Morphological and Quantitative Description of Connective, Adipose and Vascular Tissues of the Digital Torus of Horses

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INTRODUCTION

During the evolution the members of horses have undergone a selection for locomotion at high speeds, including the simplification of the distal region of the limb into a single digit (Denoon 1994). The palmar or plantar portion of the hoof is the first touching the ground, thus the structures with the greatest shock absorption capability are located on this part of the hoof. And the most part of impact in the hoof is cushioned at heel through digital tissues and acts in the proximal interphalangeal joint, helping to dissipate the impact and reducing the weight on the digit and on a cuneiform part involved in the strengthening of the central portion of the frog during the race stop (Floyd et al., 2007). For Taylor et al. (2005) fore limb digital torus in response to

The digital torus was characterized as a modified subcutaneous tissue working in shock absorption during gait and aiding the venous return from the hoof. The present study quantified the proportion of tissues compounding palmar and plantar digital torus of horses. For this, we selected fore- and hind-limbs of eight crossbred adult horses, without limb disorders. Torus sections were submitted to conventional histology, cut to a thickness of 4µm and stained with Hematoxylin/Eosin, AlcianBlue, Safranin O, and Gomori'strichrome. With digital optical microscope, the connective tissue was quantified using the image analysis software Image-Pro Plus®, to quantify the adipose tissue, myxoid tissue and blood vessels it was used the point system, and for the differentiation of collagen fibers, it was used the polarized Picrosirius red staining. In the forelimbs, the mean value and the standard error of the proportion of connective tissue was 68.7%±5.7, of the adipose tissue was 8.9%±4.60, and of blood vessels was 6.87%±3.48. Types III and I collagen fibers presented mean value and standard error of 73.24%±1.87 and 9.15%±1.6, respectively, while the myxoid tissue, 4.03%±1.90. In turn, hindlimbs presented a proportion of connective tissue of 80.52%±0.36, of adipose tissue was 6%±3.06 and of vessels was 3.88%±1.98. Types III and I collagen fibers presented mean and standard error of 75.61%±2.53 and 4.55%±0.68, respectively, whereas the myxoid tissue, 6.38±2.96. Besides that, significant difference was observed between fore and hindlimbs considering the quantification of connective tissue, as well as between collagen fibers and myxoid tissue.
vertical load respond as a restraint system in the displacement of the middle phalanx or like a passive structure ensuring a greater flexibility to the two caudal thirds of these structures in the fore limbs. Moreover, the displacement occurs on the distal palmar axis and is not affected by the degree of support on the ground.

Microscopically, according to Egerbacher et al. (2005), the shock absorption by digital torus occurs because during this process there is a deformation of adipocytes, leading to an expansion of the collagen fiber bundles, once they are closely related. In the region where the collagen fibers diverge gaps are formed, being occupied by the myxoid tissue, with a hyaluronic acid-rich matrix, which is able to retain water, making more viscous the interstitial fluid. In this way, collagen fibers are enclosed by a sort of gel that assists in balancing the mechanical pressure in the connective tissue. This allows the digital torus to compress and expand during locomotion, providing a much greater dynamics than might be supposed.

Based on the morphological diversity evident between different organic systems of horses, the present study aimed to set by means of histochemical and morphometric techniques the characterization and composition of structures comprising the digital torus of horses. This can be performed by establishing properly the architecture and arrangement of tissues forming the digital torus of fore- and hind limbs of these animals, especially in relation to connective, adipose and vascular tissues.

MATERIALS AND METHODS

It was studied eight digital torus of crossbred adult horses, four palmar and four plantar, taken from animals of both sexes used to pull carts, 334kg average weight. All the animals died in the Large Animal Veterinary Hospital of the University of Brasília due to problems not related to the locomotor system. The experiments were conducted in accordance with the guidelines of the Ethics Committee of Animal Use in the Institute of Biological Sciences of the University of Brasília, Brazil (Protocol 51203/2010).

Using a band saw, hooves were sectioned at the level of the median sagittal plane, allowing the observation and isolation of torus. These were fixed in aqueous solution of 10% formaldehyde for 48 hours. After this, eight fragments of each torus were randomly taken and subjected to dehydration in increasing ethanol, cleared in xylene, and embedded in paraffin. With a manual microtome (Leica RM 2125RT), the fragments were sectioned into slices of 4μm thickness, and stained with Hematoxylin/Eosin, Alcian Blue, Picrosirius red, Safranin O, and Gomori’s trichrome.

Photomicrographs of five random fields of each fragment were taken using a BX51 Olympus® microscope coupled to a capture and image analysis software ProRes® Capture Pro 2.5. In order to quantify the amount of connective tissue it was employed the image analysis software Image Pro Plus 6.0®. The area occupied by blood vessels, myxoid tissue and adipocytes was measured with a point system (Gundersen et al. 1988).

