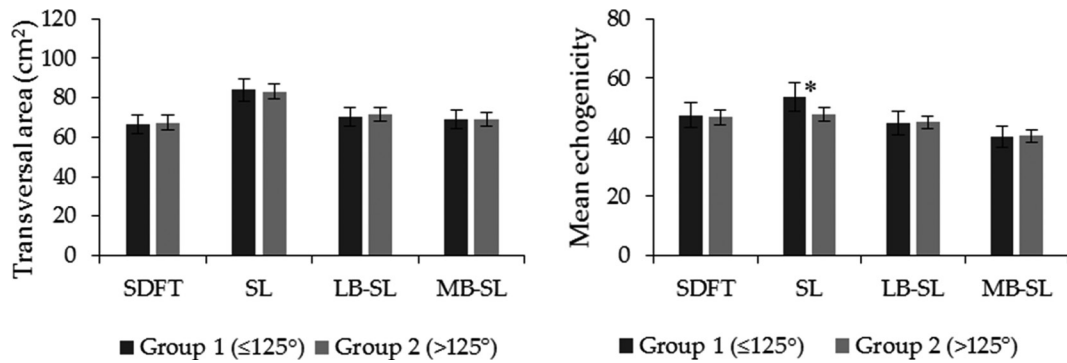
Fig. 4. Comparison of the transversal area (cm^2) and mean echogenicity of the superficial digital flexor tendon and suspensory ligament between groups of MM horses according to the dynamic angle of the metacarpophalangeal joint: group 1, 125° or smaller (n=13), and group 2, greater than 125° (n=12). SDFT, superficial digital flexor tendon; SL, suspensory ligament; LB-SL, lateral branch of the suspensory ligament; MB-SL, medial branch of the suspensory ligament. *P=0.039 according to the Mann-Whitney *U* test.
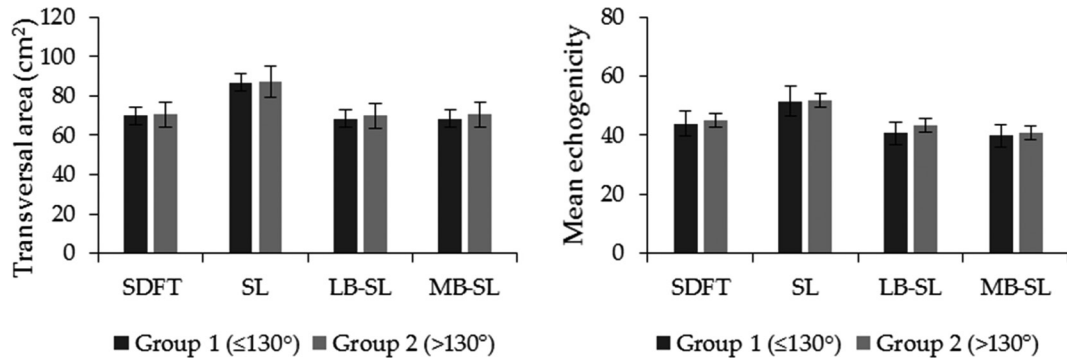
Fig. 5. Comparison of the transversal area (cm^2) and mean echogenicity of the superficial digital flexor tendon and suspensory ligament between groups of Campeiro horses according to the dynamic angle of the metacarpophalangeal joint: group 1, 130° or smaller (n=15), and group 2, greater than 130° (n=10). SDFT, superficial digital flexor tendon; SL, suspensory ligament; LB-SL, lateral branch of the suspensory ligament; MB-SL, medial branch of the suspensory ligament.

