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Original Article
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Occurrence of clinical laminitis after adaptation to confinement: effects on morphology, density, and mineral composition of the hoof of Nellore cattle after finishing[1]

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ABSTRACT.- Freitas S.L.R., Queiroz P.J.B., Fernandes J.J.R., Nascente E.P., Santos A.S., Nascimento K.S. & Silva L.A.F. 2023. **Occurrence of clinical laminitis after adaptation to confinement: effects on morphology, density, and mineral composition of the hoof of Nellore cattle after finishing**. *Pesquisa Veterinária Brasileira 43:e07131, 2023*. Departamento de Medicina Veterinária, Escola de Medicina Veterinária e Zootecnia, Universidade Federal de Goiás, Campus Samambaia, Rodovia Goiânia Km 8, Nova Veneza, Goiânia, GO 74690-900, Brazil. E-mail: sabrina.freitas@ifgoiano.edu.br

Laminitis is a disease that affects the dermis and epidermis of the bovine hoof, generating changes in the hoof capsule. This study evaluated the effects of clinical laminitis diagnosed after the adaptation phase to confinement on the morphology, density, and mineral composition of the hoof of Nellore cattle after finishing. The animals were separated in the first weeks of confinement into a sick group (SG), with clinical laminitis, and a healthy group (HG). SG animals had higher heel length, dorsal wall length, toe height, and diagonal hoof length (p<0.05) than healthy animals. The dermal laminae had similar measurements for thickness, length, and spacing between them between SG and HG. Animals with laminitis showed congestion, hemorrhage, and basement membrane irregularities on histology. Computed microtomography (μCT) revealed that the hoof density of sick animals is lower than healthy ones. According to the mineral composition by energy-dispersive X-ray fluorescence (ED-XRF) spectrometry, the hooves of animals with laminitis (SG) and healthy ones (HG) were not biochemically different. Therefore, the occurrence of clinical laminitis in Nellore cattle in the first weeks of confinement causes an increase in the morphometric parameters of the hoof capsule and a reduction in the density of the abaxial hoof wall evaluated after the finishing period. This disease does not promote changes in the histomorphometric parameters of the dermal laminae and the percentage of minerals in the abaxial hoof wall.

INDEX TERMS: Biochemistry, lameness, X-ray fluorescence spectrometry, histology, computed microtomography, hoof, Nellore, cattle.

RESUMO.- [Ocorrência de laminite clínica após adaptação ao confinamento: efeitos na morfologia, densidade e composição mineral do casco de bovinos Nelore após terminação.]

A laminite é uma doença que afeta a derme e epiderme do casco de bovinos gerando alterações no estojo córneo. O estudo avaliou os efeitos da laminite clínica diagnosticada após a fase de adaptação ao confinamento na morfologia, densidade e composição mineral do casco de bovinos da raça Nelore após terminação. Nas primeiras semanas de confinamento, os animais foram separados em um grupo doente (GD) com laminite clínica e em um grupo saudável (GS). Os animais do GD apresentaram maior comprimento de talão, comprimento da parede dorsal, altura da pinça e comprimento diagonal do casco (p<0,05) do que os saudáveis. As lâminas dérmicas tiveram medidas semelhantes para espessura, comprimento e espaçamento

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entre elas entre GD e GS. Animais doentes apresentaram congestão, hemorragia e irregularidades da membrana basal na histologia. A microtomografia computadorizada (μCT) revelou que a densidade do casco de animais doentes é menor do que o saudável. Para a composição mineral por meio da espectrometria de fluorescência de raio-X por dispersão de energia (ED-XRF), o casco dos animais doentes (GD) e dos saudáveis (GS), não se mostraram diferentes bioquimicamente. Conclui-se que a ocorrência de laminite clínica em bovinos da raça Nelore nas primeiras semanas de confinamento ocasiona aumento de parâmetros morfométricos do estojo córneo e redução da densidade da parede abaxial do casco, avaliados após o período de terminação. Essa enfermidade não promove modificações nos parâmetros histomorfométricos das lâminas dérmicas e na porcentagem de minerais da parede abaxial do casco.

TERMOS DE INDEXAÇÃO: Bioquímica, claudicação, espectrometria de fluorescência de raio-X, histologia, microtomografia computadorizada, casco, Nelore, bovinos.

INTRODUCTION

Bovine laminitis is an aseptic and diffuse inflammatory process of the dermal lamina of the hoof, which develops, in most cases, secondary to endotoxemia caused by ruminal acidosis (Nagaraja & Lechtenberg 2007). The disease can manifest clinically with pain, severe lameness, and reluctance to move. In the subclinical form, damage to the dermis for long periods causes low-quality hoof tissue synthesis and, consequently, predisposition to secondary lesions (Hoblet & Weiss 2001). The chronic phase is characterized by irregular and excessive growth of the dorsal wall of the hoof capsule associated with a reduction in heel height, which results in a change in hoof morphology popularly known as founder (Thoefner et al. 2005, Lean et al. 2013).

Several techniques have been described for studying the hoof capsule of cattle with laminitis. Morphometric evaluation is an alternative to identifying animals with chronic laminitis (Ayalp et al. 2019). However, no studies have been found in the literature that evaluated the long-term effects of clinical laminitis on morphometric parameters of the hoof of confined Zebu animals. Another important technique is the histological evaluation, which allows the diagnosis of inflammatory changes in the hoof dermis (Noronha Filho et al. 2019). However, little is known about the histomorphometry of the dermal laminae of cattle with laminitis. This technique is relevant because it allows the characterization of anatomical and histological aspects of different breeds of cattle and buffaloes (Rabelo et al. 2015, Assis et al. 2017a).

Recently, some material characterization techniques have been used to evaluate the horny epidermis of the bovine hoof, with emphasis on computed microtomography (μCT) and energy-dispersive X-ray fluorescence (ED-XRF). μCT evaluates the density of the hoof tissue and other parameters such as the diameter and concentration of horny tubules of the hoof of bovines and buffaloes (Rabelo et al. 2015, Assis et al. 2017b). ED-XRF, a non-destructive and easy-to-perform technique, allows the identification of minerals in different types of materials (Rebiere et al. 2019). This technique made it possible to determine the mineral composition of the hoof epidermis of the hoof of buffaloes (Assis et al. 2017a), heifers supplemented with biotin (Queiroz et al. 2021), and of cows with lameness associated with subclinical laminitis (Barbosa et al. 2016).

This study evaluated the effects of clinical laminitis diagnosed after the confinement adaptation phase on the morphology, density, and mineral composition of the hoof of Nellore cattle after finishing.

MATERIALS AND METHODS

Experimental design. The study was carried out from April to August 2019, after approval by the Ethics Committee for the Use of Animals of the "Universidade Federal de Goiás" (CEUA-UFG), under protocol number 066/2018. In total, 160 24-month-old intact Nellore male animals from the experimental confinement of the "Escola de Medicina Veterinária e Zootecnia" (School of Veterinary Medicine and Animal Science – EVZ) of UFG were evaluated. The animals were confined for 106 days in stalls measuring 77m^2 with an earthen floor, containing eight animals per stall, which provided 9.63m^2 of area per animal and 96.25cm of trough per animal. Access to clean water was *ad libitum* provided at Australian drinking troughs. Before confinement, all animals were dewormed, vaccinated against clostridiosis, rabies, and foot-and-mouth disease, and identified by earrings. The cattle were fed once a day, always in the morning, and leftovers, which were maintained at approximately 5%, were quantified daily. The diet composition (dry matter) was 10.05% sugarcane bagasse, 78.15% ground corn, 8.95% soybean meal, 0.75% urea, and 2.10% mineral mix.

