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A method for characterizing the mechanical behaviour of hoof horn

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Abstract The hoof plays an important role in the health and well-being of dairy animals. Consequently, mechanical properties of the hoof horn and changes in this tissue with nutrition or environmental factors are important concerns. A novel experimental approach for evaluating the mechanical behaviour of hoof horn has been developed. The process is comprised of obtaining incremental slices of hoof horn, stamping samples from selected zones of the sectioned tissue, performing uniaxial tensile tests and evaluating the mechanical response using digital image correlation (DIC). From a combination of unique methods of extraction and evaluation, the process enables hoof horn tissue to be characterized as a function of distance from the dermal-epidermal junction, within specific regions of the claw and as a function of hydration. In addition, the methods enable both the elastic and inelastic response of the tissue to be quantified. A preliminary study was performed to validate the new approach. In this manuscript the methods of evaluation are described and demonstrated through an examination of the mechanical behaviour of bovine hoof horn at two different levels of hydration.

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Introduction

Hoof horn tissue is a keratinized epithelial tissue that must transfer loads borne from the musculoskeletal system to the ground. It also serves to protect the delicate proliferative layers of the epidermis and capillary beds of the underlying dermis against injury. A change in the structure or properties of hoof horn tissue can increase the potential for opportunistic infections or can directly result in lameness of the animal. Conversely, there may be changes in the mechanical behaviour of hoof horn caused by infectious, metabolic, nutritional and/or environmental factors that diminish hoof horn function and hamper animal mobility. These issues are of significant importance to herd management and animal health.

The mechanical behaviour of hoof horn has been explored in a number of studies. Experimental evaluations of equine horn have distinguished that the mechanical properties are not uniform, and that there are large differences between the wall and sole [1–5]. Similarly, evaluations of healthy hoof horn from bovine have distinguished that the hardness is site specific [6–8]. A recent study compared properties of the white line (the division between the innermost sole and outermost wall horn) from the claws of Holstein cows [9, 10]. Interestingly, the white line of the medial claws exhibited a larger biomechanical tensile strength than that of the lateral claws. Although the evaluation did not examine the elastic behaviour, the differences in strength suggest that there are structural and mechanical differences between the claws of a single limb. If these differences have evolved as a function of weight bearing, then there may also be unique differences between the properties of horn tissue from the front and rear hooves. It remains unclear whether there are mechanical differences in hoof horn properties with respect to distance from the dermal epidermal junction (DEJ) or between the front and rear hooves of an animal. Other studies have reported that the mechanical properties of hoof horn are influenced by nutrition [11–16] and moisture [17–21]. Through these studies there is increased recognition that the dairy environment and nutrition contribute to hoof horn properties and, therefore, may play an important role on animal well-being. Nevertheless, at present the mechanical behaviour of bovine hoof horn is not completely understood.

There are obstacles to characterizing the properties of hoof horn. Namely, there are difficulties in preparing samples of tissue with size and geometry that enables an evaluation of properties limited to specific locations of the hoof. Furthermore, the methods used to characterize the properties of common engineering structural materials are not directly applicable to hoof horn due to the relatively large compliance and moisture [19, 22]. Therefore, the overall objective of the investigation was to develop and validate a new method for obtaining horn samples from different areas of bovine hooves, and for quantifying the mechanical behaviour of the sampled materials. As such, this preliminary study represents an incremental step towards an identification of the relationship between the diet of dairy animals, the dairy environment, and properties of the hoof.

Experimental methods

The experimental approach is comprised of preparing specimens from selected horn tissue and characterizing the mechanical behaviour in uniaxial tension. To emphasize important details, the methods are presented through an application to the front and rear hooves of two mature (between 1 and 3 years of age) cows. The hooves were obtained within 12 h of slaughter and a veterinarian was present to provide a clinical diagnosis at receipt; the hooves of each animal were diagnosed as sound with no ailments. Although the breed of each animal was known, there was no information available on the specific age or diet of the two animals, and therefore, are simply regarded as Animal 1 and Animal 2. The lateral and medial claws were separated and the sole of each claw was trimmed to establish a flat reference plane. Slices of uniform thickness (0.5 ± 0.1 mm) were then excised incrementally from the sole using a handheld microtome (i.e. wood plane) until reaching the DEJ (Fig. 1(a)). The thickness of each slice was measured and recorded with its relative position from the DEJ. Using the combination of thickness and slice sequence information enabled the property distribution to be described with respect to the sole exterior or with respect to the DEJ. More than 10 slices were obtained from the medial and lateral claws of each hoof using this process and approximately 80 slices were obtained from each animal (8 claws × 10 slices = 80). The total number of slices available from each claw was dependent on the last scheduled trimming and residual sole thickness. While not conducted in this preliminary study, the sequential trimming procedure can also be used to retrieve samples from live animals as well, provided that proper restraint of the hooves could be achieved.

