2018

Biomechanical analysis of bovine lamina

Ray Jeffrey Lee
Iowa State University

Recommended Citation
Lee, Ray Jeffrey, "Biomechanical analysis of bovine lamina" (2018). *Graduate Theses and Dissertations*. 16616.
https://lib.dr.iastate.edu/etd/16616

Biomechanical analysis of bovine lamina

by

Ray Jeffrey Lee

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Interdisciplinary Graduate Studies (Biological and Physical Sciences)

Program of Study Committee:
Jennifer Schleining, Major Professor
Leo Timms
Timothy Derrick

Iowa State University

Ames, Iowa

2018

DEDICATION

To my beloved wife Molly, who always encourages me to move mountains.

> “As soon as I saw you, I knew a grand adventure was about to happen.”
>
> A. A. Milne, *Winnie-the-Pooh*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

There have been numerous influences on my career to date. As a child, my parents, Jeffrey and Eunice Lee, encouraged me from a young age to be inquisitive. I remember asking the rapid-fire endless questions a small child asks and they would patiently answer until my supply of questions was exhausted. My father, being a mechanical engineer by trade and moon-lighting as a farmer, was continuously repairing broken equipment or testing designs for new manufacturing methods in his farm shop. This encouraged me to understand how all the pieces fit together to make a working piece of machinery, and I extrapolated this interest to livestock, particularly trying to understand how they move. I remember, as a young boy, being intrigued by the parallel link nature of the equine reciprocal apparatus and watching for hours trying to imagine the moving parts of this feature. My active participation in FFA encouraged me to make connections with people and to network, and I was privileged to have an advisor, Ken Bollinger, who always went the extra mile to facilitate this.

I am exceptionally fortunate to have a background in many disciplines. In different phases of my life thus far, I have functioned as a construction worker, farm hand, farrier, bovine hoof trimmer, commercial truck driver, transit bus operator, and production welder. In many of these vocations I have been fortunate to have stellar mentors and supervisors that encouraged my personal and professional growth.

As a professional farrier, I have been trained to consider all the moving parts of the limb and the how changing the way a digit contacts the ground affects the entire body. Being a farrier is a skill-based trade and requires mastery of a diverse set of techniques such as blacksmithing and hoof-trimming, along with a firm understanding of the anatomy and biomechanical function of the hoof, limb and how this affects the whole animal; Doug Russo has been a significant influence on my abilities in this aspect of my career.

Members of my study committee have stoked my enthusiasm for understanding the biomechanical nature of livestock locomotion. The trailhead of my journey to my current academic status was the introduction to Dr. Jennifer Schleining, who shares my enthusiasm for livestock biomechanics. A class with Dr. Timothy Derrick was my first foray into biomechanics, and although that first class was tough (as I had not taken a formal physics class prior to that point in my life), I realized that this way to describe movement was directly in line with my experiences and interests. Dr. Leo Timms has also joined me on my journey and has been a valuable resource as his interests span across many facets of the livestock industry. I am grateful for my committee's efforts and all that have put time and effort into my career and personal development.

ABSTRACT

Bovine lameness is an economic drain on producers and is a welfare issue. There are many causes of lameness; however, most causes involve the hoof. One of the supporting structures of the hoof is the interface between the hard epidermal layer and the sensitive dermal layer and is referred to as the lamina. Lamina consist of leaflets of tissue that interdigitate with one another to allow for constant renewal and remodeling of these hoof attachments. Systemic or traumatic insults can compromise the integrity of the lamina and may result in reduced ambulatory capabilities of the animal, pain, and a myriad of hoof lesions.

The first study in this thesis describes the biomechanical properties of the laminar junction in market weight cattle scored as having normal mobility. Following foot collection at harvest, one hoof was immediately processed for biomechanical testing and the opposite hoof was processed and tested six months later. The outcome of this study determined that duration of freezing may affect the test results of biomechanical specimens which is in contrast to what is currently published in the literature.

In the second study, cattle were observed and scored over a range of mobility using the North American Meat Institute mobility scoring system. Hooves were collected from the scored animals and biomechanical specimens were prepared and tested to determine biomechanical variables of the lamina across a range of mobility. It was determined in this study that the strain at break at the lamina for animals with reduced mobility was significantly higher than for animals with uninhibited mobility. In addition, cattle exhibiting the worst mobility scores had lamina with a signficantly lower modulus of elasticity. This would suggest that the lamina in animals with altered mobility has

decreased integrity through an increase in elasticity as observed by a dynamic increase in length of the lamina under stress. The exact mechanism by which this occurs is the focus of future research.

CHAPTER 1. THESIS OVERVIEW

This thesis seeks to explore the material nature of bovine tissues that make up the hoof, and to build the base of knowledge surrounding these tissues. We start with a review of the anatomy of the bovine hoof and a description of how these structures work together. The forces on the lamina investigated in this thesis relate to the forces while the hoof is weight-bearing. Gleaning what is known from the most current literature relating to the material properties of bovine lamina, a comprehensive description of the tissue follows. Two of these chapters are meant for submission to the American Journal of Veterinary Research. While what is written in these chapters describes the association between the material properties of the bovine lamina and mobility score, it would be irresponsible to assume a definitive causational property exists. The conclusion of this thesis is contained in the final chapter and provides a summary of our understanding to date of the tensile material properties of bovine lamina.

CHAPTER 2. REVIEW OF LITERATURE

To foster a better understanding of the research performed for this thesis, the review of current literature will include a broad overview of the anatomical features of the distal limb of the bovine, a description of the biomechanical function of the structures of interest to this thesis, a review of tensile testing terms and procedures, and a brief overview of literature relevant to biomechanical testing of the laminar junction.

Bones of the Distal Limb

In the bovine, as in all animals, the skeleton functions as a framework to support the body and enable locomotion [1]. Of interest for this thesis are the components of the distal limb, considered for purposes of this discussion to be the portion of the limb below the carpus (knee) or tarsus (hock) of a bovine [2, 3]. As the research performed in completing this thesis was performed on front hooves only, the following discussions will use only the terms relating to the thoracic (front) limbs.

Immediately distal to, and articulating with, the carpus are the fused third and fourth metacarpal bones commonly referred to as the cannon bone [2]. Distal to the metacarpals are two digits comprised of three phalangeal bones per digit that make up the two complementary claws. The first phalanges are the medial and lateral proximal phalanges (P1) with sesamoids on the caudal aspect of the metacarpophalangeal joint; also referred to as the "fetlock" [2, 3]. The pastern joint consists of the articulation of the proximal and intermediate (P2) phalanges [2]. At the most distal end of the digit is the distal phalanx (P3) which articulates with the intermediate phalanx creating the distal interphalangeal (or coffin) joint [2]. A sesamoid bone, commonly referred to as the navicular bone, is located at the caudal aspect of the distal interphalangeal joint [2], as shown in Figure 2-1.

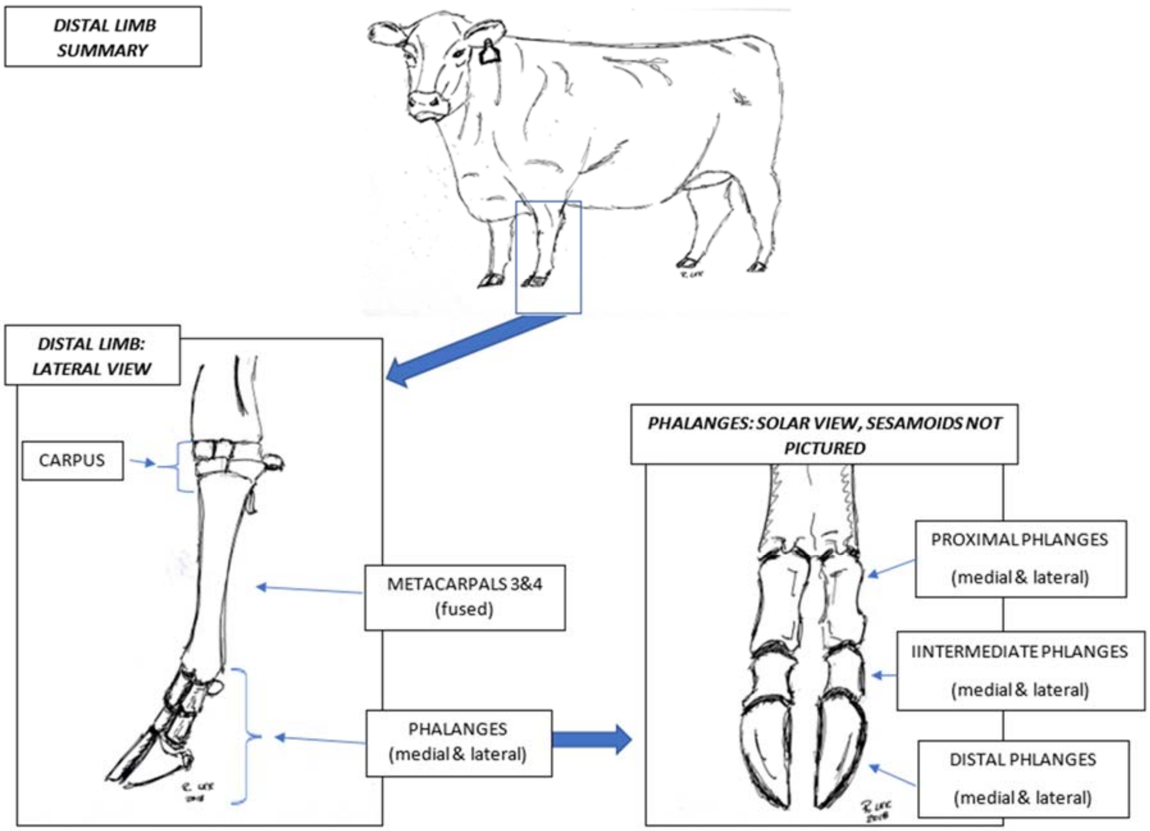


Figure 2-1 Drawing describing the distal limb and the skeletal components within

Key Structures of the Hoof

The bovine hoof has two digits or “claws;” these claws are covered with a keratin-rich material [2]. This material covers the distal phalanx, distal sesamoid, tendons and ligaments, and features a variety of suspensory mechanisms within the hoof to dissipate the ground reaction force that is a product of normal bovine behavior and locomotion [2]. Hoof wall grows from the proximal to the distal aspect of the digit [3]. The proximal border of the hoof wall is referred to as the “coronary band” [2, 3] The solar aspect of the hoof is also covered in a keratin-rich material, this is referred to as the “sole” [2]. The hoof wall and sole continuously grow, which allows the animal to have a regenerating wear surface on the end of the limb, as shown in Figure 2-2.

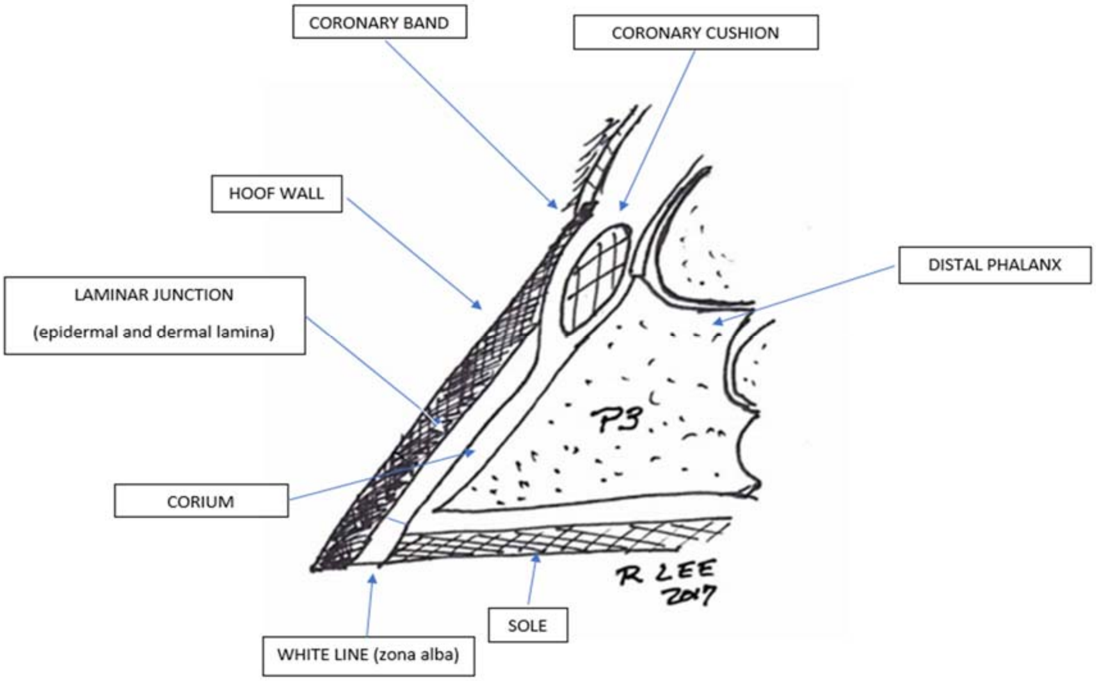


Figure 2-2 Diagram of hoof wall and supporting location of components contributing to the attachment and growth of the hoof wall and sole

The hoof wall is attached to the bone of the distal phalanx by a structure that is continuously being remodeled to allow the hoof wall to advance to the distal aspect of the digit as it accommodates new growth proximally [2]. This structure is referred to as the "lamina" [3]. The lamina consists of the insensitive epidermal lamellae which are the most superficial components of this structure, and the sensitive dermal lamellae, seen in Figure 2-3 and 2-4, which interdigitate and are complimentary to the epidermal lamellae [2, 3]. Between the lamina and the distal phalanx is the corium [3]. The corium is highly vascular and innervated and nourishes the dermal lamellae [2, 3]. At the interface between the corium and the distal phalanx is the basement membrane [3]. At the solar aspect of the hoof, the junction between the sole and the hoof wall is referred to as the "white line" or zona alba [2, 3].