A lameness test was performed on all cattle confined in a fenced area near the stalls after 20 days of confinement (Tavares et al. 2019), according to the mobility scoring proposed by the Agriculture and Horticulture Development Board (AHDB 2017). The following criteria were adopted for the diagnosis of clinical laminitis: history of consumption of a highly acidogenic diet (Bergsten 2003), degree 3 of mobility score with pain in all digits during support, and antalgic position characteristic of acute laminitis (Shearer et al. 2004; Thoefner et al. 2005). In addition to the aforementioned criteria, a macroscopic examination of the hooves was performed, according to the methodology described by Shearer et al. (2004), in which animals with laminitis were considered those without visible lesions (white line disease, corkscrew hoof, digital and interdigital dermatitis, heel erosion, infectious pododermatitis – foot-rot – sole hemorrhage, interdigital hyperplasia, ulcers – sole, toe, and heel – and vertical fissures of the wall).

Fourteen (8.75%) cattle were diagnosed with clinical laminitis, of which nine (64.28%) were randomly chosen and identified to form the sick group (SG). Nine healthy animals, which had no lameness and no macroscopic foot injury, were randomly selected at the end of the confinement period, in the week of slaughter, to compose the healthy group (HG) and act as a control. The cattle were slaughtered in a slaughterhouse inspected by the Federal Inspection Service (SIF), and the right pelvic and thoracic limbs of the animals selected from each group (SG and HG) were collected at the time of slaughter. Healthy animals had an average weight of 556 ± 36.15, and the sick ones had 530.56±49.6 at the end of confinement.

Macroscopic and morphometric evaluation of the hoof. All collected limbs were used to perform the morphometric study, totaling 18 thoracic limbs (nine SG and nine HG) and 18 pelvic limbs (nine SG and nine HG), totaling 36 medial digits (18 SG and 18 HG) and 36 lateral digits (18 DG and 18 HG). A universal caliper graduated 300mm x 0.05mm (KingTools®, Delhi, India) was used to obtain the measurements expressed in centimeters. A total of eight measurements were taken after adapting the studies by Silva et al.

(2015) and Ayalp et al. (2019) (Fig.1), namely: heel length (HL), dorsal hoof wall length (DWL), heel height (HH), toe height (TH), sole length (SL), diagonal hoof length (DHL), medial and lateral digit width (DW), and medial and lateral digit length (DL) of both limbs (thoracic and pelvic). The presence of macroscopic lesions and eventual changes in the morphology of the hoof capsule were assessed after the morphometric evaluation.

Fragment collection site. Fragments of the abaxial wall of the medial digit of the thoracic limb and the lateral digit of the pelvic limb were collected for histological, microtomographic, and mineral composition evaluations. The 1.5-cm distal limit of the coronary band was established as the collection site for the fragments after clinical laminitis. This limit was established considering the average growth of the hoof wall, which is 0.5 cm per month (Greenough 2007) (Fig.2) after 86 days of confinement.

Histology and histomorphometry. A total of 12 samples were collected for histology and histomorphometry from region 1 (Fig.2), six from sick animals (SG), and six from healthy animals (HG). In region 1, the hoof capsule was abraded using sandpaper coupled to a micro-grinding machine (Dremel® 4000-3/36, Robert Bosch Tool Corporation, São Paulo-SP, Brazil) to assist in the preparation of the slides. Subsequently, a sample (1.0 x 1.0 cm) was collected from the region of the dermis-epidermis junction of the abaxial hoof wall using a lamellotome (Mendes et al. 2018), as performed by Noronha Filho et al. (2019).

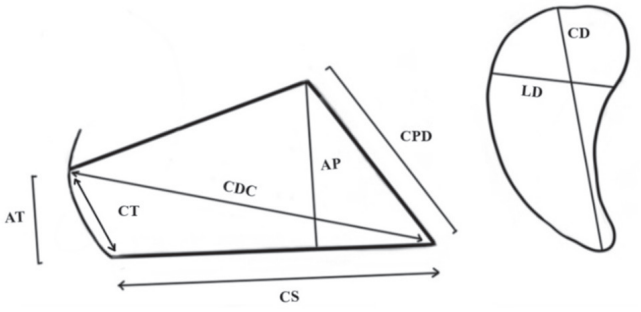

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Fig.1. Scheme of measurements for hoof morphometry in healthy and sick animals. HL = heel length, DWL = dorsal hoof wall length, HH = heel height, TH = toe height, SL = sole length, DHL = diagonal hoof length, DW = medial and lateral digit width, DL = medial and lateral digit length. Adapted from Silva et al. (2015) and Ayalp et al. (2019).

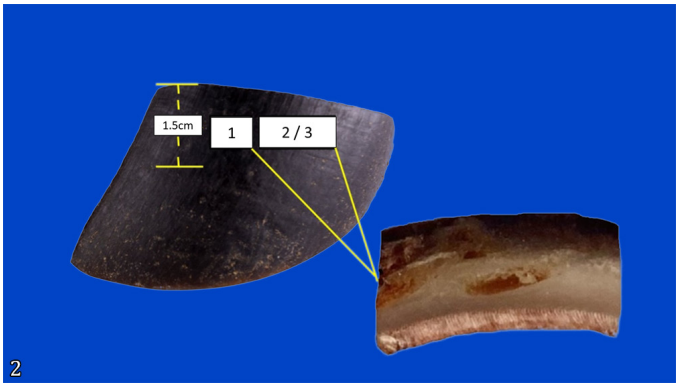

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Fig.2. Scheme showing the collection site and the fragment collected for performing the histomorphometry, µCT, and ED-XRF analyses. Fragment collected for histological evaluations, size (1.0 x 1.0cm) (1). Fragment collected for evaluation by µCT and ED-XRF, size (0.5 x 3.0 x 0.5cm) (2/3).

The fragments were fixed in a 10% buffered formalin solution, dehydrated in increasing ethyl alcohol solutions, clarified in xylol, embedded in paraffin, and laminated to 5-µm thickness (Luna 1968). The sections were stained by the routine hematoxylin and eosin (HE) technique, and the basement membrane was evaluated by periodic acid-Schiff (PAS) staining (Luna 1968, Tolosa et al. 2003). Histological evaluations were performed blindly by two evaluators using an optical microscope (Leica DM 400), and an image capture system (Leica Application – LAS) coupled to the optical microscope, using objective lenses with magnifications of 10x and 40x.

The methodology recommended by Rabelo et al. (2015) and Assis et al. (2017a) was adopted with some modifications for the histomorphometric evaluations. The length of the dermal laminae was determined by measuring the distance between the dermis and the basal layer at the apex of each lamina. The thickness of the laminae was determined by the distance from the left to the right basal layer of each lamina, while the space between the laminae was determined by the distance between the basal layer of the lamina to the basal layer of its adjacent lamina. Six intact laminar structures were measured and evaluated in the middle portion of the histological section, calculating the average of each sample at the end. Additionally, the presence or absence of congestion (dilated blood vessels), hemorrhage (presence of red blood cells in the extravascular space), and inflammatory infiltrate (presence of inflammatory cells in the perivascular or intravascular interstitium) was evaluated at the dermal-epidermal junction throughout the entire length of the histological sections stained with HE. When present, these changes were further classified according to their intensity (mild, moderate, or severe). The basement membrane in samples stained with PAS was evaluated for the presence and intensity of irregularities in its morphology throughout the entire dermal lamina at the apical, middle, and basal portions (Mendes et al. 2013).