Conventional dogbone tensile specimens were stamped from the uniform slices using specially prepared dies. The dies were designed to enable multiple specimens to be obtained within each zone of the hoof as shown in Fig. 1(b). A schematic diagram of the final specimen geometry is shown in Fig. 1(c). In evaluating hoof horn properties of the 2 animals, tensile specimens were extracted from every second or third section (as a function of distance from the DEJ) to reduce the number of specimens examined per claw. The slices were selected such that they were equidistant from each other over the total sole thickness. In general, 3 or more tension specimens were obtained from the zones of interest from each of the selected slices. Results presented herein are limited to the response of hoof horn from Zones 4 and 5 (Fig. 1(b)).

As previously mentioned, the primary objective of the study was to develop and validate a new approach for characterizing the mechanical behaviour of bovine hoof horn. The precise moisture content in the hoof horns was not of primary interest. However, to expand the conditions involved in validation, the specimens were divided into 2 groups and stored for 48 hours at 2°C in either air or submersed in distilled water; for convenience, slices stored in the two conditions are termed "dry" and "wet", respectively. These two environments provided a broad range of conditions for evaluation and were considered to roughly bound the range of hydration that may be experienced on a dairy farm. In fact, Baillie et al. [23] has suggested that soaking hoof horn in demineralized water is the most