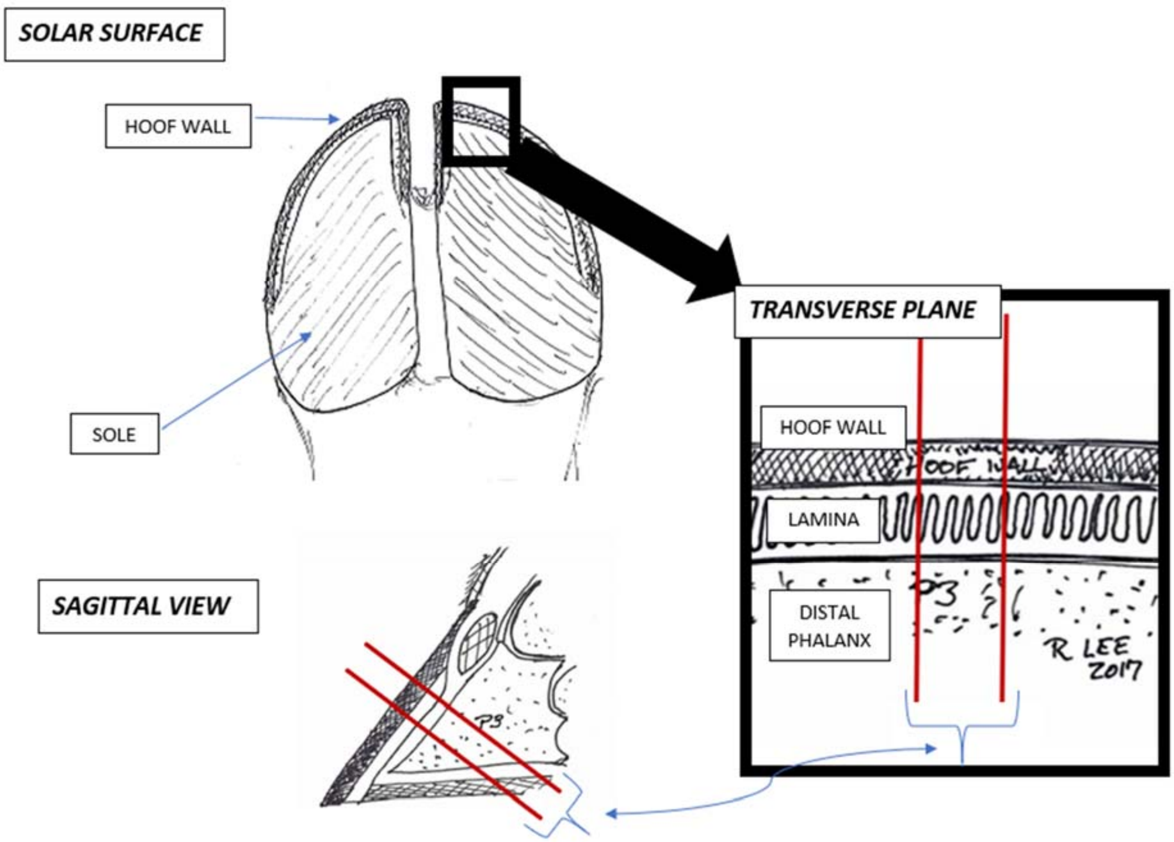


Figure 2-3 Diagram representing the structure of lamina and the location of interest to the research conducted for this thesis- equidistant from coronary band to solar surface

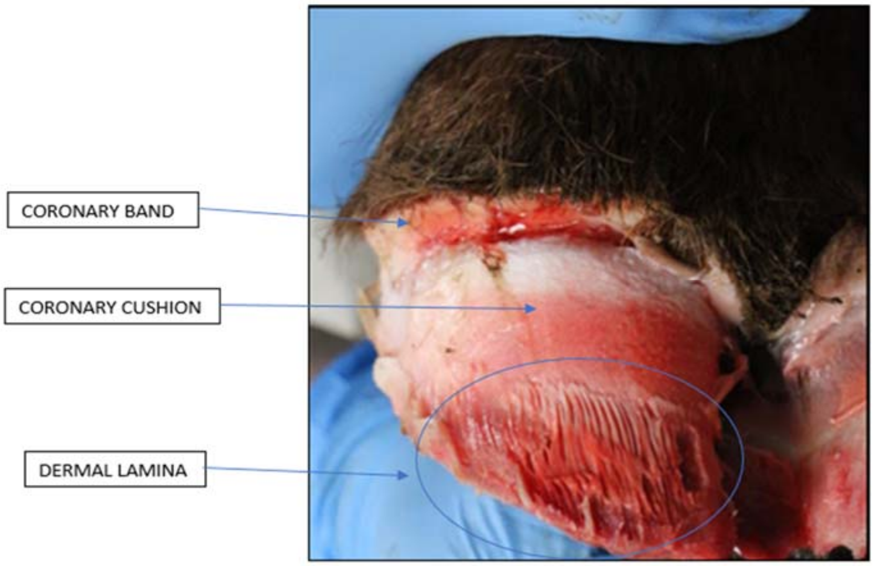


Figure 2-4 Photograph of cadaver hoof with hoof wall removed; coronary band, coronary cushion and dermal lamina labelled

While there are numerous suspensory apparatuses located within the bovine limb that also contribute to the neutralization of ground reaction forces, many of these are outside of the scope of this thesis. The structures located within the hoof that neutralize the ground reaction force will briefly be discussed including the digital cushion and the lamina.

Biomechanical Function of the Hoof

Bovine hooves are composed of two complementary claws, each having a somewhat different function in the normal gait pattern [4, 5]. Generally, the lateral claw of the pelvic limb is subjected to greater forces while weight-bearing, while the opposite is true for the thoracic limb which places more load on the medial claw [5, 4]. The function of the bovine hoof is to dissipate the ground reaction force from the mass of the animal using a variety of structures before reaching the distal limb [3]. The bovine hoof is composed of a harder outer layer and softer structures within, as seen in *Figure 2-5* [3].

The outer layer is composed of a tissue made of keratin-rich material and is referred to as the hoof wall or horn [3]. On the dorsal aspect of the hoof within the hoof wall are a group of tissues that interface and allow for continual growth to accommodate wear [3]. The interdigitating epidermal and dermal lamina (the laminar junction) hold the hoof wall onto the distal phalanx via the basement membrane. Displacement of the distal phalanx within the hoof capsule is controlled via the integrity of the laminar junction and digital cushion (described below) and functions to dissipate the ground reaction force while weight-bearing during foot impact and the phases of stance [3, 4]. Anatomically, the laminar connection on the bovine hoof has far more surface area on the abaxial wall than on the axial wall [3].

On the palmar aspect (heel) of the hoof, horn covers the sole of the hoof with a cushion between the palmar surface of the distal phalanx [6]. The cushion between the sole and the distal phalanx is called the digital cushion; this is one of the more studied materials within the bovine hoof. The digital cushion is composed of three pads which parallel one another, composed of fat, collagen, and connective tissue [7, 8, 9]. As the animal ages, and is subjected to negative energy balances, such as after parturition, the composition of these cushions has been documented to become denser and have a lower saturated fat content [6, 7].

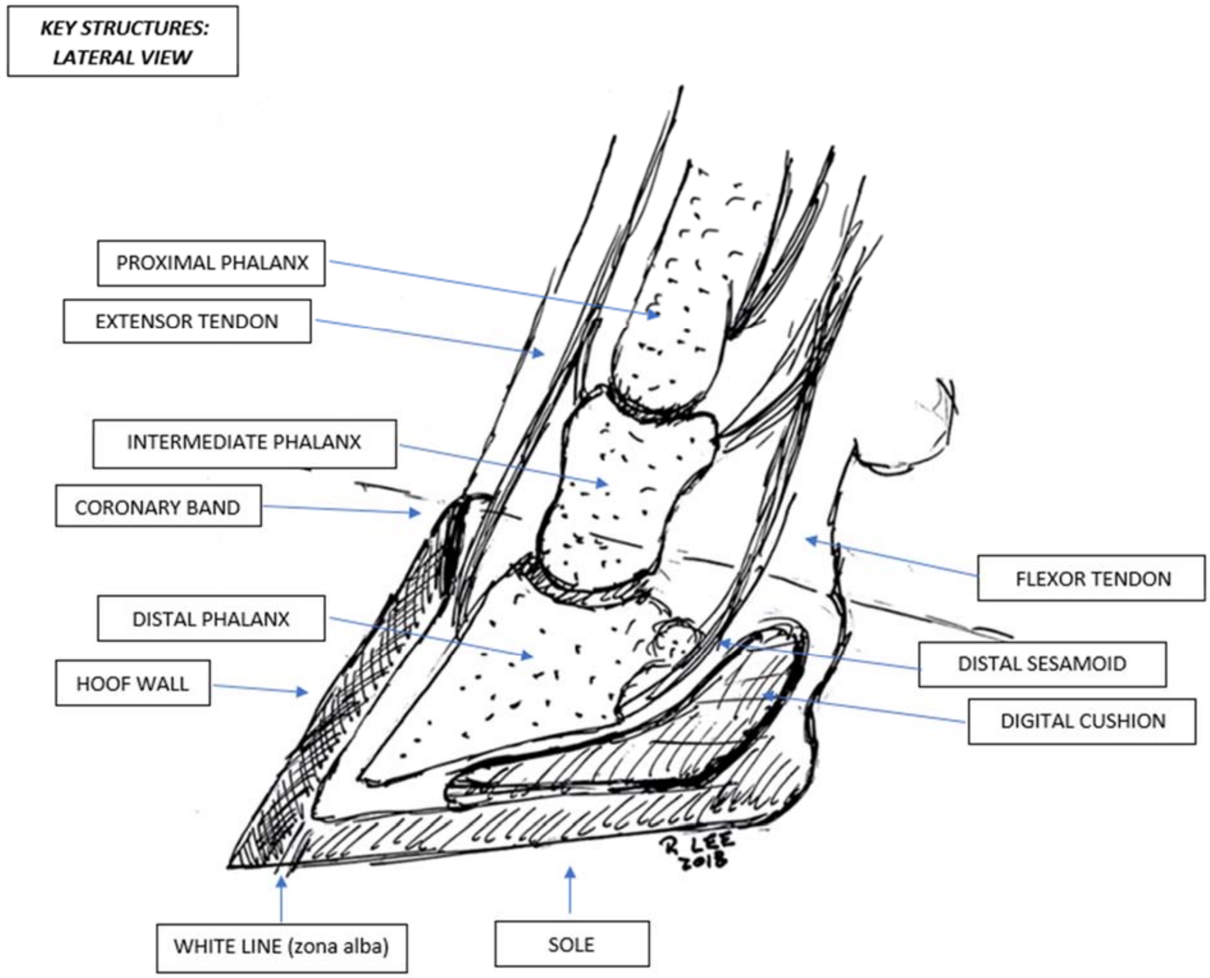
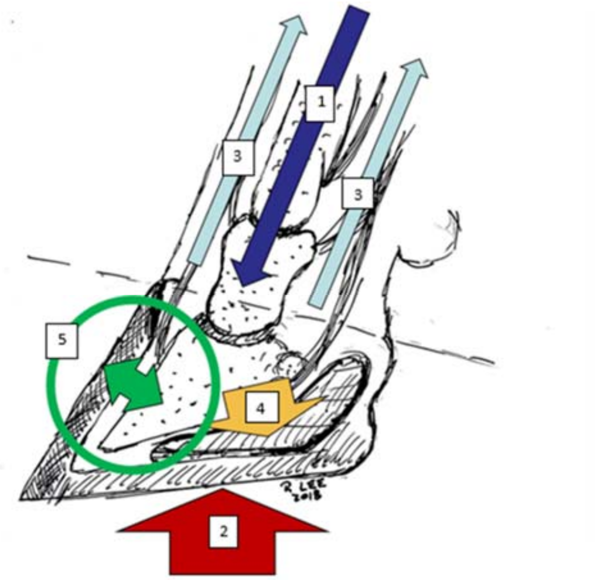


Figure 2-5 Key anatomical features of the bovine digit

While the limb is weight bearing, the force of the animal is transferred via the bony column of the distal limb to the hoof and the ground reaction force is dissipated at the level of the hoof via compression of the digital cushion and the lamina being placed into tension at the dorsal aspect of the hoof [3], as shown in Figure 2-6. Propulsive forces are provided extrinsically via tendons which course distally along the dorsal, palmar, and ventral aspect of the limb [3]. The forces experienced at the hoof level that are of interest to the research performed for this thesis are the forces experienced at the dorsal aspect of the digit (zone 5 in figure 2-6), equidistant from coronary band to solar surface.