Computed microtomography (µCT). Half of the sample from the 2/3 region (Fig.2) of four animals from the SG group and one animal from the HG was used for µCT analysis, serving as a parameter for comparison with the sick animals (control). The fragment was cut using a micro-grinding machine (Dremel® 4000-3/36, Robert Bosch Tool Corporation, São Paulo/SP, Brazil) coupled to a 38-mm diameter diamond cutting disc at a rotation speed of 20,000rpm. The cuts were deepened up to the dermal-epidermal junction and then the fragment of the abaxial wall was released from the dermis b using a metallic spatula. This fragment was divided into two, used for evaluation by µCT and ED-XRF. The samples were stored in plastic packages, identified, and frozen at -22°C. Transport for the analysis was carried out in a styrofoam box with ice. The µCT evaluation was carried out at the National Laboratory of Nanotechnology (LNNano, Campinas-SP, Brazil), using a SkyScan 1272 microtomograph (Bruker®, Kontich, Belgium). The image acquisition settings were electric voltage of 25kV, electric current of 180µA, rotation range of 180°, exposure time of 3018 ms, rotation time of 0.6 s, and pixel size of 17µm. A 0.25-mm aluminum filter was used. NRecon® software version 1.7.4.6 (Micro Photonics Inc., Allentown, USA) was used to reconstruct the obtained microtomographic images.

The density of the abaxial hoof wall was determined by evaluating the set of two-dimensional images using the DataViewer® software version 1.5.4.6 (Bruker®, Kontich, Belgium). The density of the

keratinized tissue was evaluated in the axial section. The abaxial hoof wall was divided into three regions: internal (IR), middle (MR), and external (ER) (Fig.3). In each region, 30 pixels were selected, and 30 different density values were generated. The values were averaged and expressed in attenuation coefficient (AC).

Energy-dispersive X-ray fluorescence (ED-XRF) spectrometry. The mineral composition was evaluated using the other half of the sample from the 2/3 region (Fig.2) of four animals from each group (SG and HG), totaling eight samples per group, four from the medial digit of the thoracic limb and four of the lateral digit of the pelvic limb. The evaluation of the mineral composition of the abaxial hoof wall was performed using the ED-XRF technique. The material was processed at the Analytical Center of the Physics Course of the "Universidade Federal de Jataí" (UFJ). The samples were thawed and sanitized with alcohol to remove possible contamination by elements that do not make up the horny tissue. After the samples were completely dry, a Ray Ny EDX-720 benchtop spectrometer (Shimadzu®, Columbia, USA) was used to identify chemical elements located between sodium (Na) and uranium (U) in the periodic table. Measurements were performed in a vacuum chamber, and liquid nitrogen was used to cool the photon detector. Primary X-rays were generated by a rhodium (Rh) tube at a voltage of 50kV and an electric current of 100μA. The X-ray beam was directed toward the sample through a 5.0mm collimator. The measurement time for each sample was 120 seconds and the fluorescent spectra were recorded at energies ranging from 0 to 40keV (2048 points per spectrum).

Statistical analysis. The morphometry data were expressed as mean values ± standard deviation using the SPSS statistical program version 23.0 (IBM Corp., Armonk/NY). After analyzing the parametric assumptions, the independent and paired t-test was performed to compare the experimental groups (SG and HG), limbs (thoracic and pelvic), and digits (medial and lateral) in the different variables. The statistical comparison between experimental groups when considering the lateral plus medial digits for the limbs (thoracic and pelvic) was performed using analysis of covariance (ANCOVA). Similarly, statistical differences for the histomorphometry data between and within the experimental groups (HG and SG) for limbs (thoracic and pelvic) were obtained by independent and paired t-tests, respectively. Inflammatory findings on histology were analyzed descriptively.

Hoof wall density data obtained through μCT were expressed as mean and standard deviation and analyzed by descriptive statistics. Hoof wall mineral composition data were subjected to analysis of variance (ANOVA) and Tukey's test. The R statistical program (version 3.3.1, 2016 – The R Foundation for Statistical Computing) was used for this analysis. Statistical differences in all analyses were considered when the probability value was lower than $p<0.05$.

RESULTS

The analysis of covariance (ANCOVA) for the morphometric evaluation of the hoof capsule, considering the medial and lateral digits together, showed significant differences in the parameters HL, DWL, HH, TH, and DHL (Table 1), with higher values found in animals with laminitis (SG).

Among the macroscopic changes resulting from laminitis, all (100%) of the sick animals (SG) presented more than three lines of stress in one of the hooves. In comparison, only two (22.22%) healthy animals presented this particularity. In addition, eight animals (88.88%) from SG had changes in the conformation of one of the digits of the thoracic limb or pelvic limb (Fig.4-6).

The histomorphometry data referring to the thickness, length, and space between dermal laminae comparing the

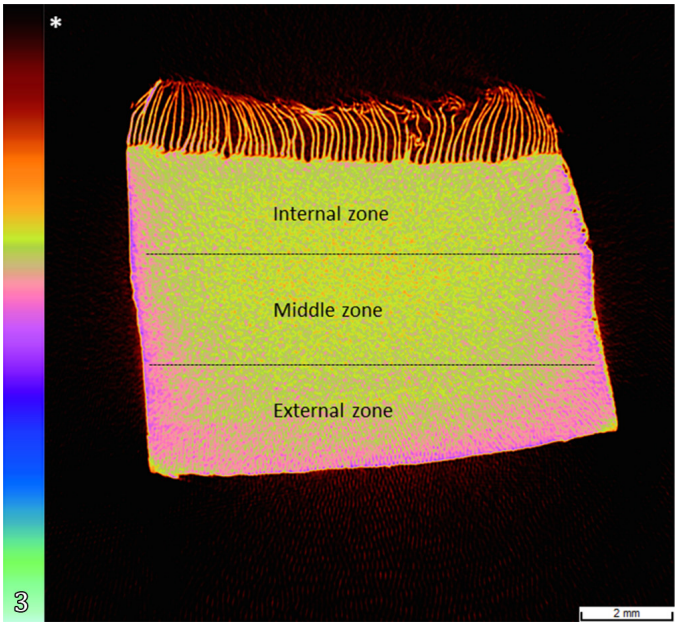

Fig.3. Axial section of a sample of the abaxial hoof wall, demonstrating the regions considered for evaluation of hoof density: internal (IR), middle (MR) and external regions (ER). Colorimetric density scaling, where black indicates the absence of density, yellow and pink indicate median density and light green indicates high density (*).

Table 1. Mean ± standard deviation of the hoof morphometry of healthy and sick Nellore cattle, considering the measurement of the medial and lateral digits of the right thoracic and pelvic limbs, confined at the Experimental Confinement of Beef Cattle, EVZ-UFG, Goiânia, Goiás, Brazil

Variable	Group	Thoracic	Pelvic	p-value	*p*-value (ANCOVA)¥
Heel length (HL)	HG	5.81±0.4^{B}	4.84±0.43^{B}	<0.001	<0.001
	SG	7.13±0.96^{A}	6.45±1.03^{A}	0.025	
Dorsal hoof wall length (DWL)	HG	7.92±0.35^{B}	7.48±0.33^{B}	<0.001	<0.001
	SG	9.11±0.55^{A}	9.02±0.43^{A}	0.362	
Heel height (HH)	HG	4.28±0.63^{A}	3.38±0.63^{B}	<0.001	0.002
	SG	4.61±0.54^{A}	4.00±0.52^{A}	0.001	
Toe height (TH)	HG	6.53±0.69^{B}	6.09±0.51^{B}	0.007	<0.001
	SG	7.21±0.67^{A}	7.07±0.44^{A}	0.304	
Sole length (SL)	HG	8.71±0.72^{A}	8.59±0.88^{A}	0.577	0.057
	SG	8.41±0.92^{A}	7.99±1.17^{A}	0.111	
Diagonal hoof length (DHL)	HG	13.41±0.57^{B}	12.41±0.57^{B}	<0.001	<0.001
	SG	14.68±0.51^{A}	13.43±0.97^{A}	<0.001	
Digit width (DW)	HG	5.13±0.36^{A}	4.46±0.34^{A}	<0.001	0.066
	SG	5.75±1.39^{A}	4.57±0.46^{A}	0.003	
Digit length (DL)	HG	9.39±0.74^{A}	8.99±0.7^{A}	0.009	0.696
	SG	9.28±1.6^{A}	8.9±0.8^{A}	0.295	