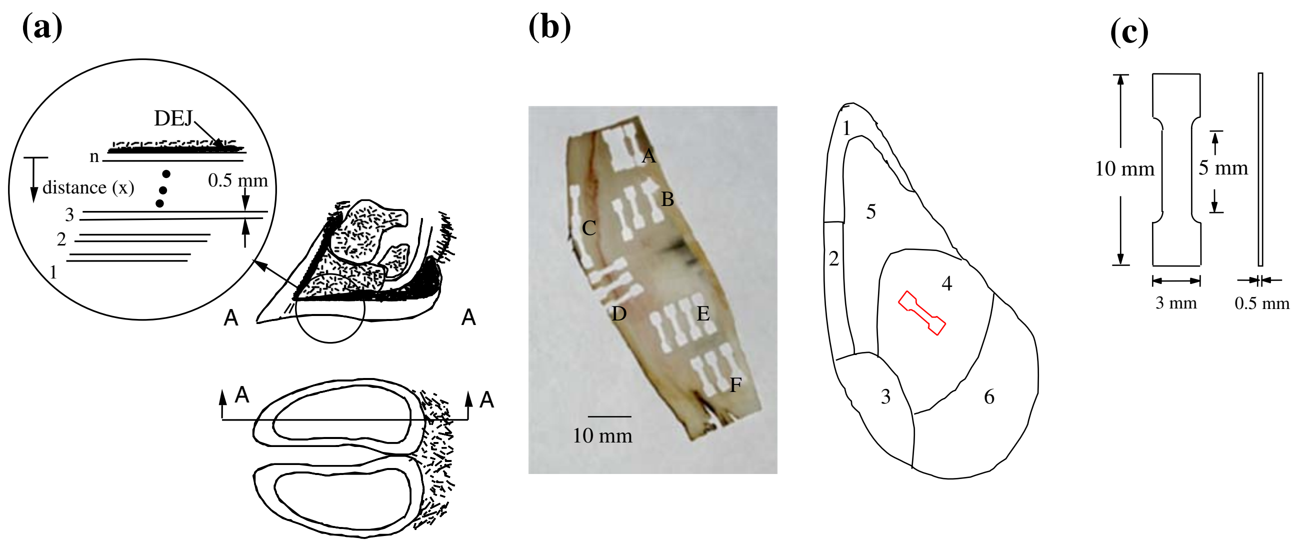

Fig. 1 Preparation of tensile specimens from hoof horn. (**a**) Sectioning of consecutive slices of hoof horn from bovine claws and terminology used in referring to the horn in reference to the DEJ. Note that 1, 2, 3, ... *n* represent the first, second, third and last slice sectioned from the sole. The sequential sections were removed using a hand-held microtome (i.e. wood plane). (**b**) A slice of bovine hoof horn with multiple specimens extracted from specific regions. Specimens denoted A and B are from Region 5 and those denoted E and F and from Regions 4 and 6, respectively. The specimens denoted C are from Region 2 (wall) and those marked as D are from Regions 2 and 4 (perpendicular to the white line) (**c**) dimensions of the stamped specimens. All dimensions are in millimeters

effective way to mimic the true moisture uptake that would occur in vivo. The mechanical behaviour of hoof horn at intermediate levels of hydration is equally relevant and will be addressed in future studies.

To quantify the moisture content, approximately 100 rectangular specimens were excised from Regions 4 and 5 of the sequential sections. A subset of the specimens obtained from each claw was stored in the two storage conditions (wet and dry); a third subset was completely dehydrated in a tissue drying oven at 60 °C until reaching a steady state weight. The average weight of the dehydrated samples was subtracted from that of the dry and wet specimens after 48 h storage. Differences in weight in each condition were divided by the average weight of the dehydrated specimens to estimate the moisture content in % mass of water present. Using this method of description, the moisture content of the fully dehydrated samples was 0% and the relative moisture content of specimens in the air-dried (dry) and submerged (wet) storage conditions was approximately 15% and 50%, respectively.

The relatively low stiffness of hoof horn and high moisture content precludes the use of an extensometer or strain gages in monitoring elongation. In addition, due to the non-uniform cross-section (width) of the tensile samples, the cross-head displacement could not be used in providing an accurate measure of the gage-section response. Thus, a non-contact optical method was adopted for measurement of elongation and strain within the sample gage section. Briefly, a thin (~25 μm) coating of white correction fluid was brushed on one surface of the gage section followed by the deposition of a peripheral coating of black enamel spray paint. Since the time required for surface preparation was approximately 2 min, the potential for hydration changes during this period was small and therefore ignored. In addition, since the applied coating was thin relative to the specimen thickness (25 vs. 500 μm), and is more compliant than hoof horn, there was negligible contribution to the mechanical response. The process resulted in a very high contrast speckle pattern on the specimen's surface, which appeared as a series of black dots of approximately 0.3 mm diameter on a matte white background. Following surface preparation the specimens were subjected to uniaxial tension to failure under displacement control actuation using a universal test center.[1] Compression grips were used to clamp both ends of the specimens leaving an average distance between grips of 8 mm (Fig. 2(a)). Axial loads were applied under displacement control actuation at a strain rate of approximately 5×10^{-4} s^{-1}. Uniaxial loading was discontinued at fracture or after achieving 30% elongation. The in-plane displacement field was

[1] Instron Dynamite Universal Testing System, Model 8841, Canton, MA.

