In vitro biomechanical properties of sole tissues: Comparison between healthy and ulcerated bovine claws

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ABSTRACT

Sole ulcers are reportedly one of the most prevalent diseases associated with lameness in dairy cattle, significantly affecting animal welfare and farm profitability. The degree to which sole soft tissues, healthy or ulcerated, are able to maintain their structure and function when subjected to compressive forces remains unknown. Therefore, the aims of the present study were to assess sole tissue biomechanics in healthy and ulcerated claws and to describe correlated histology. Cylindrical samples were harvested from zones 4 and 6, as described by the international foot map, from hind lateral healthy ($n = 12$) and ulcerated bovine claws ($n = 8$; animals $n = 12$). Tissue biomechanics and morphology were evaluated via compressive tests and hematoxylin-eosin-phloxine-saffron staining, respectively. A 2-sample *t*-test was used to compare zones' mechanical properties between healthy and ulcerated tissues, and the Cochran-Mantel-Haenszel test was used to measure the effect of claw zone on histology. The fibril modulus (E_f) and permeability (k) respectively increased and decreased in ulcerated claws ($E_f = 0.201 \pm 0.104$ MPa; $k = 0.128 \pm 0.069$ mm²/MPa·s) compared with healthy claws ($E_f = 0.105 \pm 0.050$ MPa; $k = 0.452 \pm 0.365$ mm²/MPa·s) only for zone 6. Histology scores equal to or greater than 3 were associated with macroscopic presence of ulceration. A higher proportion of adipose tissue (30% or more) was associated with zone 6 compared with zone 4, but no difference was seen between healthy and ulcerated claws. Ulcerated claws had a higher prevalence of exostoses compared with healthy ones (33% vs. 8%). Sole soft tissues showed, as

hypothesized, a viscoelastic behavior using unconfined compression testing, which, however, may not reflect in vivo loading conditions. Clinical and histological signs of sole ulceration were not associated with decreased strength of the supportive apparatus of the distal phalanx in zone 4 in this study.

Key words: dairy cow, sole ulcer, biomechanics, histology, claw supportive apparatus

INTRODUCTION

Lameness is one of the most important welfare issues in modern dairy industry worldwide, with growing concern among the general public. Claw lesions have long been recognized as the leading cause of lameness in dairy cattle (Murray et al., 1996). Among claw horn disruption lesions (CHDL), sole ulcers are reported to be the most prevalent disease associated with lameness (Murray et al., 1996; Bicalho et al., 2007; Cramer et al., 2008), significantly affecting animal welfare and farm profitability (Hultgren et al., 2004; Amory et al., 2008; Cha et al., 2010).

Despite their undeniable importance, little is yet known about the etiology and pathophysiology of sole ulcers. Nowadays it is widely accepted that sole ulcers are a consequence of contusions of the corium and germinal epithelium of the sole at the level of the flexor tuberosity of the distal phalanx within the horn capsule. Ultimately, disturbances in the microvasculature of the compressed area of the corium lead to ischemia, hypoxia, disruption of keratinization and, eventually, sole horn perforation (Mochizuki et al., 1994; Singh et al., 1994; Hendry et al., 2001).

Although in the past it has been suggested that sole ulcers are caused by subclinical laminitis (Chew, 1972; Greenough et al., 1990; Boosman et al., 1991), the evidence to support this theory in dairy cattle is limited (Lischer et al., 2002; Logue et al., 2004; Thoenes et al., 2005). The term “subclinical laminitis” has been used to explain the underlying cause of infectious claw

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lesions; however, the nomenclature has evolved. Sole ulcers in dairy cattle are now considered part of the so-called claw horn disruption syndrome. The term “claw horn disruption” regroups all disease processes that ultimately affect horn formation at the cellular level.