^{A,B} Different uppercase letters in the column have a statistical difference by the t-test ($p<0.05$); ¥ ANCOVA between experimental groups (HG and SG) with the limbs (thoracic and pelvic) as a covariate; HG = healthy group, SG = sick group.

thoracic and pelvic limbs of sick (SG) and healthy (HG) animals are shown in Table 2. The space between dermal laminae in the thoracic limb of HG was higher compared to the pelvic limb. The only difference present in the measurements of dermal laminae was the space between laminae within the healthy group, with this space being higher in the thoracic limb. The other variables showed no differences between and within groups for thoracic and pelvic limbs. Figure 7 shows the correlation for histomorphometry variables between healthy and sick animals.

Positive correlations are present for the spacing and thickness of laminae. In other words, when one increases, the other also increases. The analysis of the vectors (Pe-Do, To-Do, Pe-As, and To-As) around the variables showed their dispersion and, consequently, no standardization in the individual measurements of the animals, characterizing the individuality of the animal.

The evaluation of the inflammatory aspects showed that the animals in both groups (HG and SG) had no inflammatory infiltrate. Among healthy cattle (HG), one animal (16.66%) had mild congestion in the pelvic limb. Four animals (66.66%) from SG had congestion in the dermal laminae, all in the thoracic limb, but the degree was mild in two and moderate in the other two. Irregularities in the basement membrane were observed in four animals (66.66%) at a mild degree in SG, one in the thoracic limb, two in the pelvic limb, and one in both limbs (Fig.8-10).

Figure 11-12 shows the two-dimensional (2D) images of fragments of the abaxial hoof wall obtained by µCT. The samples from HG and SG showed a predominance of yellow color in their central region and pink in the peripheral regions, which indicates an intermediate density according to the colorimetric scale (Fig.11-12). Table 3 shows the average densities found on the abaxial hoof wall of four bovines with laminitis and one healthy bovine. The abaxial hoof wall of the healthy animal presented higher density than bovines with laminitis and the external region of the samples presented higher density than the others.

The following minerals were identified when evaluating the mineral composition of the abaxial hoof wall: sulfur (S), calcium (Ca), potassium (K), phosphorus (P), silicon (Si), copper (Cu), zinc (Zn), and iron (Fe). The microminerals Cu (copper), Zn (zinc), and Fe (iron) were only identified in the qualitative examination, as they were found at very

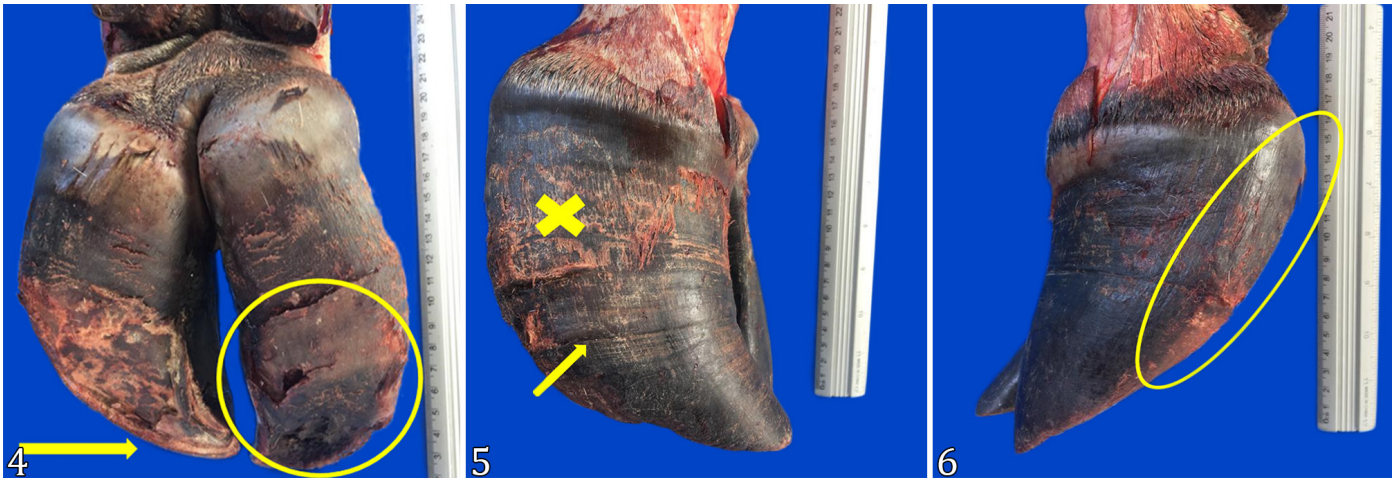

Fig.4-6. Macroscopic changes found in the digits of animals with laminitis. (4) Circle = fissures in the horny tissue of the sole, arrow = abnormal toe growth. (5) Arrow = growth of horny tissue appearing of low quality, showing greater roughness than normal horny tissue; arrow = stress lines. (6) Abnormal heel growth.

Table 2. Mean ± standard deviation of the thickness, space, and length of epidermal laminae of the hoof of the thoracic and pelvic limbs of healthy and sick male Nellore cattle. Experimental Confinement of Beef Cattle, EVZ-UFG, Goiânia, Goiás, Brazil

Variable	Limb	Group		*p*-value
		Healthy (HG)	Sick (SG)	
Lamina thickness (10x)	Thoracic	81.67 ± 19.4^{A}	79.06 ± 13.58^{A}	0.793
	Pelvic	81.49 ± 12.15^{A}	80.71 ± 13.26^{A}	0.918
Space between laminae (10x)	Thoracic	60.95 ± 8.66^{A}	52.7 ± 11.73^{A}	0.196
	Pelvic	45.77 ± 8.17^{B}	43.59 ± 9.19^{A}	0.674
Length (5x)	Thoracic	1429.78 ± 485.98^{A}	1820.41 ± 328.64^{A}	0.134
	Pelvic	1360.59 ± 361.42^{A}	1667.69 ± 377^{A}	0.180

^{A,B} Means followed by different uppercase letters in the column and lowercase letters in the row mean statistical difference (p<0.05); HG = healthy group, SG = sick group.