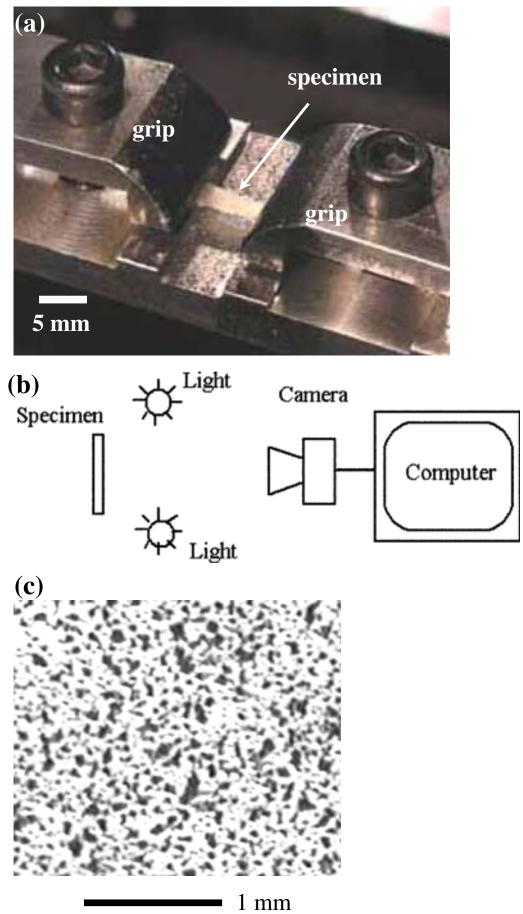

Fig. 2 Details of the experimental techniques. (**a**) Tensile specimen of hoof horn mounted between the grips, (**b**) optical arrangement, (**c**) typical speckle distribution on the surface of a tensile specimen

monitored using an optical extensometer based on Digital Image Correlation (DIC). Briefly, the optical arrangement consisted of a CCD camera with 7.5× zoom lens, an incoherent light source, and a desktop computer as described in Fig. 2(b). An image size of approximately 4 mm by 3 mm was utilized and digitized into a sample of 640 by 480 pixels with 256 gray levels. A portion of an image from the surface of a specimen is shown in Fig. 2(c) and highlights the typical speckle size and distribution. Images were captured incrementally at a frequency of between 0.5 and 1 Hz, and then synchronized with the corresponding axial load applied to the specimen.

The full-field displacement distribution, comprised of both the axial (direction of applied load) and transverse (normal to the loading axis) displacements, was obtained from the digitized speckle distributions captured at each load step. Using these quantities the strain in both the axial direction and transverse direction were quantified. Digital images acquired before and after deformation were defined in terms of the grayscale distribution at each pixel. The light intensity distribution about any particular point (x, y) can be described by the grayscale matrix $F(x, y)$ over a selected subset of the digital image. With deformation of the object, each position of the surface (x, y) is assumed to exist at a new location (x^*, y^*). The in-plane surface displacement was determined using the digital images by finding the position of the grayscale distribution $F^*(x^*, y^*)$ that most closely resembled the original distribution $F(x, y)$ according to an iterative search. The surface displacements were determined at each pixel location of the acquired images. In comparison to the magnitude of in-plane displacement resulting from uniaxial tension, the out-of-plane displacement is small and can be neglected. Thus, the location of the grayscale distribution (with highest degree of correlation) in the deformed image (x^*, y^*) is described by

$$\begin{aligned} x* &= x + u + \frac{\partial u}{\partial x}\Delta x + \frac{\partial u}{\partial y}\Delta y \\ y* &= y + v + \frac{\partial v}{\partial x}\Delta x + \frac{\partial v}{\partial y}\Delta y \end{aligned} \tag{1}$$

where u and v are the displacements of the subset center in the x and y directions, respectively. Since the displacements are much greater than the corresponding partial derivatives, the process can be simplified if only the displacements are obtained from correlation and the strain is determined according to

$$\begin{aligned} \varepsilon_x &= \frac{\mathrm{d}u}{\mathrm{d}x} \\ \varepsilon_y &= \frac{\mathrm{d}v}{\mathrm{d}y} \end{aligned} \tag{2}$$

Both the axial and transverse strains resulting from uniaxial loading were computed at each pixel location (640 by 480 pixels) over the entire window of evaluation according to Eq. (2). The strain within the gage section resulting from axial loading was estimated from the average strain over the entire documented pixel space at that load. Additional details regarding DIC are described in references [24, 25] and a discussion of applications of DIC to soft and hard tissues can be found in references [26, 27].