Extensive research has been carried out on equine laminitis in association with inflammatory processes such as carbohydrate overload and endotoxemia. What has traditionally been called laminitis in cattle appears to be primarily a degenerative rather than an inflammatory process, as dissociation between laminar corium and horn leaflets has never been demonstrated, nor has the presence of signs of repair such as hyperkeratosis and thickening of epidermal leaflets. Rather, the process in cattle is characterized by a stretching of the dermal lamellae (Lischer et al., 2002; Thoenes et al., 2005; Danscher et al., 2010). The physiological hormonal changes occurring around calving appear to affect the integrity of the distal phalanx suspensory apparatus via activation of matrix metalloproteinases, resulting in an increase in the elasticity of collagen fibers, thus allowing the distal phalanx to sink within the horn capsule (Tarleton et al., 2002; Huxley, 2012). Likewise, recognition is growing in the equine medicine field that a high proportion of laminitis cases may be associated with an underlying endocrinopathy rather than with endotoxemia and systemic inflammation. Obesity and insulin resistance are recognized as contributing to development of laminitis in animals affected by the so-called equine metabolic syndrome (Johnson et al., 2010; Durham et al., 2019). Stretching of the secondary epidermal lamellae is the earliest histological change observed in equine hyperinsulinemic laminitis models (de Laat et al., 2013).

The digital cushion, which is a modified layer of subcutis situated beneath the distal phalanx, has been investigated in more detail (Räber et al., 2004; Bicalho et al., 2009; Newsome et al., 2017a,b). Composed of adipose and connective tissue, it is considered important in dampening compression and dissipating forces at the beginning of the stance phase (Schmid et al., 2009), thus protecting the corium and germinal epithelium under the distal phalanx (Lischer et al., 2002; Räber et al., 2004). Thickness of the digital cushion beneath the flexor tuberosity of the distal phalanx has been investigated in several studies using ultrasonography (Bicalho et al., 2009; Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017a). Bicalho et al. (2009) and Newsome et al. (2017a) reported positive association between digital cushion thickness and BCS or back fat thickness, respectively. Moreover, digital cushion thinning appeared to predispose to subsequent CHDL (Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017b).

The degree to which sole soft tissue (digital cushion and corium), healthy or ulcerated, is able to maintain structure and function when subjected to compressive forces remains unknown. The link between tissue thickness and mechanical properties is also not clearly determined. Knowledge of the biomechanical properties of claw tissues will provide a basis for improved understanding of the pathogenesis of lesions and, possibly, for treatment and prevention strategies.

Therefore, the objectives of the present study were (1) to assess sole soft tissue mechanical behavior in response to compression in healthy and ulcerated bovine claws, (2) to describe sole horn mechanical behavior in response to compression in healthy and ulcerated bovine claws, and (3) to describe the corresponding histology. We hypothesized that sole soft tissue (digital cushion and corium) and horn would exhibit a viscoelastic behavior in response to compressive loading. Additionally, we hypothesized that sole soft tissue from ulcerated claws, compared with healthy claws, would show increased rigidity and decreased permeability, leading to reduced viscoelastic compression-resistance capacity. Likewise, we hypothesized that sole horn from ulcerated claws, compared with healthy claws, would show decreased elastic modulus. Moreover, we hypothesized that biomechanical changes would correlate with histological abnormalities.

MATERIALS AND METHODS

Sample Collection and Preparation

Hind feet were harvested from mature Holstein dairy cattle presented to the farm animal hospital of the Université de Montréal (Saint Hyacinthe, Canada) and subjected to euthanasia for reasons unrelated to this study. As such, approval by the institutional animal care and use committee was not required. Animals were selected on a convenience base within a 6-mo period. Information on age, BW, BCS and stage of lactation (DIM) was obtained from hospital medical records. Before euthanasia, BCS was determined for each animal according to the scoring chart developed by Edmonson et al. (1989). After euthanasia and before necropsy, BW was recorded. All feet were cleaned and the sole surface trimmed and observed for macroscopic abnormalities. Lateral claws were categorized as healthy ($n = 12$) or ulcerated ($n = 8$). Healthy claws were defined as having intact sole after trimming and being devoid of infectious lesions, such as digital dermatitis, and horn diseases including wall abnormalities, sole hemorrhages, white line disease, and heel-horn erosions. Ulcerated claws were defined as presenting a sole horn defect at the typical site [zone 4, as described by the international