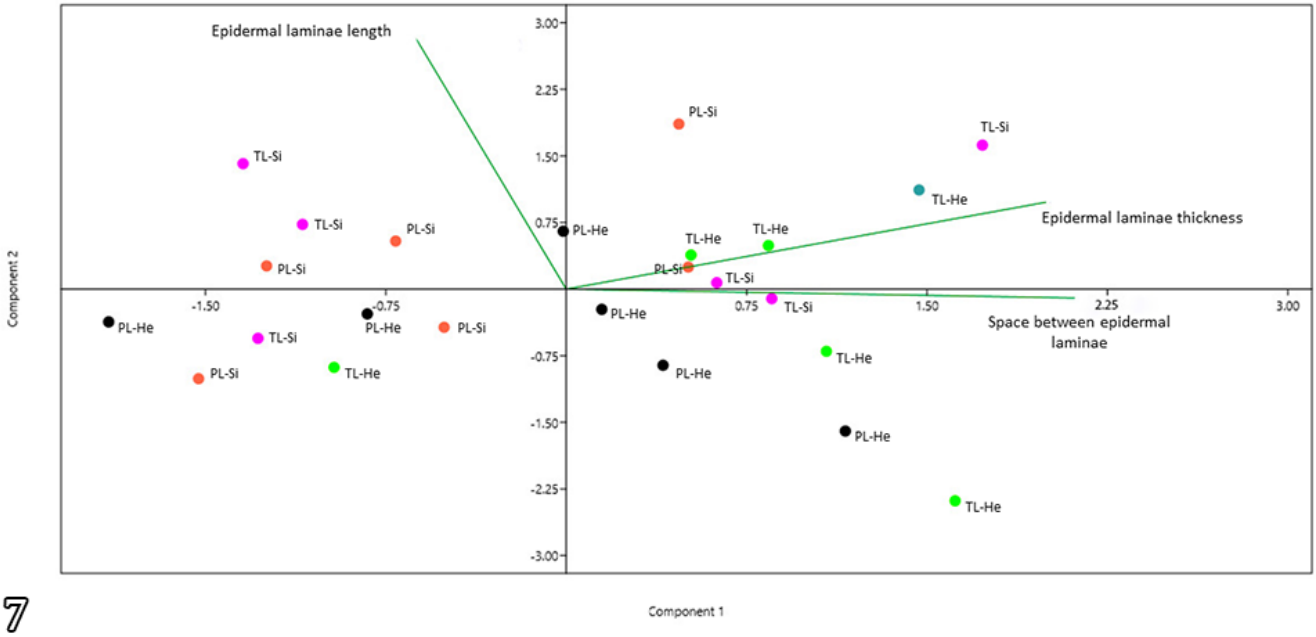

Fig.7. Analysis of the principal components of the correlation of the variables length, lamina thickness, and space between laminae used for the pelvic limb (Pe) and thoracic limbs (To) in sick (Sk) and healthy (Ht) animals.

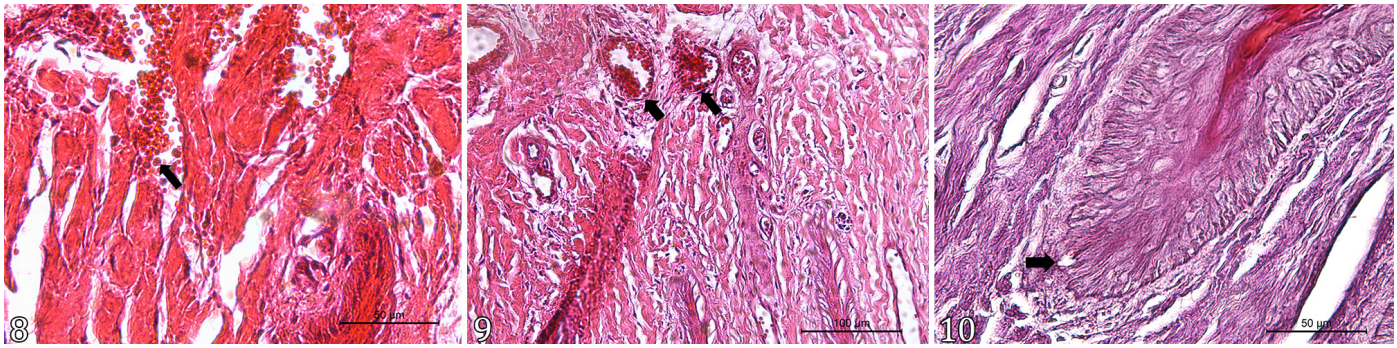

Fig.8-10. Photomicrograph of the dermal-epidermal junction of the cattle hoof (abaxial wall region) with laminitis. (8) Evidence of dilated and congested vessels (black arrow) at the dermal-epidermal junction. HE, bar = 100µm. (9) Presence of a moderate amount of red blood cells in the extravascular space (black arrow). HE, bar = 50µm.(10) Evidence of structural irregularities in the basement membrane of the dermal lamina of the bovine hoof, characterized by a focally extensive discontinuity in its basal portion (black arrow). PAS, bar = 50µm.

Table 3. Means of density of the internal (IR), middle (MR), and external regions (ER) of each hoof sample in attenuation coefficient (AC)

	Group	Internal region	Middle region	External region
Attenuation coefficient (AC)	Sick (n=4)	0.150 ± 0.008	0.159 ± 0.001	0.170 ± 0.009
	Healthy (n=1)	0.169	0.168	0.188

small concentrations in the horny tissue. Table 4 shows the percentage of the four main minerals (S, Ca, K, and P) and compares SG and HG, considering the thoracic and pelvic limbs together.

Sulfur was found in the highest percentage in the hoof of all animals and calcium was second, followed by potassium and phosphorus. The comparisons showed no differences in hoof composition between sick (SG) and healthy (HG) animals. Silicon was present and reached 3.93±1.22% for healthy animals and 5.08±2.12% for sick animals. It was not included in the composition table because it is not part of the mineral composition of the hoof, but it is a contamination element, as the animals were confined in earthen-floor stalls.

DISCUSSION

The digits of the thoracic limb had the largest measurements for SG and HG animals. Therefore, this finding may be related to the higher weight cattle support on their thoracic limbs. Sousa et al. (2020) evaluated cattle walking on a force platform and found that the thoracic limbs support 53% of the total weight. In this case, the authors justified the need for the

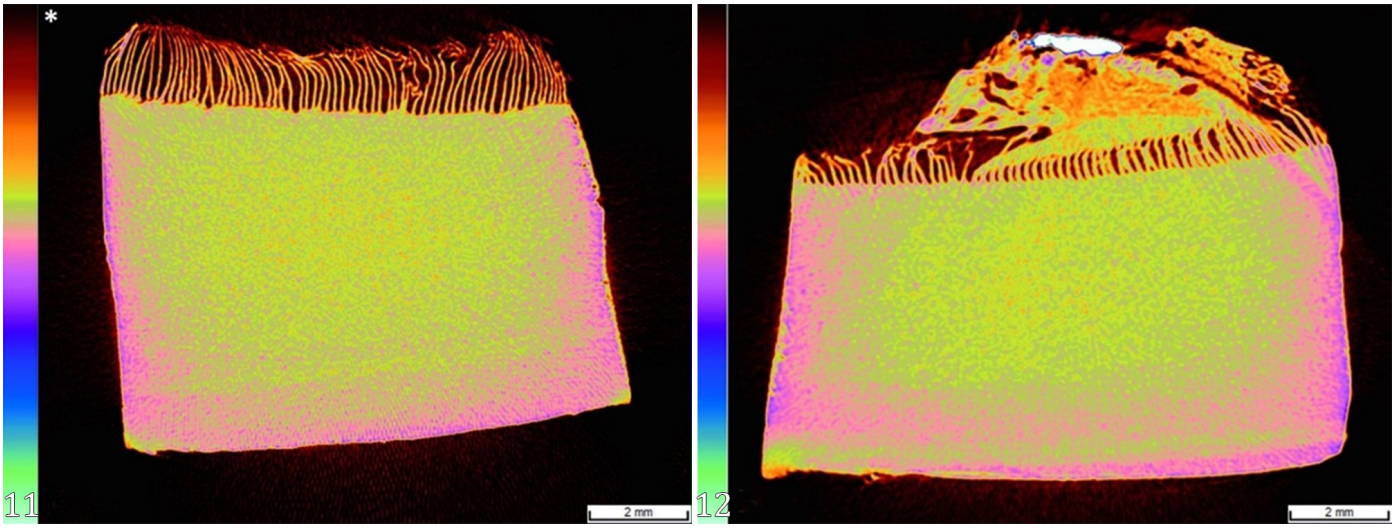

Fig.11-12. Microtomographic image of the axial section of a fragment of the abaxial hoof wall of confined Nellore cattle after finishing. Fragment of the abaxial wall of the medial hoof of the bovine thoracic limb from the (11) healthy group (HG) and (12) the sick group (SG). The colors representing the density of the horny tissue were similar in both groups, with a predominance of yellow in the central region and pink in the peripheral regions. According to the colorimetric scale (*), these colors represent areas of intermediate density.