Mechanical properties of the bovine tensile specimens were determined from the uniaxial stress (σ) strain (ε) response. In the present study the elastic behaviour was of primary interest and thus the engineering definitions of stress and strain were used in quantifying the horn properties. A change in sample dimensions occurred as a result of the storage conditions and necessitated measurement of the cross-section for an accurate determination of the gage section stress. Hence, prior to tensile testing the gage section thickness and width was measured for each specimen using a pair of digital calipers. For each specimen the axial stress was obtained by dividing the tensile load by the original cross-section area and the strain was defined according to Eq. (2). Alternate relations should be used for quantifying the mechanical behaviour for large strains. The elastic modulus (E), proportional limit stress (σ_o) and modulus of resilience (MOR) were determined from the stress-strain response of each specimen. A representative response and distinction of the specific properties analyzed is shown in Fig. 3. The elastic modulus (E) was determined using the tangent method for strains less than 1.0% and the proportional limit stress (σ_o) was identified graphically from the onset of non-linearity. It was assumed that the proportional limit stress defined the elastic limit for specimens in either the wet or dry conditions. Both E and σ_o were evaluated with respect to distance from the DEJ in the "wet" and "dry" conditions. Differences in properties were assessed using the Student's t-test. A separate evaluation was conducted using specimens extracted from the front and rear hooves. Poisson's ratio of the hoof horn was also determined from the ratio of the transverse and axial strains. Overall, the characterization of mechanical behaviour was limited to the elastic modulus and onset of "yielding". However, the stress-strain response of each specimen was examined up to failure or an elongation exceeding 30% to validate application of the optical approach for characterizing mechanical behaviour within the inelastic range.

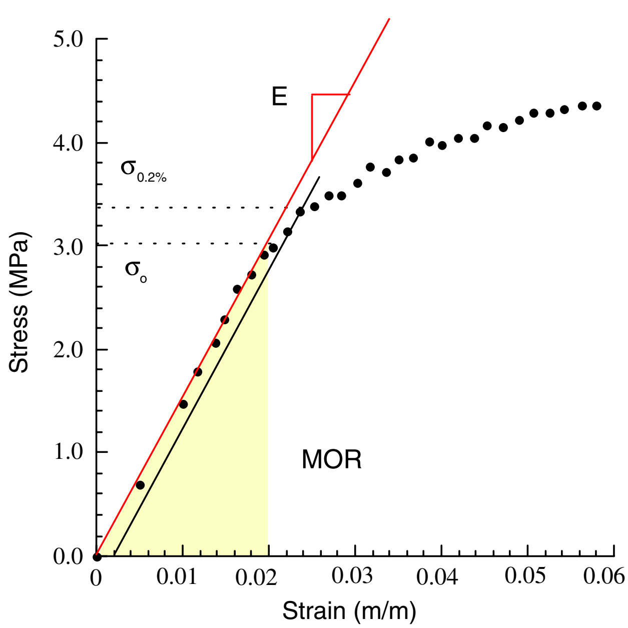

Fig. 3 Schematic diagram of a characteristic stress-strain response and the mechanical properties. In this diagram E, σ_o, $\sigma_{0.2\%}$, and MOR represent the elastic modulus, proportional limit stress, 0.2% offset yield strength, and MOR, respectively

Results and discussion

Representative stress-strain diagrams resulting from uniaxial loading of the dry and wet hoof horn are shown in Fig. 4(a) and (b), respectively. The responses presented in these figures were obtained for samples from Zone 4 (Fig. 1(b)) of the rear hooves from the same animal. While the dry horn from this region exhibited a strain to fracture of less than 10%, samples of wet horn often exceeded 40% elongation to fracture. Consequently, uniaxial tension tests with wet horn were discontinued after 30% elongation as evident from Fig. 4(b). The elastic modulus and proportional limit stress were determined for all samples. There were no significant differences in either the elastic modulus or proportional limit stress of samples from the front and rear hooves of each animal. Consequently, the properties obtained from all four hooves for each animal were pooled and the average elastic modulus and proportional limit stress for each of the two animals are listed in Table 1. Ignoring potential differences between the two animals, the overall elastic modulus of the "dry" and "wet" hoof horn was 3.02 ± 0.58 and 0.10 ± 0.03 GPa, respectively. Similarly, the average proportional limit stress (σ_o) was 30.4 ± 6.0 and 1.7 ± 0.5 MPa, respectively. As expected, there was a significant difference ($p < 0.001$) in both the elastic modulus and proportional limit stress exhibited by the wet and dry horn. These results confirm the existing knowledge that moisture plays an important role in the mechanical behaviour of hoof horn. In fact, the influence of hydration is reportedly more important than anisotropy resulting from the tubule orientation [28]. Bertram and Gosline [29] found that the elastic modulus of equine hoof wall decreased from 14.6 to 0.41 GPa after saturation, a reduction of nearly a factor of 30. Similarly, in an evaluation of donkey horn using 3-point bending, the mean elastic modulus of horn in the fully dehydrated and hydrated states were 2.17 and 0.14 GPa, respectively [19]. These earlier results compare very well with those obtained