Table 3. Correlation coefficient (r) between the static and dynamic angles of the metacarpophalangeal joint and the values for the transversal area (TA) and mean echogenicity (ME) of the superficial digital flexor tendon (SDFT), suspensory ligament (SL), and the lateral and medial branches of the suspensory ligament (LB-SL and MB-SL, respectively) for the 25 MM horses and 25 Campeiro horses

Structure	Static angle		Dynamic angle	
	TA	ME	TA	ME
SDFT	0.00	−0.26	0.17	0.09
SL	0.06	−0.04	0.11	−0.20
LB-SL	−0.24	−0.17	−0.02	0.05
MB-SL	−0.02	−0.03	0.02	−0.02

Discussion

The most sensitive points for ultrasonographic evaluation of tendon or ligament injuries are changes in echogenicity, size, contour, and definition of margins. Understanding and investigating the factors that might influence this are essential for correct diagnosis [1]. The results of this study did not confirm the hypothesis that the MCP joint angle influences the TA and ME values of the SDFT and SL in gaited horses. Factors such as physical constitution and exercise may influence the TA values and the echogenicity of the tendons and ligaments in horses [1, 22]. The influence of BMI on echogenicity has been attributed to a different organization of fibers due to the increased load over the limbs in horses with a higher BMI [1]. In turn, physical exercise is associated with an increase in TA due to the adaptative response to the tension generated during athletic performance, which results from alterations to the extracellular matrix composition [7, 10].

It is known that resistance to MCP joint extension is a primarily passive process in which the SL and the flexor tendons act as linearly elastic "springs". Therefore, the increase in the amplitude of MCP joint extension results in a rise in the tension of such structures [23], which could influence the TA and ME values. In a study by Shoemaker *et al.* [21], desmotomy of the accessory ligament of the SDFT increased the tension of the SDFT, which was attributed to the hyperextension of the MCP joint resulting from the procedure. Butcher and Ashley-Ross [9] observed greater extension of the MCP joint during gallop in two-year-old Thorough-bred horses compared with those in older age ranges. The authors attributed this difference to the smaller resistance to deformation of the suspensory apparatus in younger horses and posited that athletic training could result in an increased TA, providing greater resistance to MCP joint extension in older horses.

In the present study, sources of variation that could have influenced the results were minimized, as the acquisition of the images, as well as the measurements, was performed by a single individual [18], with a maximum VC of 5% for each variable in each structure [22]. Moreover, only healthy horses that had not been trained for at least six months were used. In this way, microlesions resulting from athletic training that could affect the TA and ME determinations were avoided [12, 17].

Photogrammetry has been used as a measuring method for linear distances and angular measurements in horses [2, 3, 20]. However, radiographic analysis is considered more precise for assessing joint angles due to the smaller variation of the results. The variations related to the photogrammetry technique are attributed to the possible dislocation or incorrect placement of the markers, which is performed subjectively and suffers from high inter- and intra-operator variation [14]. Therefore, to reduce the sources of variation in the present study, a single individual carried out the placement of the markers. For the dynamic angle, the use of radiographic images was not possible, as the angle was measured with the animal in motion.

A difference in the ME values of the SL was observed only between the groups with the smaller and larger MCP joint angles in the MM horses. Moreover, the weak correlation observed does not support the hypothesis that a smaller dorsal angle of the MCP joint (greater extension) is related to higher TA and ME values.

This study has some limitations that merit being mentioned. The division of animals into breed groups was performed due to the difference in the static and dynamic angles of the MCP joint between the breeds (Table 1), which made it impossible to form a homogeneous group with more animals from both breeds. In addition, the magnitudes of the differences between the static and dynamic angles of the fetlock in each group were small. Thus, the differences in the tension of the structures could be small and not sufficient to cause structural changes identifiable through ultrasound. However, this is the first study to assess the influence of the MCP joint angle on the TA and ME values in horses. Future studies related to the topic are necessary in other gaited horses and horses subjected to physical training, which could intensify the structural modifications of the tendons and ligaments related to the more considerable tension generated by greater extension of the MCP joint.

In conclusion, the results of this study indicate that the static and dynamic angles of the metacarpophalangeal joint do not influence the transversal area and mean echogenicity values of the superficial digital flexor tendon and suspensory ligament in gaited horses.

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Conflicts of Interest

The authors declare no conflict of interest.

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