Table 4. Mineral composition of the abaxial hoof wall of healthy and sick (with laminitis) confined Nellore cattle. Experimental Confinement of Beef Cattle, EVZ-UFG, Goiânia, Goiás, Brazil

Mineral	Group	Result (%)
Sulfur (S)	Healthy	71.35 ± 4.28[A]
	Sick	73.07 ± 12.46[A]
Calcium (Ca)	Healthy	12.23 ± 2.98[A]
	Sick	10.73± 6.44[A]
Potassium (K)	Healthy	6.96 ± 2.37[A]
	Sick	7.16 ± 1.51[A]
Phosphorus (P)	Healthy	5.51 ± 1.49[A]
	Sick	4.1 ± 0.97[A]

[A] Values followed by equal letters in the same variable do not show a statistical difference by Tukey's test (p<0.05).

digits of the thoracic limb to be larger than the digits of the pelvic limbs for a better weight distribution between limbs. Mendonça et al. (2003) and Silva et al. (2015) also showed that the digits of the thoracic limb are larger than those of the pelvic limb.

The heel length (HL), dorsal hoof wall length (DWL), toe height (TH), and diagonal hoof length (DHL) were higher in the thoracic and pelvic limbs of SG. However, heel height (HH) was higher in SG, only in the pelvic limb. Similarly, Ayalp et al. (2019) conducted a *post mortem* study and observed an increase in all hoof morphometric measurements, except for sole length and digit width, in dairy cows with subclinical laminitis. Hoof shape results from a complex balance between growth and wear of the horny tissue. Diet, age, impact, and inflammation are among the factors that increase hoof growth rate (Blowey 2015). The generalized inflammation of the hoof dermis caused by laminitis promotes increased blood flow at the early stages, stimulating the rapid growth of poor-quality horny tissue (Blowey 2015). In addition, the higher DWL and DHL observed in SG can be explained by the deepening of the third phalanx, which occurs in cattle with laminitis. According to Ossent & Lischer (1998), the hoof wall grows parallel to the dorsal surface of the third phalanx, but the deepening of this structure changes the hoof growth angle by some degrees, resulting in a concave and longer wall. Furthermore, the higher HH and TH in SG can be explained by a hypertrophic response of the epidermis caused by successive episodes of laminitis, causing an increase in height, mainly of the lateral hoof in the pelvic limb relative to the medial hoof. This height disparity causes the lateral digit to bear a higher proportion of weight and to become more hypertrophic and susceptible to injuries (Hoblet & Weiss 2001).

Considering the time of approximately 86 days that the animals remained confined after the clinical diagnosis of laminitis, the lines of stress resulting from the disease were more restricted to the segment of the hoof that grew after the onset of the disease. Other stress lines identified on the abaxial hoof wall certainly appeared as a result of different problems, such as changes in feed, pasture transfer, transport, or other situations that caused stress in the animals before confinement.

Despite the severe clinical condition observed in animals with laminitis, no visible lesions were found on the sole and white lines, with only irregularities in morphometry, stress lines, and cracks on the hoof wall being observed. It occurred due to the time interval required for circulatory damage to the corium to be identified on the sole surface (Greenough 2007, Bicalho et al. 2008, Blowey 2015), which can vary between two and three months depending on the thickness and rates of growth and wear of the sole (Blowey 2015). The maintenance of the animals on the earthen ground is a factor that may have contributed to the low wear of the sole, resulting in a long time for the superficialization of hemorrhages and sole ulcers.

The histological findings referring to the measurements of dermal laminae indicate no differences between the space, thickness, and length of these structures in animals of the two evaluated groups. However, the average length of laminae on the hoof of sick animals was higher. This finding

can be attributed to the inflammation caused by laminitis, as it reduces the onychogenic substance that forms the nail and may have caused the stretching of dermal laminae (Obel 1948). Thoefner et al. (2005) described similar findings, but the authors stated that the tapering of laminae occurred in the first hours after the clinical manifestation of laminitis and not after three months of the diagnosis of the disease, as observed in the present study. Congestion, hemorrhage, and irregularities in the basement membrane of the hoof laminae present in the dermal laminae at the end of confinement suggest signs of possible inflammation, whether chronic or perhaps of recovery in these animals. However, these findings show that, at some point, there was a release of histamine (Nocek 1997), lipopolysaccharides (Zebeli & Metzler-Zebeli 2012), and lactic acid (Concha et al. 2014) from feed rich in carbohydrates and related to ruminal acidosis. On the other hand, basement membrane irregularities imply the existence of a failure in oxygenation and nutrient supply, given the circulatory changes characteristic of laminitis (Greenough 2007, Mendes et al. 2013, Noronha Filho et al. 2019).

Computed microtomography showed that the highest density found in the fragment of the abaxial hoof wall of healthy animals and the lowest in sick animals suggest that laminitis interferes with the quality of the horny tissue formed. After laminitis, the hoof becomes weakened and predisposed to the appearance of secondary morphological changes associated with this disease. Bearing in mind that the external region of the samples presented higher density than the others, we can infer that the contact of the hoof surface with air and heat promotes higher drying of this area. According to Bertram & Gosline (1987), the fluids coming from the dermis hydrate the internal regions of the hoof and are lost on its surface, resulting in low hydration of the external region of the horny tissue. In contrast, the inner region, close to the dermis, maintains high levels of hydration and, consequently, lower tissue density.

The quantification of macro-and microminerals present in the hoof of Nellore cattle using X-ray fluorescence spectrometry was considered an important advance in the study of the microstructure of the hoof of *Bos indicus* cattle. This methodology was previously used to evaluate the mineral composition of the hoof of buffaloes (Assis et al. 2017a) and Holstein calves (Queiroz et al. 2021). Sulfur was found at a higher percentage in the hoof of all animals, regardless of whether they were sick or not and, therefore, we can infer that this mineral is important for the formation of the hoof capsule. Sulfur is present in the amino acids cysteine and methionine, interfering with the quality of the horny tissue (Nelson & Cox 2011). The mineral is directly related to the formation of disulfide bridges between keratin polypeptide chains, giving stability to this protein. Importantly, keratin is the structural protein that fills keratinocytes and is responsible for the resistance of the hoof capsule (Tomlinson et al. 2004). Thus, the higher percentage of S identified by ED-XRF compared to other minerals can be explained by its importance in the constitution of the hoof capsule.

No difference was observed in the concentration of minerals in the hoof between groups. Therefore, laminitis did not impair the transfer of minerals from the dermis to the horny epidermis. Disulfide bridges (S–S) were stabilized between the keratin molecules in the hoof, although the density of the fragment collected from sick animals was lower. This assumption has been supported by the statements of other authors (Schrooyen et al. 2001), to whom the hoof capsule density is associated with the number of sulfur bridges present in the keratin since disulfide amino acids come together to make the hoof a firm and stable structure. Importantly, the intercellular cementum is another structure that also interferes with the hoof density, as it is essential for the adhesion of keratin cells. Thus, the higher hoof capsule density identified in healthy animals may be associated with better rearrangement of these structures. According to Mulling & Hagen (2012), the intercellular cementum is rich in minerals and fat, giving higher density to the hoof. However, it needs to be better studied, especially through molecular analysis.