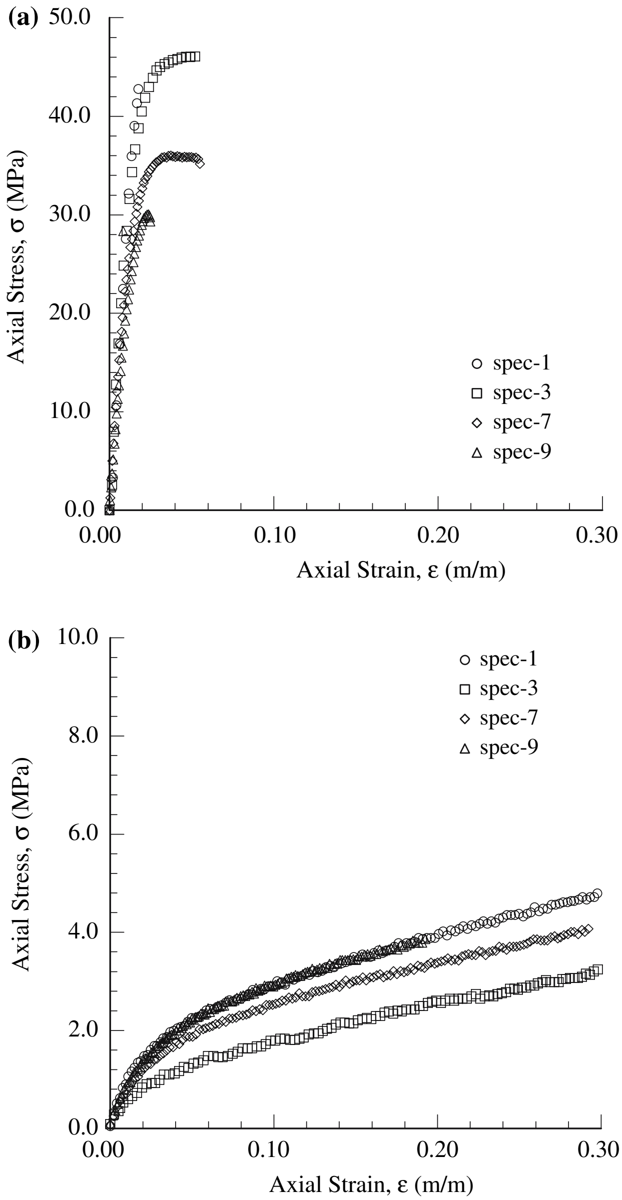

Fig. 4 Typical responses of the hoof horn from Animal 2 in uniaxial tension. Specimens are ordered (1, 3, 7, 9) from the sole towards the DEJ. Note the difference in scale for the stress and strain axes in (**a**) and (**b**) and that the responses included both the elastic and inelastic behaviour. (**a**) Dry horn, (**b**) wet horn

for the bovine hooves in the present study. In comparison to studies on the elastic modulus, there is little reported information on the effects of moisture on the tensile strength of hoof horn within specific regions of the hoof. In an evaluation of equine horn, Ley et al. [30] found that there was no distinct relationship between moisture content and tensile strength. In contrast, results for the bovine hoof horn clearly showed a decrease in strength with increasing moisture content. The disparity in results of these two studies may arise from differences in the structure between equine and bovine horn and the locations from which the tissue was extracted (i.e. specific zone).

Table 1 Summary of the mechanical properties for the hoof horn of the two animals

Animal	Elastic modulus (GPa)	Proportional limit stress (MPa)
Animal 1		
Dry	3.29 ± 0.65	33.1 ± 5.9
Wet	0.10 ± 0.03	1.9 ± 0.6
Animal 2		
Dry	2.87 ± 0.46	29.0 ± 4.8
Wet	0.10 ± 0.03	1.4 ± 0.3