1	FORCE FROM ANIMAL
2	GROUND REACTION FORCE
3	PROPULSIVE FORCES
4	COMPRESSION OF DIGITAL CUSHION
5	TENSILE FORCE ON LAMINAR JUNCTION

Figure 2-6 Diagramatic representation of forces within the bovine hoof

Due to the design of the bovine hoof, it is prone to certain maladies; for example, sole, toe and heel ulcers, which occur on regions of the claw that succumb to pressure necrosis [10]. White line disease is a defect that occurs due to damage of the junction between the sole horn and wall horn [10], and laminitis is inflammation of the laminar corium and lamellae [10].

The sensitive structures of the hoof are highly vascularized and highly innervated. Due to the complex innervation of these structures of the hoof [2], it is reasonable to assume that any type of hoof lesion or inflammation of hoof tissue, as in a case of laminitis, causes a significant amount of pain. Understanding the anatomy of the bovine foot, its function, and the pathology of disease is paramount to designing systems, treatments and measures to prevent pain associated with foot lesions.

Tensile Testing Terminology and Method

In a tensile strength materials test a specimen or material is stretched [11]. The force transmitted through the specimen is recorded as well as the degree of displacement of the specimen, usually identified as elongation [11]. This elongation is measured and divided by the original length of the specimen [11]. The initial length is referred to as the “gage length.” The displacement length compared to the gage length is referred to as the “strain,” [11] shown in Figure 2-7. As strain lacks units because it is a ratio, strain can be expressed as a percent of how much the material stretched. This description becomes “percent elongation”. The research performed for this thesis explored the laminar junction. Therefore, the thickness of the soft tissues between the hoof wall and the distal phalanx represented the gage length.

$$Strain = \frac{Difference\ in\ length}{Gage\ Length}$$

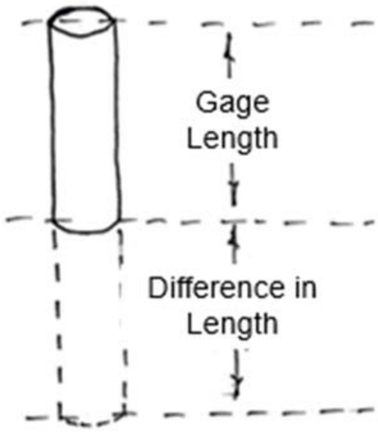


Figure 2-7 Diagram describing “strain” in tensile testing

The force transmitted through the specimen as it is placed in tension (stretched) is measured and divided by the area of the cross-section of the portion of interest of the specimen. This is referred to as “stress” [11], shown in Figure 2-8. The units of stress used in the research associated with this thesis are MPa.

$$Stress = \frac{Force}{Area}$$

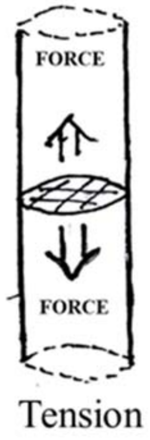
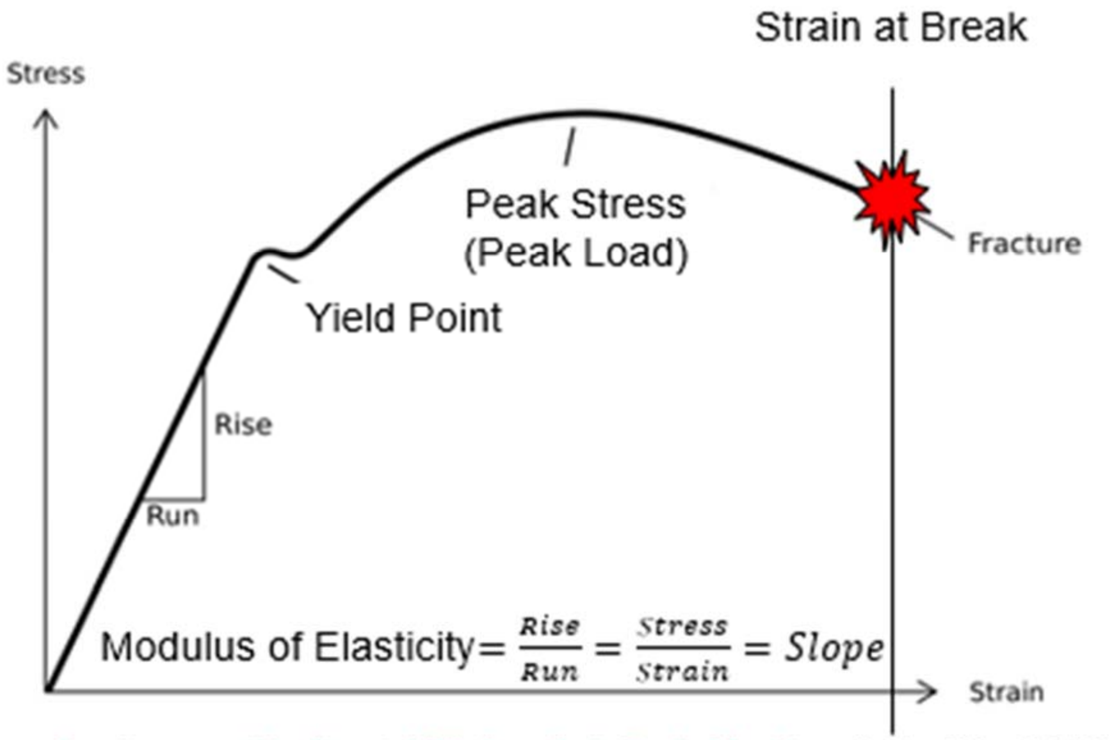


Figure 2-8 Diagram describing “stress” in tensile testing

Tensile testing explores the relationship between stress and strain as a force is applied to a material in tension [11]. Initially when a material is stretched, it behaves elastically. That is, it will return to its original shape if the force is removed [11]. The relationship between stress and strain in this stage is linear. When this relationship between stress and strain is expressed using a graph, called a stress/strain curve, one can calculate the “modulus of elasticity” of a material by finding the slope of this linear portion of the graph [11]. Modulus of elasticity is the slope of the line within the linear portion of the stress/strain curve, calculated as stress divided by strain. The less force applied to a material the more elastic the response, and the more apt it is to return to its original shape. The lower the modulus of elasticity, the more elastic the material. For example, rubber has a low modulus of elasticity, and steel has a high modulus of elasticity [12]. Stress is reported in units of force and strain is a ratio rendering it unitless; therefore, modulus of elasticity is reported in units of force [11], or in the context of this thesis, MPa.

Once a specimen is stretched beyond its elastic limit, the material will not return to its original shape when the force is removed; at this point, the material has reached its yield point [11]. The yield point is the value at which the specimen enters its plastic phase and may continue to receive more force but cannot return to its original shape [11]. The maximum amount of force that a specimen can withstand is the “peak force”. If the peak force is divided by the cross-sectional area of the specimen this value becomes the “peak stress” [11]. The maximum amount the material elongates prior to failure divided by the gage length (original length of the material) is the “strain at break.” This value represents the maximum amount of displacement a specimen can undergo before the specimen fails. Stress/strain curves for a ductile material, and for the specimen tested for this research, are described in Figures 2-9 and 2-10, respectively.



https://commons.wikimedia.org/wiki/File:Stress_Strain_Ductile_Material.png (Retrieved Nov. 20, 2017)

Figure 2-9 Typical stress/strain curve of a ductile material

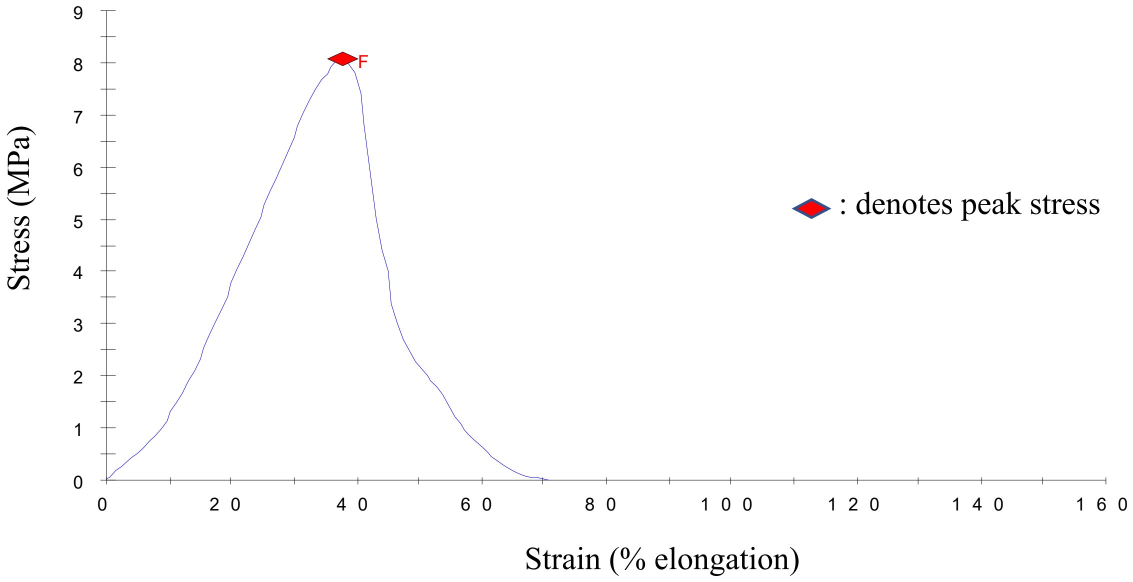


Figure 2-10 Stress/strain curve of specimen tested for research project

Biomechanical Testing in Current Literature

To date, there has been little work completed regarding biomechanical analysis of bovine hoof suspensory tissues and structures. The work that has been reported only describes analysis of the feet of dairy cattle [13, 14]

Research by Danscher et al. [13] used a laminitis model through induction of laminitis with oligofructose after which the animals were euthanized, the hooves were harvested and the laminar junction was evaluated biomechanically and histologically to determine if signs of laminitis were associated with decreased strength of the laminar junction. An oligofructose overload was administered to nongravid dairy heifers as a treatment; tap water served as the control. Animals were observed to have laminitic signs and were euthanized 24 and 72 hours after oligofructose overload. Using a bandsaw, biomechanical specimens were prepared and were kept on ice prior to tensile testing. Hooves were biomechanically tested to failure and the amount of force needed to displace the bone portion of the biomechanical specimen 1mm was recorded as well as the force exerted on the specimen at failure. This research determined that there was not a significant difference between the two groups, meaning the strength of the laminar junction was similar when comparing the force required to displace the bone portion of the specimen 1mm and the force exerted at failure.

Research performed by Tarleton et al. examined the effects of calving on the integrity of the laminar junction in Holstein cattle [14]. The cattle used in this research were euthanized either two weeks prior to the expected calving date, four weeks after calving, or twelve weeks after calving. Hooves were collected after euthanasia, and biomechanical specimens were produced. Nongravid heifers that were of the same age as the research animals functioned as the control. As in the work by Danscher, biomechanical specimens were produced with a bandsaw and included hoof wall, corium and bone. These specimens were tensile tested, and the overall stress/strain gradient, the strain at initial support, the strain at maximum support, the stress at 2mm of displacement, and the peak stress were recorded. It was concluded that there was reduced rigidity at 2mm of displacement and reduced peak stress progressively to 12 weeks post-calving. This research supports that calving alters the biomechanical properties of the connective tissue of the laminar junction and corium.

The equivalent structures of the equine hoof have also not been widely tested in the same manner as the bovine structures of interest in this thesis. This is likely due to the fact that laminitis in horses has different physiological, anatomical, and biochemical properties directing research efforts away from laminar strength and focusing on etiology and prevention strategies. Most tensile testing projects have been focused on the biomechanical properties of the hoof wall [15, 16], however the laminar junction of the equine hoof has been tested in tension and in shear [17]. Douglas *et al.* explored the biomechanical properties of different regions of the equine hoof. Specimens were prepared from equine cadaver hooves obtained from an equine abattoir. Results from this project found the region of the hoof the specimen was prepared from significantly affected the biomechanical properties of the specimen. Other research interests on the equine hoof have implemented finite element modeling of the hoof [18], which is a mathematical model to describe the interaction of the structures within the hoof that allows the use of theoretical tests rather than attempting to collect data on an actual hoof that may not be possible for practicable or ethical reasons.

References

[1] D. W. Sommerfeldt and C. T. Rubin, "Biology of bone and how it orchestrates the form and function of the skeleton," European Spine Journal, vol. 10, no.p. , 2001.

[2] A.Desrochers and D. E. Anderson, "Anatomy of the Distal Limb," Veterinary Clinics of North America-food Animal Practice, vol. 17, no. 1, pp. 25-38, 2001.

[3] P.Greenough, "Bovine Laminitis and Lameness," , 2007. [Online]. Available: http://librosdeveterinaria.com/bovine-laminitis-and-

lameness_9780702027802_50799. [Accessed 11 7 2018].

[4] P. v. d. Tol, B. Beerda, W. Ouweltjes and H. Hopster, "Locomotory behaviour in dairy cattle: the impact of walking surfaces on claw loading and locomotion," 2006. [Online]. Available: https://narcis.nl/publication/recordid/oai:library.wur.nl:wurpubs/356763. [Accessed 23 5 2018].