The statistical analysis was run in the software SigmaStat®. First, the homoscedasticity was checked by the Kolmogorov–Smirnov test, followed by the non-parametric Mann–Whitney test for independent samples, by comparing the tissues constituent of digital torus between fore- and hind limbs, at a significance level of 5%.

RESULTS AND DISCUSSION

The digital torus of horses, of both fore- and hind limbs, were predominantly formed by dense connective tissue (Table 1, Fig.1). These structures presented a complex architecture, predominantly constituted of collagen fibers arranged in parallel thick and irregular bundles, oriented in different directions and intermingled with elastic fibers, fibrocartilage and myxoid tissue in both limbs (Fig.2B,C and 3C).

The distal ends of fore- and hind limbs of horses support the body at rest or when it moves forward when in motion, by distributing the forces resulting from the interaction between the animal and the ground. However, there is a clear difference in the weight distribution between limbs. The fore limbs support around 60% of the entire weight of the horse, whereas the hind limbs support only about 40% of weight (Dyce et al. 2004). With our results, despite this uneven distribution of weight between fore- and hind limbs, the constitution of the torus was not different, corroborating Bowker et al. (1998) who described a greater amount of this tissue in fore limbs.

Small areas of fibrocartilage and hyaline cartilage were observed in the stroma of torus. The myxoid tissue was arranged among collagen fibers as a dense connective tissue, intermingled with areas of fibrocartilage and hyaline cartilage. This matrix was abundant, poorly vascularized and reagent with Safranin O and Alcian Blue (Fig.3D,E).

Collagen is a protein with vital importance in the constitution of the extracellular matrix of connective tissue, responsible for great part of its physical properties. The Picrosirius red staining is based on the red color of collagen, and reticular fibers, cartilages and basal membranes, which when associated with polarizing microscopy enables the observation of collagen by the parallel arrangement of tropocollagen molecules (Junqueira et al. 1979). Type I collagen fibers have presented color from red to bright yellow with intense birefringence, while type III fibers presented greenish color.

Adipocytes were found as lobes isolated by
elastic fibers or in small groups delimited by irregular bundles of collagen fibers, which form a support network (Fig. 2D,E), coinciding with results of Egerbacher et al. (2005). In carnivores, digital torus were comprised of great amount of lobes of connective tissue, completely involved by connective tissue fibers. When compressed, disperses and absorbs mechanical impacts (Egerbacher et al. 2005). The distribution of lobes and small groups of adipocytes was different between fore- and hind limbs, the shape of single lobes was predominant in the fore limbs, and small groups were more frequent in the hind limbs.

**Conclusion:**

The tissue organization of digital torus has played an important role on distribution the forces during weight bearing and on the storage or absorption of mechanical impacts. It presented a complex architecture, predominantly formed by collagen fibers arranged in parallel thick and irregular bundles, oriented in different directions and intermingled with elastic fibers, fibrocartilage and myxoid tissue in both limbs, besides scarce lobes of fat presented as adipocytes isolated or in small groups.

**Table 1:** Mean and standard error of connective, adipose, and vascular tissues of digital cushions of horses in fore and hindlimbs.

|                  | Connective tissue | Adipose tissue | Vascular tissue |
|------------------|-------------------|----------------|-----------------
| Forelimbs        | 80.73% + 0.60     | 0.88% + 0.21   | 0.86% + 0.19   |
| Hindlimbs        | 80.50% + 0.36     | 0.75% + 0.15   | 0.72% + 0.19   |
| *p*              | 0.111             | 0.605          | 0.655          |

**Fig. 1:** Charts relative to the proportion of connective, adipose, and vascular tissues of digital cushions of horses.
Fig. 2: Constitution of the digital cushion. Gomori'strichrome. (A) Overview of the cushion evidencing connective (asterisk), adipose (broken arrow) and vascular (full arrow) tissues. (B) Connective tissue with fibers oriented in different directions. (C) In the region where fiber bundles have diverged the gap was filled by myxoid tissue (asterisk), fibrocartilage and elastic fiber. (D,E) Single adipocytes (arrow) were mainly found in the digital cushion of the fore limb, while small groups were found in higher frequency in the hind limbs (F) Vascularization interwoven fibers of connective tissue and adipose tissue lobules.
Fig. 3: Constitution of the digital cushion. Safranin O, Alcian Blue and Picrosirius Red. (A) In the region where fiber bundles have diverged, the gap was filled by myxoid tissue (arrow), fibrocartilage and elastic fiber. Picrosirius Red. (B) elastic and reticular fibers. Safranin O. (C) Myxoid tissue (broken arrow) with small areas of fibrocartilage (arrow) and connective tissue (asterisk).Safranin O. (D,E) Myxoid tissue with matrix abundant and reagent with Alcian Blue(D), with areas of hyaline cartilage differentiating into interstitial connective tissue and adipose tissue. Alcian Blue. (F) Cells of the myxoid tissue (arrows). Alcian Blue.

REFERENCES


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and cartilage but only little unilocular fat tissue. 