foot map by Shearer et al. (2004)] with exposure of the corium or of granulation tissue. When a sole ulcer was present on one of the hind lateral claws, the contralateral lateral claw was excluded if healthy. Lateral claws of each hind foot were separated with a band saw and frozen. Then zones 4 and 6 of each claw, as described by the international foot map, were identified, and 2 samples (8-mm diameter) were obtained from each zone by cutting the frozen tissues with a hand-made biopsy punch. When sole ulceration was present, samples from zone 4 were taken at the ulcer border, to obtain soft tissue and horn. Samples from zone 6 were harvested as near as possible to the border with zone 4. Samples from zone 6 were used as negative controls, because this zone is normally exempt of sole ulcers. Each cylindrical sample was composed of approximately 3 mm of cortical bone of the distal phalanx, the full thickness of digital cushion and corium (sole soft tissues), and the sole horn (Figure 1). One of the 2 samples from each zone was randomly assigned to either mechanical testing or histological evaluation. Samples for mechanical testing were wrapped in a 4-square-inch gauze moistened with 3 mL of saline solution and individually stored in small sealed plastic containers to prevent dehydration. Samples were kept at -20°C for a maximum of 30 d before mechanical testing.

Mechanical Testing

Samples for mechanical testing were allowed to thaw at room temperature for 2 h. Sole soft tissues were isolated from bone (and deep digital flexor tendon, if present in samples harvested from zone 6) and horn using a scalpel blade. Two types of mechanical tests were completed: soft tissues (digital cushion and corium) underwent a stress–relaxation test under unconfined compression, and horn tissues underwent uniaxial compression until failure. For soft tissues, testing was performed in a hydrated environment at room temperature, using a micromechanical testing system (Mach-1, model MA410, Biomomentum, Biomomentum Inc., Laval, QC, Canada; load range 10 N). The cylindrical sample was placed between the 2 impermeable platens, allowing for its lateral expansion, in a cylindrical bath filled with PBS to maintain hydration during testing. The distance between the platens was recorded electronically and defined as the sole soft tissue thickness. Loads were recorded with a load cell, whose range and resolution were, respectively, 10 and 0.5 mN. Axial displacements were controlled via an encoder of 100-mm range and 0.5-mm resolution. After an initial pre-strain at 5% strain, samples were loaded at 20% strain at a strain rate of $1.5 \times 10^{-2}/\text{s}$. A relaxation criterion of 0.025 g/min was used to allow samples to reach equilibrium.

Figure 1. Cylindrical sample obtained from sole zone 4, composed of cortical bone of the distal phalanx, the full thickness of digital cushion and corium (sole soft tissue), and the full thickness of the epidermis, including living epidermis and dead cornified epidermis (horn).

librium. For each sample, stress–relaxation curves were recorded, from which the maximum compressive stress ($\sigma_{\max}$) was located at the time at which the applied 20% maximum strain was reached, and the equilibrium stress (σ_{eq}) upon reaching the relaxation criterion. The matrix equilibrium modulus (E_m) was directly evaluated based on the applied strain and relaxed stress values obtained from the experimental curves. The fibril network reinforced biphasic model (Korhonen et al., 2003) was fitted to the data to obtain the fibril modulus (E_f) and permeability (k) using the Mach-1 analysis software (Biomomentum Inc.).

Horn samples were subjected to a uniaxial compression test under controlled displacement in a quasi-static condition at 1 mm/min until failure. Before compression, samples were preloaded at 50 N to ensure good contact between the sample and the smooth platens. Compression tests were performed at room temperature using a micromechanical testing system [MTS/Insight electromechanical testing system 50; load range 50 kN (resolution of 2.5 kN); displacement range 1,100 mm (resolution of 0.001 mm); MTS Systems Corporation, Eden Prairie, MN]. The distance between the platens and the compression force were recorded electronically; the first was defined as the horn sample thickness. For each sample, stress (force over sample cross-sectional area) versus strain (displacement over tissue thickness) curves were recorded, from which the elastic modulus (E) was extracted at 20% of strain.

Histology

Samples for histological evaluation were fixed in 10% buffered formalin solution for at least 48 h and subsequently decalcified in Rapid Decalcifier (Apex, Aurora, IL) and dekeratinized in 10% NaOH, then embedded

in paraffin. Sections of 3- μ m thickness were mounted on glass slides and stained with hematoxylin-eosin-phloxine-saffron, following standard technique.