[5] P. v. d. Tol, J. Metz, E. Noordhuizen-Stassen, W. Back, C. Braam and W. Weijs, "The Vertical Ground Reaction Force and the Pressure Distribution on the Claws of Dairy Cows While Walking on a Flat Substrate," Journal of Dairy Science, vol. 86, no. 9, pp. 2875-2883, 2003.

[6] M. Räber, C. Lischer, H.Geyer and P. . Ossent, "The bovine digital cushion--a descriptive anatomical study.," Veterinary Journal, vol. 167, no. 3, pp. 258-264, 2004.

[7] C. İzci, M. Erol and E. Gökşahin, "A Study About Determining the Changes in the Structural Characteristics of the Digital Cushion in Heifer and Multipar Dairy Cows: A Preliminary Report," Kafkas Universitesi Veteriner Fakultesi Dergisi, vol. 17, no. 1, pp. 159-162, 2011.

[8] M. Räber, V. Letter, A. Chassot, H. Geyer and M. R. L. Scheeder, "The influence of feeding regimen on the composition of the fat pads in the bovine digital cushion.," Agricultural sciences, vol. 06, no. 9, pp. 889-899, 2015.

[9] R. Newsome, M. J. Green, N. Bell, N. Bollard, C. Mason, H. Whay and J. Huxley, "A prospective cohort study of digital cushion and corium thickness. Part 2: Does thinning of the digital cushion and corium lead to lameness and claw horn disruption lesions?," Journal of Dairy Science, vol. 100, no. 6, pp. 4759-4771, 2017.

[10] J. K. Shearer, P. J. Plummer and J. A. Schleining, "Perspectives on the treatment of claw lesions in cattle," Veterinary Medicine: Research and Reports, vol. 6, no, pp. 273-292, 2015.

[11] D. R. Askeland, "Mechanical Testing and Properties," , 1996. [Online]. Available: https://link.springer.com/content/pdf/10.1007/978-1-4899-2895-5_6.pdf. [Accessed 11 7 2018].

[12] "Modulus of Elasticity or Young's Modulus - and Tensile ...," , . [Online]. Available: http://www.engineeringtoolbox.com/young-modulus-d_417.html. [Accessed 11 7 2018].

[13] A. Danscher, T. Toelboell and O. Wattle, "Biomechanics and histology of bovine claw suspensory tissue in early acute laminitis.," Journal of Dairy Science, vol. 93, no. 1, pp. 53-62, 2010.

[14] J. F. Tarlton, D. Holah, K. Evans, S. Jones, G. Pearson and A. Webster, "Biomechanical and histopathological changes in the support structures of bovine

hooves around the time of first calving.," Veterinary Journal, vol. 163, no. 2, pp. 196-204, 2002.

[15] A. M. Goodman and L. Haggis, "Regional variation in the flexural properties of the equine hoof wall," Comparative Exercise Physiology, vol. 5, no, pp. 161-168, 2008.

[16] L. Dh and Z. Gc, "Mechanical properties of equine hoof wall tissue.," American Journal of Veterinary Research, vol. 44, no. 11, p. 2190, 1983.

[17] J. E. Douglas, T. L. Biddick, J. J. Thomason and J. C. Jofriet, "Stress/strain behavior of the equine laminar junction," The Journal of Experimental Biology, vol. 201, no. 15, pp. 2287-2297, 1998.

[18] M. Jansova, L. Ondoková, J. Vychytil, P. Kochová, K. Witter and Z. Tonar, "A Finite Element Model of an Equine Hoof," Journal of Equine Veterinary Science, vol. 35, no. 1, pp. 60-69, 2015.

CHAPTER 3. THE EFFECT OF FROZEN STORAGE ON BIOMECHANICAL TEST RESULTS OF BOVINE LAMINAR JUNCTION OBTAINED FROM NORMAL CATTLE

To be submitted to the American Journal of Veterinary Research

Ray J. Lee, BS; Jennifer A. Schleining, DVM, MS; Paul J. Plummer, DVM, PhD; and Jan A. Shearer, DVM, MS

From the Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

Abstract

Objective – To evaluate the effect of frozen storage on the biomechanical properties of hooves obtained from normal cattle.

Design - Randomized Controlled Trial

Animals – 26 cattle of market weight exhibiting normal, uninhibited mobility

Procedures – Animals were observed during antemortem exam and marked; both front hooves were collected within the packing plant and frozen for transportation to the research facility. Using a randomization chart left or right hooves were selected and biomechanically tested using a test frame. The contralateral hoof was tested after six months of frozen storage in the same manner and the test results were compared.

Results – Strain at break and the modulus of elasticity were not significantly different, however there was a significant reduction in peak stress in the specimens following six months of frozen storage.

Conclusions and Clinical Relevance - The results of this study suggest further investigation of the effects of the duration of freezing on the results of biomechanical testing is warranted.

Introduction

The bovine hoof is an intricate and functional structure that has evolved to help cattle thrive in a multitude of environments. Despite this resiliency, cattle experience a relatively high prevalence of lameness [1]. Lameness is an economic drain on producers [2,3,4,5,6], results in decreased reproductive capacity [7] and is a one of the leading factors of culling in a dairy environment [8]. There are many contributing factors to lameness, including age and parity of the animal, housing and bedding type [9], genetics [10,11], and pathologic conditions [12,13]. Due to the complex innervation of the hoof, it is reasonable to assume that hoof lesions cause a significant amount of pain. Prevention of pain is necessary to provide an adequate level of comfort and is a key component to providing an animal with proper wellbeing. Prevention of lameness is less costly than treatment and the loss of production that the condition incurs [14,15].

There are many tissues and structures within the hoof that can be compromised because of a hoof lesion or a systemic or traumatic insult. One specific example of a structure that is especially sensitive to insult is the lamina [16]. To better understand the physiologic and structural causes of lameness, it is crucial to understand the properties of healthy tissue. The comparable structure in the equine hoof has been more widely studied [17,18,19,20]. Understanding the structural properties of the lamina in cattle is crucial to understanding the forces the hoof capsule can endure with various flooring types, trailering methods, handling methods, and disease processes. The use of frozen specimens for biomechanical testing is preferable to fresh specimens to transport specimens from the collection site and in the preparation of the biomechanical specimens from the bovine hoof wall.

The primary purpose of this study was to determine how lamina from market weight cattle with normal mobility reacts when subjected to biomechanical testing. An additional objective was to determine if there is a difference in the biomechanical test results of specimens that were frozen for six months.

Materials and Methods

Cattle

Cattle used for this study were of beef-type, without signs of blindness, respiratory disease, or neurologic disturbances. The animals were observed at a packing plant during antemortem examination, were mobility scored using the North American Meat Institute mobility scoring system and were marked during the antemortem exam using a food-safe, water-based livestock marking paint. Animals were re-marked on the left shoulder in the alley with a brightly colored spray chalk product for easier animal identification by the personnel within the plant responsible for collecting hooves. The front hooves from 26 animals were collected.

Hooves were collected and immediately assigned a specimen identification number. They were immediately placed into bags labeled with the specimen identification number and transferred to a cooler with dry ice. At the end of the shift, coolers were placed into the blast freezers at the packing plant. Hooves were transported to the research facility packed with dry ice and stored at -20°F until processing.

The lateral claw of the left or right hoof from each animal was selected using a randomization chart to be processed immediately; the opposite hoof was processed and tested six months later using the same methods following frozen storage.

Biomechanical Specimens and Testing

All biomechanical specimens were prepared from frozen hooves. Briefly, hooves were sectioned using a reciprocating saw to fit into a drill press vise. A measurement was made so the specimen was produced from the dorsal hoof wall equidistant from the coronary band to the solar surface of the hoof. The sectioned foot was then placed in a vise attached to the work table of a drill press so that the dorsal hoof wall was perpendicular to the axis of the spindle of the drill press. A 14 mm arbor-type hole saw with approximately 3 degrees of positive tooth rake without a pilot bit was mounted in the chuck. The spindle speed of the drill press was 575 revolutions per minute, resulting in a cutting tool speed of 25.9 meters per minute. The feed rate of the hole saw into the hoof was approximately 0.043mm per revolution or approximately 25mm per minute. The hole saw and vise used for this research is shown in Figure 3-1.

Figure 3-1 Drill press tooling- featured in photograph: 14mm hole saw and vise

Specimens were processed in a frozen state to prevent tissue damage secondary to the torque applied to the tissues as the cut was made. Specimens were placed inside a labeled conical-bottomed tube, returned to the freezer, and kept frozen until thawed for biomechanical testing. This method produced specimens 10mm in diameter consisting of a portion of the hoof wall, lamina, corium, and distal phalanx (bone), as seen in Figure 3-2.

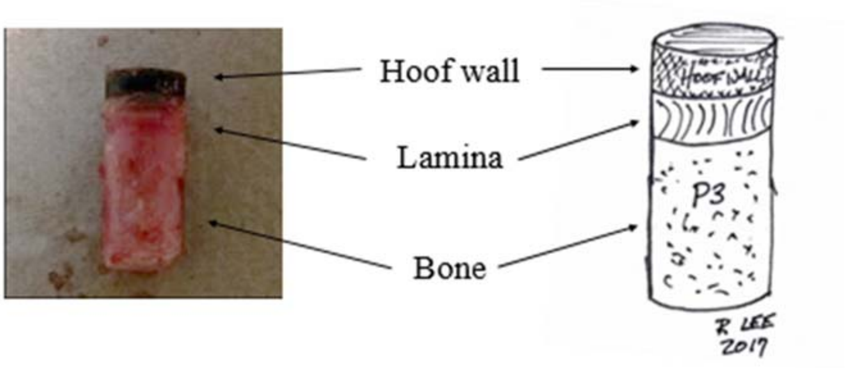


Figure 3-2 Photograph and illustration of prepared specimen

Testing was performed with a test frame (Instron Model 4502, Norwood, Massachusetts) equipped with a 1kN load cell, seen in Figure 3-3; tests were performed in tension with the moving crosshead placed below the specimen.

Figure 3-3 Test Frame: Instron 4502

Specimens were affixed to the grips by set screws that were placed through the grip into either hoof wall or a portion of the distal phalanx, as shown in Figure 3-4.

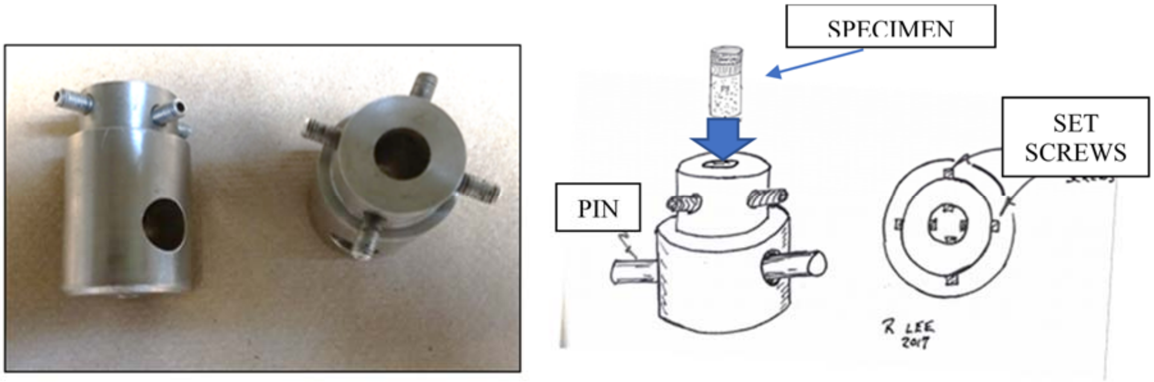


Figure 3-4 Photograph and illustration of prepared specimen

For testing, the biomechanical specimens were removed from the freezer and allowed to thaw by resting at room temperature for approximately 25 minutes while contained in labeled conical-bottomed tubes. After thawing, specimens were removed from the labeled tubes, and measurements were taken of the laminar thickness and the diameter of the laminar portion of the specimen. The surface temperature was measured to verify the specimens were room-temperature.

The specimen was then secured in custom grips with one grip affixed to the load cell, the other to the moving crosshead.

Time from removal of specimens from the freezer to insertion into the grips for biomechanical testing averaged 35 minutes. Specimens were trimmed when inserted into the grips if there was extra material protruding beyond what was necessary for fastening the specimen within the grips. The testing procedure for each specimen was approximately four minutes.

Specimens were inserted into the bore of the grip manually and set screws were tightened manually using a hex key. Cross pins attached the grips to the moving cross-head and the load cell, and specimens were placed in tension during a single cycle to failure at a speed of 25mm per minute.