Using horn samples obtained from the incremental sections, the mechanical properties were also evaluated with respect to distance from the DEJ. The distribution in E and σ_o of the hooves from an animal in the dry condition is shown in Fig. 5(a) and (b), respectively. Though there appeared to be an increase in elastic modulus and strength with increasing distance from the DEJ, there was no distinct trend for horn in either the wet or dry condition. Investigations conducted with equine horn [1–3, 31, 32] have reported that the stiffness (and corresponding elastic modulus) of horn tissue decreased with proximity to the underlying dermis. But according to results of this preliminary study, some of the regional and orientation dependent mechanical property variations may be attributed to differences in relative water content [33]. Based on the limited preliminary results, it would appear that a dairy environment could have substantial impact on the properties of hoof horn (and susceptibility of an animal to injury) through the presence of moisture and the potential reduction in strength and stiffness. For an accurate and objective identification of structure-property relationships for bovine horn, it is imperative to quantify the moisture content. Our future studies will explore the relative changes in mechanical behaviour of hoof horn with careful control of moisture content.

According to the method of displacement measurement in DIC, the optical extensometer enabled determination of the transverse and axial strain distributions. Consequently, Poisson's ratio was estimated from results of the uniaxial tension experiments and was characterized as a function of the axial elongation. Representative Poisson's ratio distributions as a function of specimen elongation are shown for specimens from the left rear lateral claw of Animal 1 in Fig. 6. In general, Poisson's ratio increased with axial strain from near 0.20 at the onset of deformation to approximately

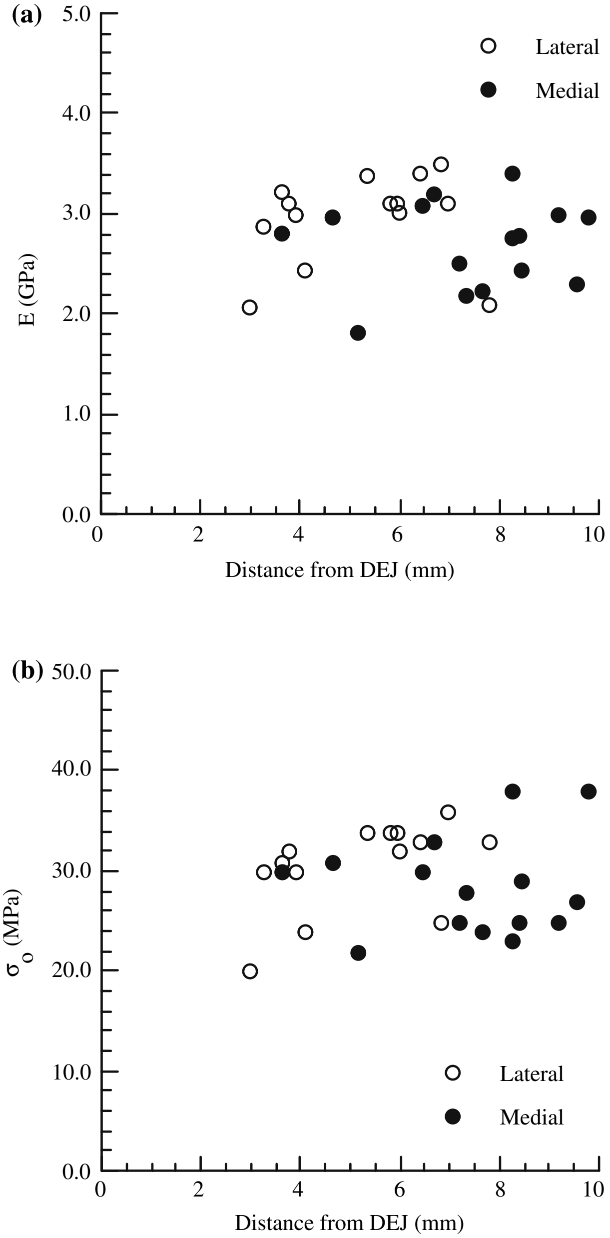

Fig. 5 Variation in mechanical properties of "dry" horn with respect to distance from the DEJ. (**a**) Elastic modulus of the hooves from Animal 2, (**b**) strength of the hooves from Animal 2