Calcium was the second mineral found at the highest percentage and was not different between animals that had or did not have laminitis. This element is one of the most important for synthesizing keratinized tissue, as it is directly related to the activation of important biochemical pathways that culminate in the differentiation of keratinocytes, including the terminal distinction, also called cornification (Mulling et al. 1999, Kalinin et al. 2001). Phosphorus, the third mineral present in the highest quantity in the hoof wall of animals, both in the group of sick animals and in the group composed of healthy cattle, is related to calcium in bone mineral metabolism. The two minerals are believed to promote proper hoof growth at the correct proportions. According to Langova et al. (2020), the change in the proportion between calcium and phosphorus can cause the formation of worse quality and more fragile horny tissue. Importantly, the equity in the amount of phosphorus in the hoof of sick and healthy animals proves that laminitis did not prevent the formation of keratinocytes. Phosphorus participates in the supply of energy for cellular metabolism (Voet & Voet 2011). Therefore, it cannot be held responsible for producing a worse-quality hoof after laminitis.

Potassium also showed no difference between groups and little information is known about its participation in hoof quality. However, this mineral element, along with sodium, is essential for maintaining balance in the intra-and extracellular environment (Reece 2006). Although silicon was also found in small amounts in hoof capsule fragments of both groups, this mineral is not part of the hoof composition (Baggott et al. 1988). Its presence is attributed to the fact that the animals are managed in pens with an earthen floor with consequent incorporation of this atom in the superficial layers of the horny tissue due to the constant friction of the hoof with the soil. Although undesired, the presence of Si did not compromise the results.

Microminerals zinc and copper were identified only in the qualitative examination, as they were found in very small amounts to be quantified by spectrometry. Zinc is essential in activating metalloenzymes, which help form structural proteins during keratinization (Lean et al. 2013). Copper is also important since it is necessary for activating the enzyme sulfhydryl oxidase, responsible for forming disulfide bridges, a structure that unites cysteine amino acids in keratin filaments (Langova et al. 2020). The study of minerals in cattle hoof after the occurrence of clinical laminitis was important to show that changes in digit conformation in animals with the disease and the higher density of the hoof wall in healthy animals did not interfere with the number of minerals. Hoof resistance and

growth are maintained under these circumstances even after laminitis. Barbosa et al. (2016) studied the hoof of animals with laminitis and also found similar levels of minerals when compared to healthy animals, but the minerals evaluated were different from those studied here.

Finally, analyzing the methodology used in the present study and the results found, we can infer that microtomography and energy-dispersive X-ray fluorescence spectrometry represent new perspectives for studying diseases that affect cattle hooves. Studies involving new technologies are of paramount importance for understanding the microstructure of the hoof and, consequently, the genesis of several foot diseases in cattle. Therefore, further research involving evaluations of other regions of the hoof of animals with clinical laminitis and after their recovery from the disease is important.

CONCLUSION

Under the conditions of this study, the occurrence of clinical laminitis in Nellore cattle in the first weeks of confinement causes an increase in the morphometric parameters of the hoof capsule and a reduction in the density of the abaxial hoof wall, evaluated after the finishing period. The disease promoted no changes in the histomorphometric parameters of the dermal laminae and the percentage of minerals in the abaxial hoof wall.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

AHDB 2017. Hoof care field guide. Agriculture and Horticulture Development Board, Kenilworth, England. 44p. Available at <https://projectblue.blob.core.windows.net/media/Default/Dairy/Publications/HoofCareGuide_180925.pdf> Accessed on May 18, 2022.

Assis B.M., Silva L.A.F., Lima C.R.O., Gouveia R.F., Vulcani V.A.S., de Sant'Ana F.J.F. & Rabelo R.E. 2017a. Microstructure and hardness of buffalo's hoofs. Anat. Hist. Embryol. 46(5):439-445. <https://dx.doi.org/10.1111/ahe.12288> <PMid:28762238>

Assis B.M., Silva L.A.F., Lima C.R.O., Sant'Ana F.J.F., Santos G.P., Vulcani V.A.S. & Rabelo R.E. 2017b. Microtomographic parameters and nanoindentation of the hoof of Girolando cattle. Anat Histol Embryol. 46(5):456-463. <https://dx.doi.org/10.1111/ahe.12290> <PMid:28857262>

Ayalp G.C., Gulsum U.C. & Demir A.A. 2019. Comparison of clinic – histopathologic findings and morphometric measurements of subclinical laminitic claw in Dairy Cattle. Turkish J. Agricult. 7(8):1113-1117. <https://dx.doi.org/10.24925/turjaf.v7i8.1113-1117.2225>

Baggott D.G., Bunkc K.J. & Gill K.R. 1988. Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. Br. Vet. J. 144(6):534-542. <https://dx.doi.org/10.1016/0007-1935(88)90023-1>

Barbosa A.A., Luz G.B., Rabassa V.R., Corrêa M.N., Martins C.F. & Del Pino F.A.B. 2016. Concentração de minerais no estojo córneo do casco em vacas leiteiras sadias e com claudicação. Semina, Ciênc. Agrárias 37(3):1423-1430. <https://dx.doi.org/10.5433/1679-0359.2016v37n3p1423>

Bergsten C. 2003. Causes, risk factors, and prevention of laminitis and related claw lesions. Acta Vet. Scand. 98(Supl.):157-166. <https://dx.doi.org/10.1186/1751-0147-44-S1-S157> <PMid:15259788>

Bertram J.E.A. & Gosline J.M. 1987. Functional design of horse hoof keratin: the modulation of mechanical properties through hydration effects. J. Exp. Biol. 130:121-136. <https://dx.doi.org/10.1242/jeb.130.1.121> <PMid:2442283>

Bicalho R.C., Warnick L.D. & Guard C.L. 2008. Strategies to analyze milk losses caused by diseases with potential incidence throughout the lactation: a lameness example. J. Dairy Sci. 91(7):2653-2661. <https://dx.doi.org/10.3168/jds.2007-0744> <PMid:18565924>

Blowey C.W. 2015. Cattle Lameness and Hoofcare. 3rd ed. 5M Publishing, Sheffield. 164p.

Concha C., Carretta M.D., Alarcon P., Conejeros I., Gallardo D., Hidalgo A.I., Tadich N., Caceres D.D., Hidalgo M.A. & Burgos R.A. 2014. Oxidative response of neutrophils to platelet-activating factor is altered during acute ruminal acidosis induced by oligofructose in heifers. J. Vet. Sci. 15(2):217-224. <https://dx.doi.org/10.4142/jvs.2014.15.2.217> <PMid:25013355>

Greenough P.R. 2007. Bovine Laminitis and Lameness: a hands-on approach. Saunders Elsevier, St. Louis. 328p. <https://dx.doi.org/10.1016/B978-0-7020-2780-2.X5001-0>

Hoblet K.H. & Weiss W. 2001. Metabolic hoof horn disease claw horn disruption. Vet. Clin. N. Am., Food Anim. Pract. 17(1):111-127. <https://dx.doi.org/10.1016/S0749-0720(15)30057-8>

Kalinin A., Marekov L.N. & Steinert P.M. 2001. Assembly of the epidermal cornified cell envelope. J. Cell Sci. 114(Pt 17):3069-3070. <https://dx.doi.org/10.1242/jcs.114.17.3069> <PMid:11590230>

Langova L., Novotna I., Nemcova P., Machacek M., Havlicek Z., Zemanova M. & Chrast V. 2020. Impact of nutrients on the hoof health in cattle. Animals, Basel, 10(10):1824. <https://dx.doi.org/10.3390/ani10101824> <PMid:33036413>