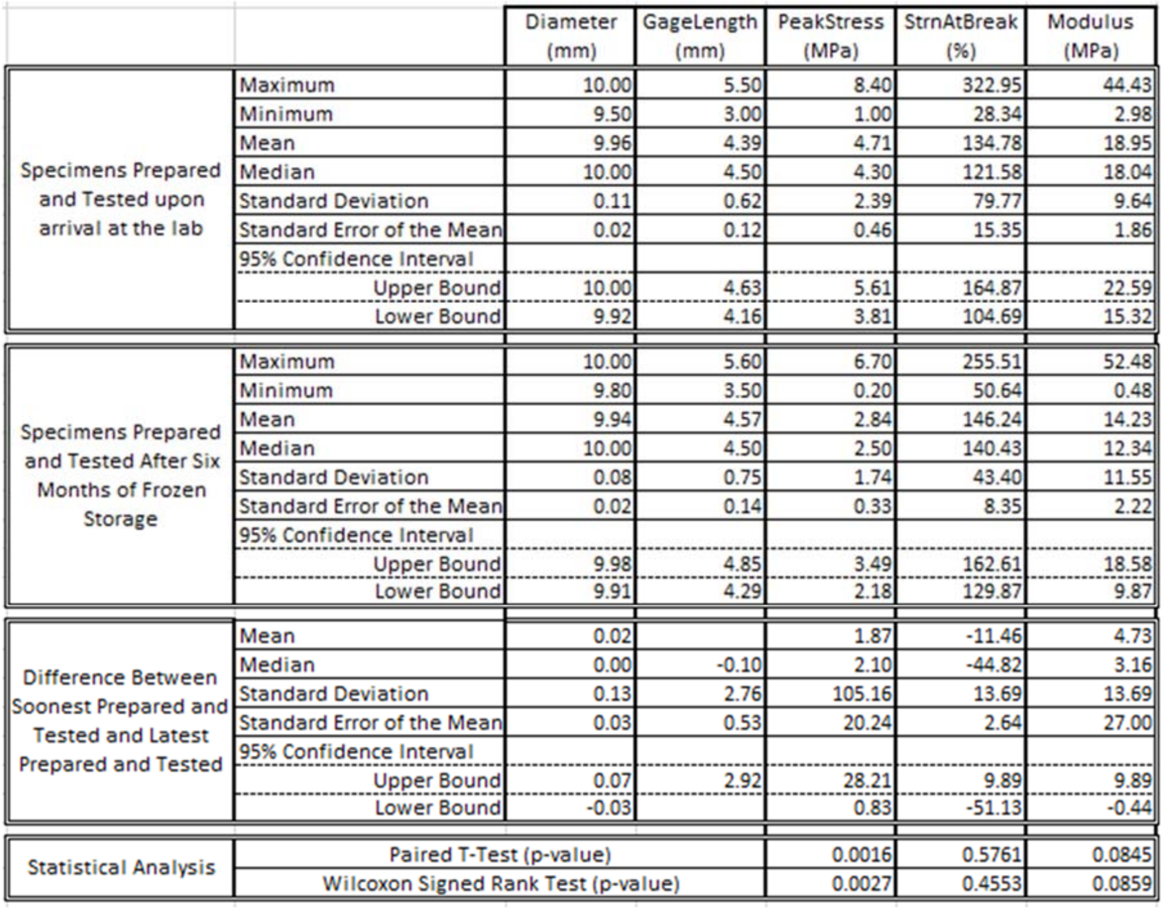
Software measured the force transferred through the specimen and displacement of the crosshead. Diameter and laminar thickness of each specimen was entered manually into the program. The software then calculated stress, strain, modulus of elasticity, strain at break, and the yield point of each specimen. These values were exported into a spreadsheet for further evaluation.

A Wilcoxon signed-rank test was performed to determine even distribution between the test results from the different sampling times. A two-tailed paired t-test was then used to compare results of the biomechanical tests from the same animal at both time points. Significance was set at $p < 0.05$.

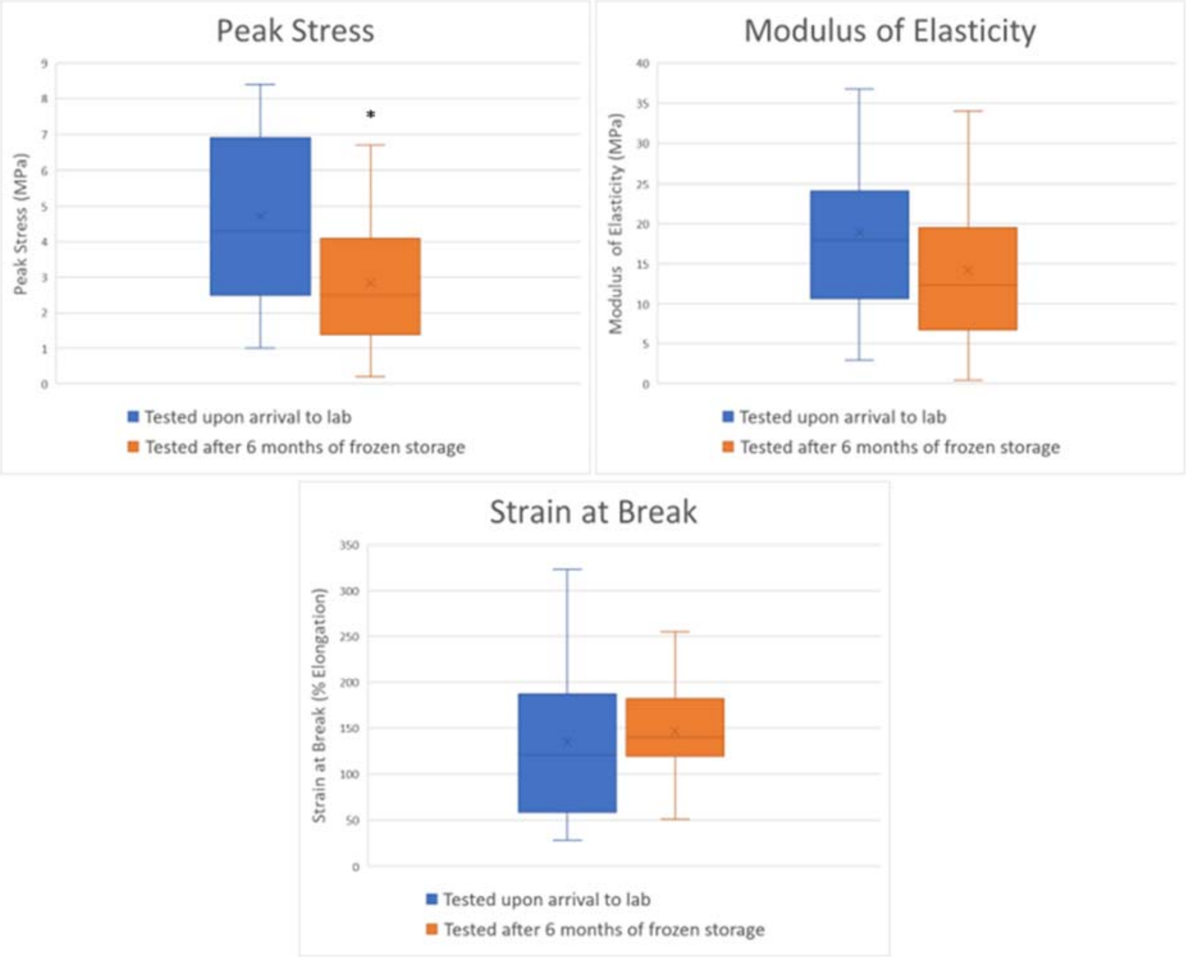
Results

The method used for producing specimens for biomechanical testing in this study resulted in specimens with uniform dimensions; the diameter of the specimens had a minimum diameter of 9.5mm and a maximum diameter of 10mm. Thickness of the lamina varied with a range of 3.0 to 5.6mm, with the mean values being 4.4mm for the specimens tested as upon arrival at the lab and 4.6mm for those tested after six months of frozen storage. All specimens failed at the junction of the dermal and epidermal lamina. Summarized test results can be found in Table 3-1. Graphs depicting the test results can be found in Figure 3-5.

Table 3-1 Summary of biomechanical test results from specimens prepared upon arrival at the lab compared to specimens prepared and tested after 6 months of frozen storage



		Diameter (mm)	GageLength (mm)	PeakStress (MPa)	StrnAtBreak (%)	Modulus (MPa)
Specimens Prepared and Tested upon arrival at the lab	Maximum	10.00	5.50	8.40	322.95	44.43
	Minimum	9.50	3.00	1.00	28.34	2.98
	Mean	9.96	4.39	4.71	134.78	18.95
	Median	10.00	4.50	4.30	121.58	18.04
	Standard Deviation	0.11	0.62	2.39	79.77	9.64
	Standard Error of the Mean	0.02	0.12	0.46	15.35	1.86
	95% Confidence Interval					
	Upper Bound	10.00	4.63	5.61	164.87	22.59
	Lower Bound	9.92	4.16	3.81	104.69	15.32
Specimens Prepared and Tested After Six Months of Frozen Storage	Maximum	10.00	5.60	6.70	255.51	52.48
	Minimum	9.80	3.50	0.20	50.64	0.48
	Mean	9.94	4.57	2.84	146.24	14.23
	Median	10.00	4.50	2.50	140.43	12.34
	Standard Deviation	0.08	0.75	1.74	43.40	11.55
	Standard Error of the Mean	0.02	0.14	0.33	8.35	2.22
	95% Confidence Interval					
	Upper Bound	9.98	4.85	3.49	162.61	18.58
	Lower Bound	9.91	4.29	2.18	129.87	9.87
Difference Between Soonest Prepared and Tested and Latest Prepared and Tested	Mean	0.02		1.87	-11.46	4.73
	Median	0.00	-0.10	2.10	-44.82	3.16
	Standard Deviation	0.13	2.76	105.16	13.69	13.69
	Standard Error of the Mean	0.03	0.53	20.24	2.64	27.00
	95% Confidence Interval					
	Upper Bound	0.07	2.92	28.21	9.89	9.89
	Lower Bound	-0.03		0.83	-51.13	-0.44
Statistical Analysis	Paired T-Test (p-value)			0.0016	0.5761	0.0845
	Wilcoxon Signed Rank Test (p-value)			0.0027	0.4553	0.0859



"*" denotes statistical significance

Figure 3-5 Box and whisker plots summarizing and comparing biomechanical test results from specimens prepared and tested upon arrival at the lab and specimens prepared and tested after six months of frozen storage

The median peak stress decreased by 1.87 MPa between the specimens prepared and tested on arrival at the lab and those prepared and tested after six months of frozen storage . This difference was significant.

The median strain at break value for the specimens tested on arrival at the lab was 121.58% elongation and the median strain at break value for the specimens tested after six months of storage was 146.24% elongation. This difference was not statistically significant.

Median modulus of elasticity values was 18.04 MPa for specimens prepared and tested on arrival at the lab and 12.34 MPa for specimens prepared and tested after six months of frozen storage. This difference was not statistically significant.

Discussion

The preparation technique of the specimens using a hole saw resulted in a consistent measurable circular diameter, and a uniform cross-sectional area was determined to exist amongst specimens in this sample. This is important because having a reliable cross-sectional area directly affects the stress value as any error in the dimensions of the specimen obtained by less reliable methods would result in a discrepancy amongst the other specimens in the sample. This discrepancy would directly affect the calculation of stress, which is calculated by force divided by area. For instance, if the specimen is cut at any angle other than a right angle, yielding a parallelogram rather than a perfect square, discrepancies may exist in the area as well as introduction of shear forces when tested in tension. The area of these discrepancies could be accounted for by measuring the dimensions of the specimen and calculating the cross-sectional area for each specimen, as was performed in this study. However, utilizing a round specimen as performed in this study, prevented these discrepancies.

Having an accurate measurement of the laminar thickness was also crucial for this study, as this measurement represents the “gage length” of the specimen. Strain is expressed as “percent elongation.” Thus, even small errors are compounded dramatically when comparing the percent change between two values resulting in significant error. The use of a calibrated calipers in this study decreased the likelihood of error in measurement.

All specimens were frozen before processing, and this was expected to have little effect on the performance of the lamina when tested biomechanically as reported by Tarlton et al in 2002 [21]. However, surprisingly, we found that the hooves stored frozen for six months had significantly less peak stress than the initial samples which had only been frozen for 10 days. The reasons for this could be explained by the possibility that specimens may have been prepared in a slightly different location on the dorsal hoof wall of each animal. Although care was taken to prepare specimens equidistant from both the solar surface and the coronary band, since this measurement was made manually and due to anatomical variation, there could be enough error to have produced some bias. Bias may have resulted because the laminar junction has been determined to have different biomechanical properties in different regions of the hoof wall [19]; thus, if specimens were consistently prepared in a slightly different place, this could affect the test results. Additionally, the torque supplied by the hole saw may have had an impact on the specimen integrity; however, the convenience and reliability of the specimen diameters and measurements and the fact that the tissue was frozen completely during processing likely negate any of the effects that there may have been on the tissue.

The modulus of elasticity, or the stiffness of the laminar junction, was not significantly different between specimens tested on arrival at the lab and those tested after six months of frozen storage. There was also no significant difference between the strain at break of samples before complete failure.

In conclusion, the results of this research indicate that the elastic properties of the suspensory tissues are not significantly affected by the duration of freezing. The peak stress in the biomechanical specimens prepared from the hooves frozen for six months was significantly decreased. Further research on the effects of the duration of freezing on hooves to be processed into biomechanical specimens is suggested.

References

[1] G. Cramer, K. Lissemore, C. Guard, K. Leslie and D. F. Kelton, "Herd-and Cow-Level Prevalence of Foot Lesions in Ontario Dairy Cattle," *Journal of Dairy Science,* vol. 91, no. 10, pp. 3888-3895, 2008.

[2] J. Davis-Unger, E. A. Pajor, K. S. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman and K. Orsel, "Economic impacts of lameness in feedlot cattle," 2017. [Online]. Available: https://animalsciencepublications.org/publications/tas/articles/0/0/tas2017.0052. [Accessed 30 5 2018].

[3] J. A. Willshire and N. J. Bell, "An economic review of cattle lameness.," *Cattle Practice,* vol. 17, no. 2, pp. 136-141, 2009.