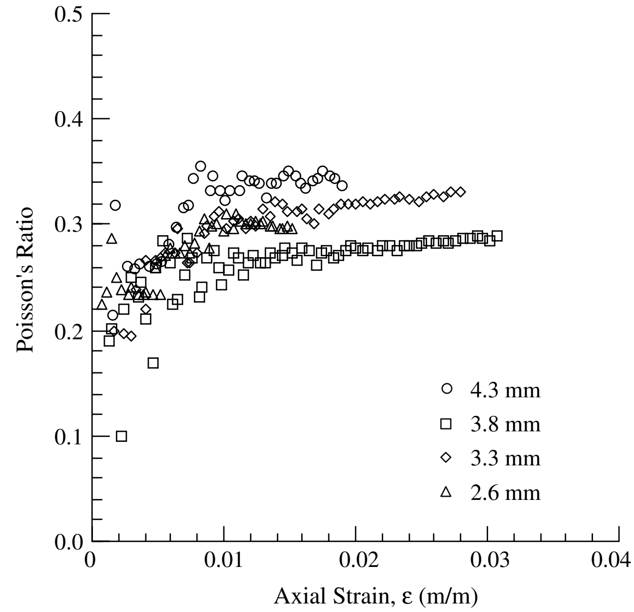

Fig. 6 Change in Poisson's ratio with axial strain for the left rear lateral claw of Animal 1in the "dry" condition. Four responses are shown, which distinguish the response at specific distances from the DEJ

0.32 at approximately 4% strain. Surprisingly, there was no distinct difference in Poisson's ratio of the horn in the wet and dry condition, or between hooves from the two animals. The average Poisson's ratio calculated from results of the two cows was approximately 0.28, regardless of moisture content. In the author's best knowledge, these measures represent the first reported Poisson's ratio of hoof horn.

The aforementioned procedures represent a new and valuable approach for quantifying the mechanical behaviour of bovine hoof horn. There were no apparent difficulties or limitations in application of DIC for evaluating the gage section elongation. Yet, there are a number of recognized limitations and concerns related to the present evaluation of bovine hoof horn. For example, the evaluation was limited to uniaxial tension experiments with specimens excised with an orientation perpendicular to the tubules. Tension occurs near the DEJ as a result of localized contact loads on the sole that cause bending deformation and curvature of the proximal surface. As a result, the horn undergoes tensile stresses parallel to the proximal surface of the sole and perpendicular to the keratin tubules. The magnitude of stress increases with distance from the point of contact. Compressive stresses develop in the horn parallel to the axis of contact and essentially parallel to the tubule orientation. The elastic modulus of equine hoof wall has been found to be larger in compression than tension [1] and the strength and elastic behaviour is also a function of the keratin tubule orientation [28]. Thus, future work should be conducted in compression with samples obtained parallel to the tubules. Uniaxial loading was conducted under displacement control actuation at a single constant rate (5×10^{-4} s^{-1}) that was considerably lower than that expected to exemplify natural gait. The strain rate was selected to enable comparison of results with those of other studies performed on similar or dissimilar materials. There is an increase in elastic modulus of equine hoof wall with strain rate [31]. Consequently, it appears important to perform future evaluations of bovine hoof horn using the new approach at physiologically relevant strain rates. Perhaps the largest limitation of the study is the limited number of animals included in the study. A robust understanding of the mechanical properties of bovine hoof horn requires a controlled study comprised of an analysis of results from many more animals. Nevertheless, the methods developed and utilized in this report will be instrumental in future studies aimed at the development of new knowledge on the mechanical behaviour of hoof horn.

Summary

A new method for examining the mechanical behaviour of hoof horn was developed. The process involves obtaining incremental slices of horn tissue, stamping samples from selected zones of the hoof, performing uniaxial tensile tests and evaluating the mechanical response using DIC. The methods were adopted to examine the properties of bovine hoof horn, which were stamped from incremental sections of each claw that were obtained proximally towards the dermal-epidermal junction (DEJ). The specimens were examined in uniaxial tension and at relative moisture content of 15% (dry) or 50% (wet). Mechanical behaviour of the horn was quantified as a function of moisture and distance from the DEJ in terms of the elastic modulus (E) and proportional limit (σ_o) stress. It was found that the elastic modulus and strength of the wet bovine hoof horn was significantly lower than that in the dry condition. The methods will enable future studies on the influence of environment factors and diet on the mechanical behaviour of hoof horn and its importance to bovine lameness

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