Lean I.J., Westwood C.T., Golder H.M. & Vermunt J.J. 2013. Impact of nutrition on lameness and claw health in cattle. Livest. Sci. 156(1/2):71-87. <https://dx.doi.org/10.1016/j.livsci.2013.06.006>

Mendes H.M.F., Casagrande F.P., Lima I.R., Souza C.H., Gontijo L.D., Alves G.E.S., Vasconcelos A.C. & Faleiros R.R. 2013. Histopathology of dairy cows' hooves with signs of naturally acquired laminitis. Pesq. Vet. Bras. 33(5):613-619. <https://dx.doi.org/10.1590/S0100-736X2013000500011>

Mendes H.M.F., Rocha Júnior S.S., Paz C.F.R., Valadares R.C., Ribeiro R.M., Azevedo D.S.D., Vasconcelos A.C. & Faleiros R.R. 2018. Testing a new surgical instrument to obtain bovine hoof biopsy samples. Pesq. Vet. Bras. 38(6):1077-1084. <https://dx.doi.org/10.1590/1678-5150-PVB-5073>

Mendonça A.C., Silva L.A.F., Fioravanti M.C.S., Moraes J.O.R., Almeida C.F., Oliveira K.S., Oliveira M.P. & Silva L.M. 2003. Aspectos morfológicos dos dígitos de bovinos das raças Gir e Holandesa. Ciênc. Anim. Bras. 4(1):53-60.

Mulling C. & Hagen J. 2012. Importance of claw disorders and functional anatomy of the claw. Prakt. Tierarzt 93:4-10.

Mulling C.K.W., Bragulla H.H., Reese S., Budras K.-D., Steinberg W. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anat. Histol. Embryol. 28(2):103-108. <https://dx.doi.org/10.1046/j.1439-0264.1999.00180.x.> <PMid:10386004>

Nagaraja T.G. & Lechtenberg K.F. 2007. Acidosis in Feedlot Cattle. Vet. Clin. N. Am., Food Anim. Pract. 23(3):333-350. <https://dx.doi.org/10.1016/j.cvfa.2007.04.002>

Nelson D.L. & Cox M.M. 2011. Princípios de Bioquímica de Lehninger. 5ª ed. Editora Artmed, Porto Alegre. 1304p.

Nocek J.E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80(5):1005-1028. <https://dx.doi.org/10.3168/jds.S0022-0302(97)76026-0> <PMid:9178142>

Noronha Filho A.D.F., Freitas S.L.R., Rodrigues D.F., Mendes F.F., Miguel M.P., Cunha P.H.J., Fioravati M.C.S. & Silva L.A.F. 2019. Characterization of ruminal acidosis and initial phase of laminitis inducted by oligofructose in crossbred calves. Pesq Vet. Bras. 39(2):99-106. <https://dx.doi.org/10.1590/1678-5150-PVB-5619>

Obel N. 1948. Studies on the Histopathology of Acute Laminitis. Almquist and Wiskells, Sweden. 95p.

Ossent P. & Lischer C.H.J. 1998. Bovine laminitis: the lesions and their pathogenesis. Practice 20(8):415-427. <https://dx.doi.org/10.1136/inpract.20.8.415>

Queiroz P.J.B., Assis B.M., Silva D.C., Noronha-Filho A.D.F., Pancotti A., Rabelo R.E., Borges N.C., Vulcani V.A.S. & Silva L.A.F. 2021. Mineral composition and microstructure of the abaxial hoof wall in dairy heifers after biotin supplementation. Anat. Hist. Embryol. 50(1):93-101. <https://dx.doi.org/10.1111/ahe.12605> <PMid:32815592>

Rabelo R.E., Vulcani V.A.S., Sant'Ana F.J.F., Silva L.A.F., Assis B.M. & Araújo G.H.M. 2015. Microstructure of Holstein and Gir breed adult bovine hooves: histomorphometry, threedimensional microtomography and microhardness test evaluation. Arq. Bras. Med. Vet. Zootec. 67(6):1492-1500. <https://dx.doi.org/10.1590/1678-4162-8282>

Rebiere H., Kermaïdic A., Ghyselinck C. & Brenier C. 2019. Inorganic analysis of falsified medical products using X-ray fluorescence spectroscopy and chemometrics. Talanta 195:490-496. <https://dx.doi.org/10.1016/j.talanta.2018.11.051> <PMid:30625574>

Reece W.O. 2006. Dukes – Fisiologia dos Animais Domésticos. 12ª ed. Guanabara Koogan, Rio de Janeiro. 926p.

Schrooyen P.M.M., Dijkstra P.J., Oberthür R.C., Bantjes A. & Feijen J. 2001. Stabilization of solutions of feather keratins bysodium dodecyl sulfate. J. Colloid Interface Sci. 240(1):30-39. <https://dx.doi.org/10.1006/jcis.2001.7673> <PMid:11446783>

Shearer J., Anderson D., Ayars W., Belknap E., Berry S., Guard C., Hoblet K., Hovingh E., Kirksey G., Langill A., Mills A., Miskimins D., Osterstock J., Price R., Prigel D., Roussel A., van Amstel S., Wallace R., Wasson J., Cook N., Garrett E.F., Hostetler D.E. & Schugel L. 2004. A record-keeping system for capture of lameness and foot-care information in cattle. Bovine Practitioner 38(1):83-92. <https://dx.doi.org/10.21423/bovine-vol38no1p83-92>

Silva L.A.F., Campos S.B.S., Rabelo R.E., Vulcani V.A.S., Noronha Filho A.D.F. & Freitas S.L.R. 2015. Análise comparativa da morfometria do casco de bovinos das raças Nelore, Curraleira e Pantaneira e de bubalinos e sua relação com a etiopatogenia das enfermidades digitais. Pesq. Vet. Bras. 35(4):377-384. <https://dx.doi.org/10.1590/S0100-736X2015000400011>

Sousa R.S., Oliveira F.L.C., Dias M.R.B., Minami N.S., Amaral L., Minervino A.H.H., Shecaira C.L., Bombardelli J.A., Benesi F.J. & Ortolani E.L. 2020. Evaluation of infrared thermography, force platform and filmed locomotion score as non-invasive diagnostic methods for acute laminitis in zebu cattle. PLoS One 15(7):e0235549. <https://dx.doi.org/10.1371/journal.pone.0235549> <PMid:32628702>

Tavares N.C., Barbosa A.A., Bermudes R.F., Rechsteiner S.M.E.F., Cruz L.A.X., Bruhn F.R.P., Silva P.M. & Martins C.F. 2019. The impact of high-energy diets on the rumen environment and digital cushion in confined cattle. Pesq. Vet. Bras. 39(12):970-977. <https://dx.doi.org/10.1590/1678-5150-PVB-6230>

Thoefner M.B., Wattle O., Pollit C.C., French K.R. & Nielsen S.S. 2005. Histopathology of oligofructose-induced acute laminits in heifers. J. Dairy Sci. 88(8):2774-2782. <https://dx.doi.org/10.3168/jds.S0022-0302(05)72957-X>

Tolosa E.M.C., Rodrigues C.J., Behmer O.A. & Freitas Neto A.G. 2003. Manual de Técnicas para Histologia Normal e Patológica. Editora Manole, São Paulo. 341p.

Tomlinson D.J., Mulling C.H. & Fakler T.M. 2004. Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. J. Dairy Sci. 87(4):797-809. <https://dx.doi.org/10.3168/jds.S0022-0302(04)73223-3>

Voet D. & Voet J.G. 2011. Biochemistry. 4th ed. John Wiley and Sons, New York. 1428p.

Zebeli Q. & Metzler-Zebeli B. 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Res. Vet. Sci. 93(3):1099-1108. <https://dx.doi.org/10.1016/j.rvsc.2012.02.004> <PMid:22370295>