[4] L. Ózsvári, "Economic Cost of Lameness in Dairy Cattle Herds," *Journal of Dairy, Veterinary & Animal Research,* vol. 6, no. 2, pp. 1-8, 2017.

[5] B. Selmeczy, "Economic losses in dairy cattle herds due to lameness," 2012. [Online]. Available: http://huveta.hu/handle/10832/801. [Accessed 30 5 2018].

[6] G. Verhoef, "Economic impact of foot disorders in dairy cattle," , 2012. [Online]. Available: https://dspace.library.uu.nl/handle/1874/289377. [Accessed 30 5 2018].

[7] J. F. Ettema and S. D. Østergaard, "Economic decision making on prevention and control of clinical lameness in Danish dairy herds," *Livestock Science,* vol. 102, no. 1, pp. 92-106, 2006.

[8] H. Barkema, J. . Westrik, K. v. Keulen, Y. Schukken and A. Brand, "The effects of lameness on reproductive performance, milk production and culling in Dutch dairy farms," *Preventive Veterinary Medicine,* vol. 20, no. 4, pp. 249-259, 1994.

[9] N. Cook, "Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface," *Journal of The American Veterinary Medical Association,* vol. 223, no. 9, pp. 1324-1328, 2003.

[10] O. M. Obike, "Genetics of health and lameness in dairy cattle," , 2009. [Online]. Available: https://era.lib.ed.ac.uk/handle/1842/4113. [Accessed 30 5 2018].

[11] P. Boettcher, J. C. M. Dekkers, L. D. Warnick and S. Wells, "Genetic analysis of clinical lameness in dairy cattle.," *Journal of Dairy Science,* vol. 81, no. 4, pp. 1148-1156, 1998.

[12] J. K. Shearer, P. J. Plummer and J. A. Schleining, "Perspectives on the treatment of claw lesions in cattle," *Veterinary Medicine : Research and Reports,* vol. 6, no. , pp. 273-292, 2015.

[13] G. G. Miguel-Pacheco, H. J. Thomas, J. Huxley, R. Newsome and J. Kaler, "Effect of claw horn lesion type and severity at the time of treatment on outcome of lameness in dairy cows," *Veterinary Journal,* vol. 225, pp. 16-22, 2017.

[14] R. J. Esslemont and M. A. Kossaibati, "Culling in 50 dairy herds in England," Veterinary Record, vol. 140, no. 2, pp. 36-39, 1997.

[15] J. F. Ettema and S. D. Østergaard, "Economic decision making on prevention and control of clinical lameness in Danish dairy herds," Livestock Science, vol. 102, no. 1, pp. 92-106, 2006.

[16] N. Cook, K. V. Nordlund and G. Oetzel, "Environmental Influences on Claw Horn Lesions Associated with Laminitis and Subacute Ruminal Acidosis in Dairy Cows," *Journal of Dairy Science,* vol. 87, 2004.

[17] G. D. Ramsey, P.J. Hunter, and M. Nash. (2011). The effect of hoof angle variations on dorsal lamellar load in the equine hoof. *Equine Veterinary Journal*. 43. 536-42.

[18] T. Kameya, K. Kiryu, M. Kaneko, and H. Satoh, "[Histopathogenesis of thickening of the hoof wall laminae in equine laminitis (author's transl)].," *The Japanese journal of veterinary science,* vol. 42, no. 3, p. 361, 1980.

[19] S. M. Sarratt and D. M. Hood, "Evaluation of architectural changes along the proximal to distal regions of the dorsal laminar interface in the equine hoof," *American Journal of Veterinary Research,* vol. 66, no. 2, pp. 277-283, 2005.

[20] E. D. Roberts, R. Ochoa and P. F. Haynes, "Correlation of dermal-epidermal laminar lesions of equine hoof with various disease conditions.," *Veterinary Pathology,* vol. 17, no. 6, pp. 656-666, 1980.

[21] J. F. Tarlton, D. Holah, K. Evans, S. Jones, G. Pearson and A. Webster, "Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving.," *Veterinary Journal,* vol. 163, no. 2, pp. 196-204, 2002.

CHAPTER 4. THE RELATIONSHIP BETWEEN MOBILITY SCORE AND BIOMECHANICAL TEST RESULTS OF BOVINE LAMINA

To be submitted to the American Journal of Veterinary Research

Ray J. Lee, BS; Jennifer A. Schleining, DVM, MS; Paul J. Plummer, DVM, PhD; and
Jan A. Shearer, DVM, MS
From the Department of Veterinary Diagnostic and Production Animal Medicine,
College of Veterinary Medicine, Iowa State University, Ames, IA 50011

Abstract

Objective – To evaluate the relationship between the mobility score of market weight cattle and the biomechanical properties of the lamina.

Design - Case-control study

Animals – 85 cattle of market weight exhibiting different scores of mobility as described by the North American Meat Institute (NAMI)

Procedures – Animals were observed, mobility scored, and marked using a color code during antemortem exam at a packing plant. Both front hooves were collected at harvest and frozen for transportation to the research facility. Using a randomization chart, left or right hooves were selected and biomechanically tested using a test frame. The test results were then compared between mobility scores.

Results – The laminar strain at break values were significantly increased amongst biomechanical specimens produced from cattle with mobility scores 3 and 4, and the modulus of elasticity values were significantly less for the animals with the most reduced mobility. Peak stress was not significantly altered.

Conclusions and Clinical Relevance – There is a relationship between both strain at break and modulus of elasticity and altered mobility. The results of this study suggest the need for further research of the relationship between mobility and biomechanical properties of bovine lamina.

Introduction

The bovine hoof is an intricate and functional structure that has evolved to help cattle thrive in a multitude of environments. Despite this resiliency, cattle experience a relatively high prevalence of lameness [1]. Lameness is an economic drain on producers [2], decreases the reproductive capacity of cattle [3,4], and is a welfare issue [5]. There is a high prevalence of lameness, as reflected in the fact that lameness has been shown to be one of the leading causes of culling in a dairy environment [6]. Prevention of pain is necessary to provide an adequate level of comfort and is a key component to providing an animal with proper wellbeing. Prevention of lameness is less costly than treatment and the loss of production the condition incurs [7,8].

There are many tissues and structures within the hoof that can be compromised because of a lesion, a systemic insult, or trauma. One specific example of a structure that is especially sensitive to insult is the lamina [9]. To better understand the physiologic and structural causes of lameness, it is crucial to understand the properties of healthy lamina and compare these properties to lamina from animals with compromised mobility. The hoof of the equine has a similar structure that has been more widely researched than the bovine equivalent to date [10], including finite element modelling [11], which is a mathematical model to describe the interaction of the structures within the hoof that allows the use of theoretical tests rather than attempting to collect data on an actual hoof that may not be possible for practicable or ethical reasons.

Lameness has many causes including genetics [12], housing type, age and parity of the animal [13], and pathologic conditions [14]. Due to the complex innervation of the tissues and structures that the hoof is comprised of, it can be assumed that hoof lesions could cause a significant amount of pain. A better understanding of the pathogenesis of lameness can guide industry to establish appropriate parameters that the tissues and structures can withstand; for instance, guiding flooring types, trailering methods, and handling methods, based on the forces that the hoof capsule can endure.

There are many bone and soft tissue interfaces within the structure of the hoof that can be compromised because of a systemic or traumatic insult resulting in a variety of lesions. Of specific interest to this study is the laminar junction [15]. The lamina functions as an interface between the hoof wall and the distal phalanx. The hoof wall continually grows from the coronary band to the distal aspect of the hoof and is constantly being remodeled as the hoof wall descends. Due to this complex interface of the tissues comprising the lamina it is crucial to understand its normal properties in addition to examining the properties and conditions of diseased lamina.

Limiting the focus to the dorsal hoof wall, the lamina is comprised of several tissues including the epidermal or insensitive lamina, dermal or sensitive lamina, corium, and basement membrane. The epidermal lamina is the most interior layer of the epidermal layer of the hoof and the most superficial layer of the laminar junction. This portion of the lamina consists of leaflets that interdigitate with the corresponding structure of the sensitive lamina which are extensions of the corium. The interface of these leaflets allows for the continuous renewal of the hoof from the coronary band to the weight bearing surface. The corium is highly vascular and nourishes the leaflets that compose the dermal lamina. The basement membrane is the final connection to the bone of the third phalanx.

Another significant purpose of the lamina is its importance in neutralizing the ground reaction force within the hoof capsule by effectively suspending the third phalanx in the hoof capsule. A lack of laminar integrity is linked to a variety of hoof pathologies in cattle suggesting the distal phalanx may be displaced distally when the strength of the laminar junction is compromised.

The purpose of the research conducted in this study was to investigate the strength of the laminar junction across all four NAMI mobility scores. This consisted of placing a core of hoof tissue under increasing tensile strain until failure and recording the parameters under which it failed. The goal was to examine the biomechanical properties of bovine lamina in states of altered mobility to gain insight into the relationship between mobility and the biomechanical properties of the lamina. It was hypothesized that the animals with higher mobility scores would have lamina that exhibited decreased peak stress, increased strain at break, and decreased modulus of elasticity due to a compromise of the laminar junction.

Materials and Methods

Cattle

Cattle used for this study were of beef-type, without signs of blindness, respiratory disease, or neurologic disturbances. The animals were observed during antemortem examination at a packing plant, were scored based on mobility and were marked with a color-code during the antemortem exam using a food-safe, water-based livestock marking paint. Animals were re-marked on the left shoulder in the alley with a brightly colored spray chalk product for easier animal identification by the personnel within the plant responsible for collecting hooves. Hooves from 85 animals were collected. This included 24 animals in mobility score 1, 24 animals in mobility score 2, 17 animals in mobility score 3, and 20 animals in mobility score 4. Forty-five were steers and 40 were heifers.

Table 4-1 Table describing the distribution of cattle by mobility score and sex

Mobility Score	Heifer	Steer	Total
1	15	9	24
2	14	10	24
3	6	11	17
4	5	15	20

Cattle were scored using the North American Meat Institute (NAMI) mobility scoring system. Using this system, a score of “1” represents a normal animal that is moving freely without pain, a mobility score of “2” represents an animal that lags slightly behind the herd as the animals are moved in front of the observer, a score of “3” represents an animal that is lagging significantly and will only move when encouraged to do so by the handler, and a mobility score of “4” is awarded to an animal reluctant to move, and that a handler has significant difficulty provoking the animal to move; mobility score “4” animals are described as ‘statue-like’[16].

Front hooves were collected and immediately assigned a specimen identification number. They were immediately placed into bags labeled with the specimen identification number and transferred to a cooler with dry ice. At the end of the shift, coolers were placed into the blast freezers at the packing plant. Hooves were transported to the research facility packed with dry ice and stored at -20°F until processing.

Biomechanical Specimens and Testing

The lateral claw from the left or right front foot from each animal was selected using a randomization chart to be processed into biomechanical specimens and these specimens were prepared from frozen hooves. Briefly, hooves were sectioned using a reciprocating saw to fit into a drill press vise. A measurement was made so the specimen was produced from the dorsal hoof wall equidistant from the coronary band to the solar surface of the hoof. The sectioned foot was then placed in a vise attached to the work table of a drill press so that the dorsal hoof wall was perpendicular to the axis of the spindle of the drill press. A 14 mm arbor-type hole saw with approximately 3 degrees of positive tooth rake without a pilot bit was mounted in the chuck. The hole saw and vise used for this research is shown in Figure 4-1. The spindle speed of the drill press was 575 revolutions per minute, resulting in a cutting tool speed of 25.9 meters per minute. The feed rate of the hole saw into the hoof was approximately 0.043mm per revolution or approximately 25mm per minute.

Figure 4-1 Drill press tooling- featured in photograph: 14mm hole saw and vise

Specimens were processed in a frozen state to prevent tissue damage secondary to the torque applied to the tissues as the cut was made. Specimens were placed inside a labeled conical-bottomed tube, returned to the freezer, and kept frozen until thawed for biomechanical testing. This method produced specimens 10mm in diameter consisting of a portion of the hoof wall, lamina, corium, and distal phalanx (bone), as seen in Figure 4-2.

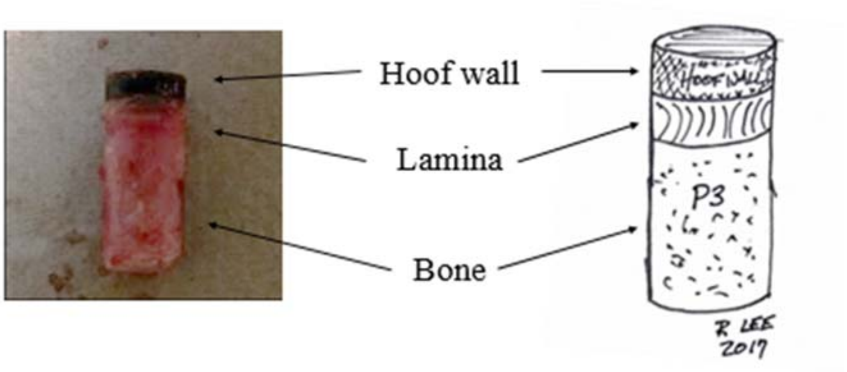


Figure 4-2 Photograph and illustration of prepared specimen

Testing was performed with a test frame (Instron Model 4502, Norwood, Massachusetts) equipped with a 1kN load cell, seen in Figure 4-3; tests were performed in tension with the moving crosshead placed below the specimen.