Samples for histology were evaluated blindly by 2 of the authors (NW, trained pathologist, and EM) at a 3-wk interval. A 4-grade evaluation system, where grade 1 was considered normal, was adapted from the 5-grade scale previously reported by Mochizuki et al. (1994) and was based on the severity of epidermal, microvascular, and collagenic abnormalities (Table 1; Figure 2). Epidermal and dermal papillae were evaluated for their shape and pattern as regular or flattened and irregular. The living epidermal thickness was described as normal or decreased (epidermal atrophy). Dyskeratosis, defined as abnormal keratinization occurring prematurely within groups of cells in the suprabasal layers, and spongiosis, defined as intercellular edema, were classified as absent, mild, moderate, or severe. Microvascular abnormalities were defined as thrombosis, dilation, endothelial hypertrophy, or increased number of dermal vessels, and were classified as absent, mild, moderate, or severe. Collagen was described as organized or disorganized, with or without basophilic degeneration.

In addition, the degree of soft tissue mineralization, the presence or absence of exostoses, and the proportion of adipose tissue (visual estimation) were assessed. Soft tissue mineralization was classified as absent, mild, moderate, or severe, and the proportion of adipose tissue categorized from 1 to 5, where 1 was <10%, 2 between 10 and 30%, 3 between 30 and 50%, 4 between 50 and 75%, and 5 >75% of the dermis or subcutaneous tissue.

Statistical Analysis

Statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC). Data sets were tested for normality and homogeneity of variances, and a logarithm transformation was applied when needed. A 2-sample *t*-test was used to compare mechanical properties of each zone between healthy and ulcerated claws. Weighted kappa was used to evaluate inter- and intra-rater agreement for histology evaluation. The Cochran-Mantel-Haenszel test for repeated measures was used to evaluate the effect of claw zone and macroscopic appearance on histology grade, proportion of adipose tissue, and presence of exostoses. Results were considered significant with $P < 0.05$.

RESULTS

Twenty lateral hind limb claws were harvested from 12 mature Holstein dairy cows with mean age of 6.5 yr

Table 1. Grading system for histology evaluation

Grade	Dermo-epidermal junction				Corium		
	Papillae	Living epidermis thickness	Dyskeratosis	Spongiosis	Microvasculature abnormalities	Collagen	Mineralization
1	Regular	Normal	Absent	Absent	Absent	Organized	Absent
2	Regular	Decreased	Mild	Mild	Mild	Organized	Mild
3	Irregular and flattened	Decreased	Moderate	Moderate	Moderate	Disorganized without basophilic degeneration	Moderate
4	Irregular and flattened	Decreased	Severe	Severe	Severe	Disorganized with basophilic degeneration	Severe

old ± 2 yr (SD), mean BW of 636 kg ± 90 kg, mean BCS of 3.5 ± 0.5 , and median DIM of 16 d (range 3 to 305 d). Claws were categorized as healthy (12) or ulcerated (8) based on macroscopic evaluation after trimming. The BCS in the healthy group was 3.46 ± 0.25 , compared with 3.44 ± 0.4 of the ulcerated group.

Sole soft tissue (digital cushion and corium) thickness was significantly higher in zone 6 ($7.40 \text{ mm} \pm 0.25 \text{ mm}$) compared with zone 4 ($6.14 \text{ mm} \pm 0.25 \text{ mm}$; $P = 0.004$). Horn thickness after trimming was not related to claw zone (zone 4: $6.04 \text{ mm} \pm 1.74 \text{ mm}$; zone 6: $6.35 \text{ mm} \pm 1.68 \text{ mm}$).

Mechanical Properties

A typical experimental stress-relaxation curve for sole soft tissue and a typical uniaxial compression curve for sole horn are presented in Figure 3. The corresponding fibril network reinforced biphasic curve showed good agreement with the experimental data of the stress-relaxation tests (Figure 3).