Figure 4-3 Test Frame: Instron 4502

Specimens were affixed to the grips by set screws that were placed through the grip into either hoof wall or a portion of the distal phalanx.

For testing, the biomechanical specimens were removed from the freezer and allowed to thaw by resting at room temperature for approximately 25 minutes while contained in labeled conical-bottomed tubes. After thawing, specimens were removed from the labeled tubes, and measurements were taken of the laminar thickness, the diameter of the laminar portion of the specimen, and the surface temperature.

The specimen was then secured in custom grips with one grip affixed to a 1kN load cell, the other to the moving crosshead, shown in Figure 4-4.

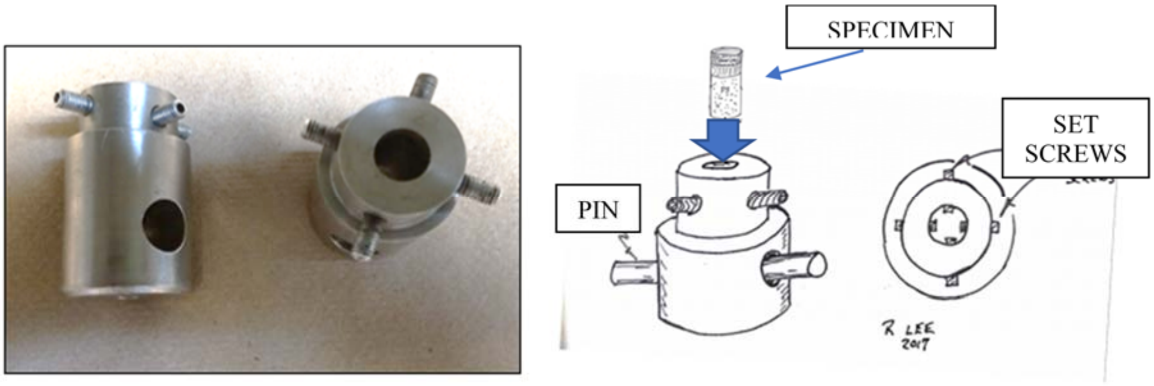


Figure 4-4 Photograph and illustration of grips demonstrating specimen insertion

Time from removal of specimens from the freezer to insertion into the grips for biomechanical testing averaged 35 minutes. Specimens were trimmed when inserted into the grips if there was extra material protruding beyond what was necessary for fastening the specimen within the grips. Specimens were inserted into the bore of the grip manually and set screws were tightened manually using a hex key. Cross pins attached the grips to the moving cross-head and the load cell, and specimens were placed in tension during a single cycle to failure at a speed of 25mm per minute. The testing procedure for each specimen was approximately four minutes.

Software measured the force transferred through the specimen and displacement of the crosshead. Diameter and laminar thickness of each specimen was entered manually into the program. The software then calculated stress, strain, modulus of elasticity, strain at break, and the yield point of each specimen. These values were exported into a spreadsheet for further evaluation.

The tensile test results from specimens produced from hooves of cattle that were observed to have each mobility score were compared to the tensile test results of the other mobility scores. Statistical significance was determined using ANOVA and Tukey's multiple comparisons test with significance set at $p < 0.05$.

Results

All specimens failed at the junction of the dermal and epidermal lamina. Summarized test results for biomechanical specimens for each mobility score can be found in Table 4-1, and graphs depicting the test results can be found in Figure 4-5.

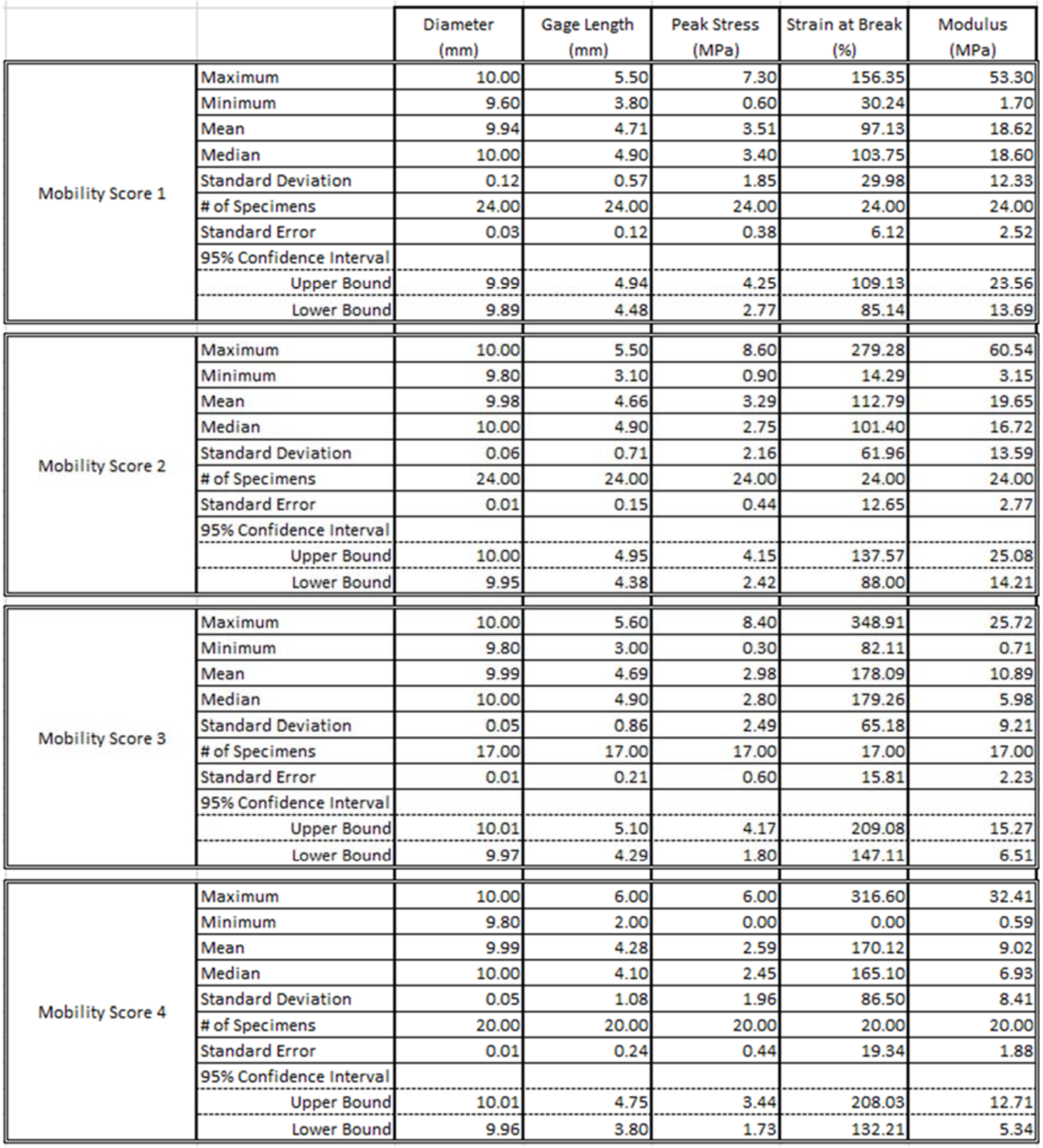
Biomechanical specimens were prepared from a total of 85 cattle hooves. Refer to Table 4-1 for the distribution of animals within each mobility score. There was a downward trend for mean peak stress as mobility score increased, but this was not found to be statistically significant.

The mean values for peak stress for mobility score 1 was 3.51MPa, mobility score 2 was 3.29MPa, mobility score 3 was 2.98MPa, and mobility score 4 was 2.59MPa. There was no statistical significance between groups (ANOVA p-value: 0.5091), Table 4-3.

The strain at break had significant differences between the mobility scores; ANOVA analysis was used to determine overall significance (p value = 0.0001). The mean strain at break for mobility score 1 was 97.13%, mobility score 2 was 112.79%, mobility score 3 was 178.09%, and mobility score 4 was 170.12%. Significance was found between specimens collected from mobility scores 1 and 2 when compared to mobility scores 3 and 4. However, mobility scores 1 and 2 were not statistically significant nor were mobility scores 3 and 4. Table 4-4.

The median modulus of elasticity for mobility score 1 was 18.60MPa, mobility score 2 was 16.67 MPa, mobility score 3 was 5.98 MPa, and mobility score 4 was 6.93 MPa. There was statistical significance within the group as evidenced by ANOVA (p value = 0.0043). The modulus of elasticity was statistically different between mobility score 1 and 4, and mobility score 2 and 4. There was no statistical difference between the modulus of elasticity of mobility score 1 and 2, or between mobility score 2 and 3, Table 4-5.

Table 4-2 Summary of biomechanical test results from specimens prepared from hooves of animals that were mobility scored during antemortem examination



		Diameter (mm)	Gage Length (mm)	Peak Stress (MPa)	Strain at Break (%)	Modulus (MPa)
Mobility Score 1	Maximum	10.00	5.50	7.30	156.35	53.30
	Minimum	9.60	3.80	0.60	30.24	1.70
	Mean	9.94	4.71	3.51	97.13	18.62
	Median	10.00	4.90	3.40	103.75	18.60
	Standard Deviation	0.12	0.57	1.85	29.98	12.33
	# of Specimens	24.00	24.00	24.00	24.00	24.00
	Standard Error	0.03	0.12	0.38	6.12	2.52
	95% Confidence Interval					
	Upper Bound	9.99	4.94	4.25	109.13	23.56
	Lower Bound	9.89	4.48	2.77	85.14	13.69
Mobility Score 2	Maximum	10.00	5.50	8.60	279.28	60.54
	Minimum	9.80	3.10	0.90	14.29	3.15
	Mean	9.98	4.66	3.29	112.79	19.65
	Median	10.00	4.90	2.75	101.40	16.72
	Standard Deviation	0.06	0.71	2.16	61.96	13.59
	# of Specimens	24.00	24.00	24.00	24.00	24.00
	Standard Error	0.01	0.15	0.44	12.65	2.77
	95% Confidence Interval					
	Upper Bound	10.00	4.95	4.15	137.57	25.08
	Lower Bound	9.95	4.38	2.42	88.00	14.21
Mobility Score 3	Maximum	10.00	5.60	8.40	348.91	25.72
	Minimum	9.80	3.00	0.30	82.11	0.71
	Mean	9.99	4.69	2.98	178.09	10.89
	Median	10.00	4.90	2.80	179.26	5.98
	Standard Deviation	0.05	0.86	2.49	65.18	9.21
	# of Specimens	17.00	17.00	17.00	17.00	17.00
	Standard Error	0.01	0.21	0.60	15.81	2.23
	95% Confidence Interval					
	Upper Bound	10.01	5.10	4.17	209.08	15.27
	Lower Bound	9.97	4.29	1.80	147.11	6.51
Mobility Score 4	Maximum	10.00	6.00	6.00	316.60	32.41
	Minimum	9.80	2.00	0.00	0.00	0.59
	Mean	9.99	4.28	2.59	170.12	9.02
	Median	10.00	4.10	2.45	165.10	6.93
	Standard Deviation	0.05	1.08	1.96	86.50	8.41
	# of Specimens	20.00	20.00	20.00	20.00	20.00
	Standard Error	0.01	0.24	0.44	19.34	1.88
	95% Confidence Interval					
	Upper Bound	10.01	4.75	3.44	208.03	12.71
	Lower Bound	9.96	3.80	1.73	132.21	5.34

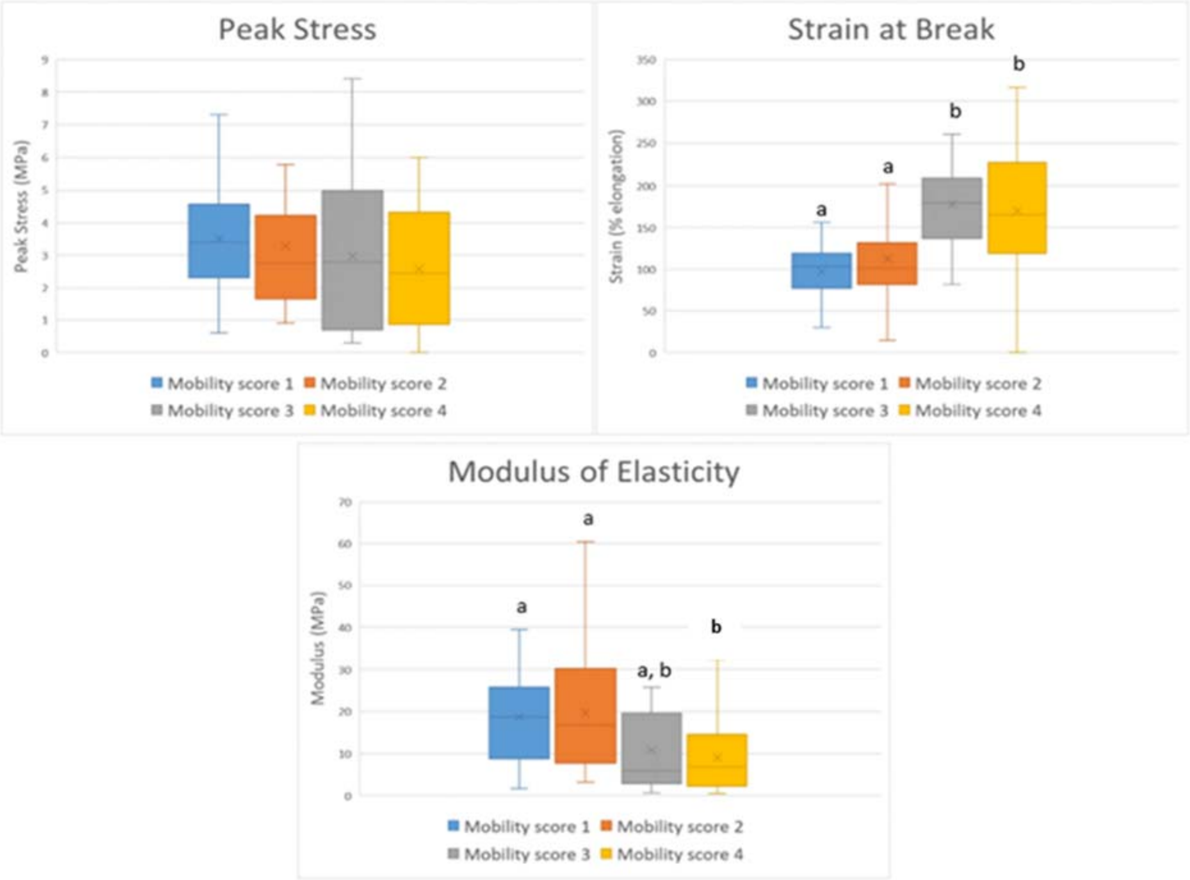


Figure 4-5 Box and whisker plots summarizing and comparing biomechanical test results from specimens prepared from hooves of animals that were mobility scored during antemortem examination. [a-b] Mobility scores with different letters are significantly ($p < 0.05$) different.