Data for mechanical properties are presented in Table 2. Data from 3 healthy samples for zone 6 and 1 ulcerated sample for zone 4 were excluded from the statistical analysis because of aberrant values. The

Figure 2. (A) Healthy claw, hematoxylin-eosin-phloxine-saffron (HPS) stain, $1.25\times$, 1-mm scale bar, showing horn layer (a), living epidermis with tall and regular papillae (b), corium with well-organized loose collagen (c), and well-developed digital cushion (d) underneath the distal phalanx bone (e). (B) Ulcerated claw, HPS stain, $1.25\times$, 1-mm scale bar, showing horn layer (a), living epidermis with irregular papillae (b), corium containing disorganized and dense collagen with basophilic degeneration (c), digital cushion (d), and distal phalanx bone (e) with an exostosis (*). (C) Ulcerated claw, HPS stain, $40\times$, $50\text{-}\mu\text{m}$ scale bar, showing moderate intercellular edema (spongiosis). (D) Ulcerated claw, HPS stain, $10\times$, $50\text{-}\mu\text{m}$ scale bar, showing mild dyskeratosis. (E) Ulcerated claw, HPS stain, $10\times$, $50\text{-}\mu\text{m}$ scale bar, showing disorganized collagen and moderately increased number of dermal vessels ($\blacktriangle$).

Table 2. Mechanical properties for sole soft tissue (E_m , k , E_f) and sole horn (E) in zones 4 and 6, expressed as mean $\pm$ SD¹

Item	No. of healthy vs. ulcerated claws	Sole soft tissue			Sole horn
		E_m , MPa	k , mm ² /MPa·s	E_f , MPa	E , MPa
Zone 4	Healthy: n = 12	0.008 $\pm$ 0.003	0.624 $\pm$ 0.542	0.095 $\pm$ 0.041	36.191 $\pm$ 8.513
	Ulcerated: n = 7	0.008 $\pm$ 0.002	0.313 $\pm$ 0.285	0.112 $\pm$ 0.044	29.360 $\pm$ 9.306
Zone 6	Healthy: n = 9	0.008 $\pm$ 0.114	0.452 $\pm$ 0.365*	0.105 $\pm$ 0.050*	31.899 $\pm$ 12.875
	Ulcerated: n = 8	0.051 $\pm$ 0.114	0.128 $\pm$ 0.069*	0.201 $\pm$ 0.104*	34.490 $\pm$ 6.958

¹ E_m = matrix equilibrium modulus; k = permeability; E_f = fibril modulus; E = elastic modulus.

* $P < 0.05$.

2-sample t -test showed that only the fibril modulus (E_f) and the permeability (k) were significantly increased and decreased, respectively, in ulcerated claws ($E_f = 0.201 \pm 0.104$ MPa; $k = 0.128 \pm 0.069$ mm²/MPa·s) compared with healthy claws ($E_f = 0.105 \pm 0.050$ MPa; $k = 0.452 \pm 0.365$ mm²/MPa·s) for zone 6 ($P = 0.03$). All other tested mechanical properties were not statistically different between healthy and ulcerated claws for either claw zone.

Histology

Samples for histology were classified as grade 1 (n = 11), 2 (n = 13), 3 (n = 13), or 4 (n = 3). The strength of inter- and intra-rater agreement was considered fair to very good (weighted kappa ranged between 0.534 and 0.896). The Cochran-Mantel-Haenszel test indicated association between histology scores equal to or greater than 3 and macroscopic presence of ulceration ($P = 0.005$). A higher proportion of adipose tissue (30% or more) was associated with zone 6 compared with zone 4 ($P = 0.008$), but no difference was found in the proportion of adipose tissue in healthy and ulcerated claws. Ulcerated claws had a higher prevalence of

exostoses (33%) compared with healthy ones (8%; $P = 0.04$). Exostoses were identified on the sole surface of the distal phalanx.

DISCUSSION

Despite macroscopic and histological signs of sole ulceration, only some differences in mechanical properties of sole soft tissue and sole horn between healthy and ulcerated claws could be demonstrated in the present study. Mechanical weakening of the claw supportive apparatus might be delayed relative to the onset of histological changes. In the current study, no information was available on previous trimming and possible presence of claw lesions; thus, the chronicity of the lesions could not be assessed. Therefore, it is possible that the ulcerated claws used in the current study were well into the healing process and that, consequently, no differences in mechanical properties could be detected when compared with healthy claws for zone 4. Soft tissue mineralization and presence of exostoses observed histologically in some of the ulcerated samples do suggest chronicity. Moreover, biological tissues are composed of different material with specific mechanical properties,

Figure 3. Representative experimental curves obtained from mechanical testing. (A) Stress-relaxation curve for sole soft tissue (black solid line) with corresponding fibril network biphasic curve (gray dotted line). (B) Uniaxial compression stress-strain curve for sole horn.

meaning that the mechanical properties may vary from one point to another inside the same tissue. Our test samples were of small diameter and thus may not be representative of the entire tissue structure.

As hypothesized, the fibril network modulus (E_f) and the permeability (k) of the sole soft tissue were significantly increased and decreased, respectively, in ulcerated claws compared with healthy tissues. However, this was true only for zone 6. A similar trend could be identified also for zone 4, although the finding was not statistically significant. The increased fibril modulus, which quantifies the material's resistance to elastic tensile deformation, and the decreased permeability in ulcerated claws may both be explained by the dense packing of collagen fibers in ulcerated soft tissues. Previous studies on cartilage mechanical properties have shown a decrease in the fibril modulus associated with a higher permeability following collagen degradation, which can be related to sparse packing of collagen fibrils resulting in a decreased resistance to fluid flow (Korhonen et al., 2003). It has also been suggested, in human patients, that excessive loading and repetitive stress concentration may tear collagenous fibers and decrease the integrity of the fatty chambers of the heel pad, ultimately altering the mechanical behavior of the tissue (Hsu et al., 2002). This process may also be involved in sole ulcer pathogenesis in cattle, but further histological and mechanical studies are warranted to verify this postulate.

Horn in zone 6 has been reported to be softer and more elastic and therefore may not represent a perfect negative control for zone 4, when compared with horn in zone 5. However, the digital cushion in zone 6 is of similar thickness to that of zone 4, when compared with the thin interdigitating digital cushion in zone 5 that would have precluded the possibility of performing mechanical tests on sole soft tissues. Selection of zone 6 of the same claw, rather than zone 4 of the medial ipsilateral claw, allowed the testing of zones believed to be subjected to the same forces, because the medial claw bears less weight than the lateral claw. This selection, however, constitutes a limitation for the interpretation of the results of the present study.

As hypothesized, sole soft tissue of the bovine claw behaved as a viscoelastic material, showing stress-relaxation typical of biologic soft tissues. Viscoelasticity implies an initial elastic deformation followed by a time-dependent viscous deformation until equilibrium is reached. Stress-relaxation describes how tissues relieve stress under constant strain and depends on deformation magnitude and rate. Deformation applied to the tissues was defined in a non-destructive range (20% strain), which simulates physiological circumstances. Unconfined compression has been the traditional tech-

nique employed to measure mechanical characteristics of soft tissues and was thus used in this study. The digital cushion is a complex structure composed of collagenous elements connecting the distal phalanx to the dermis and epidermis, compartmentalizing the fat of the digital cushion, and enclosed in a semi-confined environment, the horn claw capsule. Confined compression testing configuration might therefore be more similar to the *in vivo* loading state of the sole soft tissue, but concerns due to friction may arise in this case. The results presented in this study suggest that unconfined compression, together with the application of the fibril network reinforced biphasic model, is a suitable technique for assessing the mechanical properties of sole soft tissue in bovine claws. It must be noted, however, that other mathematical models may be suited to the characterization of claw soft tissue, such as the viscohyperelastic model used to predict the mechanical behavior of the human heel pad (Miller-Young et al., 2002; Natali et al., 2011).

The effects of freezing the samples, as well as the temperatures at which mechanical tests were performed in the present study, must be taken into consideration. No data are available regarding the effect of freezing on the mechanical properties of the supportive apparatus of the bovine claw. Boettcher et al. (2014) and Tarlton and Webster (2000) reported that sample storage at either -18°C or -20°C had no effect on the biomechanical strength (Young's modulus) of the suspensory tissue in bovine claws, compared with fresh samples. Equally, Bennett and Ker (1990) showed similar mechanical properties between human heel pads tested within 1 h from harvesting or after frozen storage (temperature not specified). However, it has been shown that freezing may negatively influence mechanical properties measured in other viscoelastic soft tissues in other animal species (Menard et al., 2014). All mechanical tests in the present study were performed at room temperature. Fat pads are presumably structured to ensure that their properties are optimal at normal body temperature. Consequently, the lower room temperature at which tissues were tested might influence the viscoelastic properties of these tissues such that present results may not be true *in vivo*. Furthermore, it has been shown that *in vitro* and *in vivo* mechanical tests of the human heel pad yield different results in terms of stiffness and energy dissipation, which are primarily explained by the influence of the entire lower leg in *in vivo* tests (Aerts et al., 1995). Thus, results presented here must be interpreted cautiously.

We were not able, in the present study, to show a difference in the sole horn elastic modulus between healthy and ulcerated claws. Previous studies testing sole horn under similar conditions have shown a marked

reduction in the elastic modulus of sole horn of claws affected by chronic laminitis (Hinterhofer et al., 2007). Sole horn elastic modulus values registered in the present study are more similar to those reported by Franck et al. (2006), than those reported by Hinterhofer et al. (2005, 2007). Yet it must be noted that horn humidity and dry matter content were not accounted for in this study.

Unfortunately, no information was available regarding housing of the animals enrolled in the present study. More than 80% of Quebec's dairy farms are based on tiestalls (<https://dairyinfo.gc.ca/eng/dairy-statistics-and-market-information/farm-statistics/dairy-barns-by-type/?id=1502467060775>). Although no data are available for adult dairy cows, Gard et al. (2015) stated that exercise and terrain may increase the volume and surface area of the digital cushion of the feet of dairy calves, thus reducing the prevalence of lameness associated with CHDL. It would appear, intuitively, that the prevalence of sole ulcers would be better controlled in freestall barns, considering animals thus housed exercise more than do those in tiestalls. Nevertheless, prevalence of sole ulcers at both cow and herd level is higher in freestalls compared with tiestalls (Sogstad et al., 2005; Cramer et al., 2008). This can be explained by the interaction between claws and hard surfaces, such as concrete, commonly present in freestall barns; excessive horn wear and standing long hours on concrete may predispose to CHDL. Indeed, the odds of sole ulcers and white line disease are more than double among cows housed in conventional freestalls than among those housed in deep-bedded packs (Solano et al., 2016). All these data corroborate the influence of the type of terrain and floor system on claw pathologies; thus, the interaction between sole soft tissue and floor warrants further investigation. In this direction, Telezhenko et al. (2019) made progress on evaluating the differences between the force and pressure distribution inside the claw (using sensors inserted inside the sole at the edge of the corium-claw horn junction) compared with those measurable externally in an ex vivo model where the distal limb was tested on 3 different surfaces (solid concrete, rubber mat, and grazed pasture).

The histology of claw tissues presents several challenges. Decalcification of bone and dekeratinization of horn subjectively affected the quality of the stained samples in the present study. Paraffin embedding is widely used in morphological studies, but its high melting temperature may cause heat-induced changes to the tissues. Additionally, the presence of hard and soft tissues with hardness and density different from those of the paraffin complicates microtome cutting. The use of

methyl methacrylate for the embedding of mineralized, soft, and horny tissues may have multiple advantages, such as elimination of the need for decalcification and the preservation of tissue morphology without shrinkage (Velde et al., 1977). This material was not available in our laboratory, but further investigation of its use as an embedding material for claw tissues is warranted.

Histology results of this study are similar to those reported by Mochizuki et al. (1994), in which high prevalence of tissues graded as 2 and 3 were reported. Microscopic evaluation of sole soft tissues confirmed that a higher proportion of adipose tissue is present in zone 6 compared with zone 4, reflecting the same findings as for sole soft tissue thickness. In both cases, however, we failed to detect a difference between healthy and ulcerated claws. To our knowledge, the present study is the first to evaluate the bone–corium and corium–epidermis interfaces simultaneously. High prevalence of exostoses in ulcerated claws may suggest chronicity of lesions. Concomitant evaluation of the bone–corium interface of the wall suspensory apparatus and of the sole warrants further investigation, in an effort to elucidate the pathophysiology of sole ulceration. Immunohistochemical studies on collagen fiber types of the suspensory and supportive apparatus of the claw, and their possible differences in healthy and ulcerated tissues, are needed.

CONCLUSIONS

Sole soft tissue of the bovine claw behaved as a viscoelastic material, showing stress–relaxation typical of soft tissues during unconfined compression testing, which, however, may not reflect in vivo loading conditions. Clinical and histological signs of sole ulceration were not associated with decreased strength of the supportive apparatus of the distal phalanx in zone 4 in this study. Further investigation of sole soft tissue strength is needed, especially with regard to lesions' stage, and concomitant evaluation of the suspensory apparatus.

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