Table 4-3 Tukey multiple comparison test summary with p-values for Peak Stress

Mobility Score	**Median Difference (MPa)**	**p-value**
1 vs 2	0.65	0.8999
1 vs 3	0.60	0.8425
1 vs 4	0.95	0.4733
2 vs 3	-0.05	0.8999
2 vs 4	0.30	0.6677
3 vs 4	0.35	0.8999

Table 4-4 Tukey multiple comparison test summary with p-values for Strain at Break

Mobility Score	**Median Difference (% elongation)**	**p-value**
1 vs 2	2.35	0.8008
1 vs 3	-75.52	0.0010
1 vs 4	-61.35	0.0014
2 vs 3	-77.87	0.0081
2 vs 4	-63.70	0.0176
3 vs 4	14.16	0.8999

Table 4-5 Tukey multiple comparison test summary with p-values for Modulus of Elasticity

Mobility Score	**Median Difference (MPa)**	**p-value**
1 vs 2	1.88	0.8999
1 vs 3	12.62	0.1467
1 vs 4	11.67	0.0325
2 vs 3	10.74	0.0791
2 vs 4	12.72	0.0143
3 vs 4	-0.95	0.8999

Discussion

The maximum amount of load transferred through the specimen divided by the cross-sectional area of the specimen is referred to as peak stress. This value represents the maximum amount of force that a specimen can withstand after it has stretched beyond its ability to return to its original form. While there was an observed downward trend in the peak stress of these specimens as mobility score increased, the difference was not deemed significant.

Strain at break is defined as the maximum amount the material elongates prior to failure divided by the original length of the portion of interest within the specimen. The significant difference between strain at break in animals with higher mobility scores as compared to those with lower mobility scores suggests that a structural change has occurred within the junction of the lamina for those animals with higher mobility scores resulting in decreased laminar integrity. This alteration in the biomechanical properties of animals with lower mobility scores could possibly contribute to the decreased mobility of these animals although the exact cause of this is yet to be determined.

Modulus of elasticity describes the stiffness of a material and is determined by the slope of the line within the linear portion of the stress strain curve while the material is behaving elastically and is calculated as stress divided by strain. There was a statistically significant difference found in the modulus of elasticity between the specimens obtained from mobility scores 1 and 4, and 2 and 4. Lamina from cattle with higher mobility scores had a significantly lower modulus of elasticity than cattle with normal mobility and mildly altered mobility. This finding supports our hypothesis that the modulus of elasticity in mobility impaired animals would be less than animals with normal mobility. This also supports the strain at break results and, when interpreted together, indicates that the laminar junction has higher elastic properties in cattle with high mobility scores than normal cattle. This translates into mobility impaired cattle having decreased integrity of the laminar junction which leads to increased likelihood of displacement of the third phalanx within the hoof capsule, decreased ability to dissipate ground reaction forces normally due to altered properties of the lamina, and pain arising from a compromised laminar junction. The exact mechanism, or multifactorial mechanisms, that causes this increased elasticity was not identified in this study and should be the direction of further research.

Limitations of the study would include having a mixed gender population. It is unknown whether hooves obtained from steers and heifers contain the same biomechanical properties. Additionally, variation may exist within the biomechanical properties of the bovine hoof based upon breed and genetic differences; although all animals used for this research were of beef type, specific breeds and crossbreeds were not selected. All cattle for this research came from approximately the same geographic location; all feedlots were within approximately 300 miles. Nutrition prior to slaughter was also unknown and could influence hoof quality.

In conclusion, the results of this study suggest that in cattle with high mobility scores as assessed with the NAMI scoring system the integrity and the elastic properties of the laminar junction are compromised. There is a relationship between strain at break and modulus of elasticity with the mobility score of finished cattle. As the mobility score of cattle increases the laminar junction becomes more elastic which could explain the degree of discomfort observed in cattle with high mobility scores. Further research on the relationship between mobility score and the biomechanical properties of bovine lamina is warranted.

References

[1] G. Cramer, K. Lissemore, C. Guard, K. Leslie and D. F. Kelton, "Herd-and Cow-Level Prevalence of Foot Lesions in Ontario Dairy Cattle," *Journal of Dairy Science,* vol. 91, no. 10, pp. 3888-3895, 2008.

[2] M. A. Kossaibati and R. J. Esslemont, "The costs of production diseases in dairy herds in England.," *Veterinary Journal,* vol. 154, no. 1, pp. 41-51, 1997.

[3] J. Davis-Unger, E. A. Pajor, K. S. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman and K. Orsel, "Economic impacts of lameness in feedlot cattle," , 2017. [Online]. Available: https://animalsciencepublications.org/publications/tas/articles/0/0/tas2017.0052. [Accessed 2 6 2018].

[4] D. Sprecher, D. E. Hostetler and J. Kaneene, "A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance.," *Theriogenology,* vol. 47, no. 6, pp. 1179-1187, 1997.

[5] F. Bosma, "The impact of reduced mobility score in the pre-breeding period on the fertility of dairy cattle in a seasonally breeding pasture-based system," , 2013. [Online]. Available: https://dspace.library.uu.nl/handle/1874/289487. [Accessed 2 6 2018].

[6] J. Rushen, "Assessing the Welfare of Dairy Cattle," *Journal of Applied Animal Welfare Science,* vol. 4, no. 3, pp. 223-234, 2001.

[7] R. J. Esslemont and M. A. Kossaibati, "Culling in 50 dairy herds in England," *Veterinary Record,* vol. 140, no. 2, pp. 36-39, 1997.

[8] J. F. Ettema and S. D. Østergaard, "Economic decision making on prevention and control of clinical lameness in Danish dairy herds," *Livestock Science,* vol. 102, no. 1, pp. 92-106, 2006.

[9] F. Beleg, A. Muste, N. Mates, L. Oana, C. Ober and A. Donisa, "Lameness, Hoof Care, and Functional Trimming in Cows- an Actual Review," *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine,* vol. 64, pp. 359-364, 2007.

[10] N. Cook, K. V. Nordlund and G. Oetzel, "Environmental Influences on Claw Horn Lesions Associated with Laminitis and Subacute Ruminal Acidosis in Dairy Cows," *Journal of Dairy Science,* vol. 87, 2004.

[11] B. Faramarzi, "Morphological and Biomechanical Properties of Equine Laminar Junction," *Journal of Equine Veterinary Science,* vol. 34, no. 5, pp. 589-592, 2014.

[12] G. Ramsey, P. J. Hunter and M. P. Nash, "The effect of hoof angle variations on dorsal lamellar load in the equine hoof," *Equine Veterinary Journal,* vol. 43, no. 5, pp. 536-542, 2011.

[13] P. Boettcher, J. C. M. Dekkers, L. D. Warnick and S. Wells, "Genetic analysis of clinical lameness in dairy cattle.," *Journal of Dairy Science,* vol. 81, no. 4, pp. 1148-1156, 1998.

[14] N. Cook, "Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface," *Journal of The American Veterinary Medical Association,* vol. 223, no. 9, pp. 1324-1328, 2003.

[15] B. W. Newcomer and M. F. Chamorro, "Distribution of lameness lesions in beef cattle: A retrospective analysis of 745 cases," *Canadian Veterinary Journal-revue Veterinaire Canadienne,* vol. 57, no. 4, p. 401, 2016.

[16] L. N. Edwards-Callaway, M. S. Calvo-Lorenzo, J. A. Scanga and T. Grandin, "Mobility Scoring of Finished Cattle," *Veterinary Clinics of North America- Food Animal Practice,* vol. 33, no. 2, pp. 235-250, 2017.

[17] J. F. Tarlton, D. Holah, K. Evans, S. Jones, G. Pearson and A. Webster, "Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving.," *Veterinary Journal,* vol. 163, no. 2, pp. 196-204, 2002.

CHAPTER 5. CONCLUSION

General Discussion

While there is yet much that is unknown and needs to be researched regarding the biomechanical properties of lamina within the hooves of cattle with reduced mobility, this thesis provides evidence that there are changes occurring within the structure of the lamina resulting in a decrease in the strain at break and increased elasticity of the laminar junction as mobility score increases. While the causes of the weakening of this structure in generalized lameness likely has a multifaceted etiology, when the integrity of the lamina is compromised the prevalence of other hoof lesions increases [1]. Understanding the biomechanical properties of bovine lamina is a vital element to understanding its mode of failure and how its breakdown may affect the other suspensory and support structures within the claw, the hoof, and other structures of the affected limb. The lamina plays a leading role in the stability of the distal phalanx; if compromised, the quality of the hoof and the likelihood of lesions increases. Any cause of lameness is an economic drain on producers and reduces the quality of life for that animal [2,3].

Discussion of Results

In biomechanically testing the laminar junction of the hoof, all specimens failed at the junction of the dermal and epidermal lamina. The specimen production technique described in this thesis yielded specimens with a uniform cross-sectional area allowing the calculation of stress to be a simple calculation.

The effect of long-term frozen storage on the bovine laminar junction may influence its peak tensile stress, however other properties examined in this work—modulus of elasticity and strain at break—were determined to be unaffected.

The biomechanical test specimens prepared from hooves obtained from animals with all states of mobility according to the standards designed by the North American Meat Institute were determined to have altered tensile test results. Most notably strain at break was significantly increased with decreased mobility. The modulus of elasticity was decreased amongst specimens prepared from the least mobile animals compared to the most mobile animals. The decreased peak stress values for the least mobile animals was determined not to be statistically significant.

Comparison to Previous Literature

In the work by Tarleton et al. [4] it was noted that the rigidity of the suspensory structures of the hoof were decreased in dairy cows that were post-partum. In the research performed for this thesis a significant increase in strain at break was found in animals with low mobility scores; both the research performed by Tarleton et al. and the research performed to produce this thesis indicate that there is a biological mechanism that may alter the composition and integrity of the suspensory tissues and of the bovine hoof. Research completed by Danscher et al. [5] indicated no decrease in strength of suspensory structures between animals where laminitis was induced and control animals. However, these results were obtained using a model of laminitis whereas the animals reported in this thesis had naturally occurring disease. The values reported in the research where mobility score was compared to the peak stress for this thesis also agree with the research by Danscher *et al.*; however, additional significant changes in the biomechanical nature of the laminar junction were found in the elasticity of our specimens, especially the strain at break and modulus of elasticity.

Direction for Future Studies

There may be an effect on the duration of freezing on the response of bovine hoof tissues and structures to biomechanical testing as this work suggests. There may also be additional relationships between other factors that may contribute to statistically significant different test results. Our research further suggests a relationship between mobility score and the strain at break and modulus of elasticity for bovine lamina. Additional investigation into this relationship in the future may lead to further elucidations of the relationship between peak load and mobility. Further research will be required to better understand and characterize these biomechanical properties in feet of cattle affected by altered mobility scores as well as biochemical and physiologic factors that may interface with the biomechanical properties of the laminar junction in mobility impaired cattle

References

[1] R. Smilie, M.S. Hoblet, W. Eastridge, Schnitkey, & Moeschberger. (1999). Subclinical laminitis in dairy cows: Use of severity of hoof lesions to rank and evaluate herds. Veterinary Record, 144(1), 17-21.

[2] M. A. Kossaibati and R. J. Esslemont, "The costs of production diseases in dairy herds in England.," Veterinary Journal, vol. 154, no. 1, pp. 41-51, 1997.

[3] J. Davis-Unger, E. A. Pajor, K. S. Schwartzkopf-Genswein, S. Marti, C. Dorin, E. Spackman and K. Orsel, "Economic impacts of lameness in feedlot cattle," 2017. [Online]. Available: https://animalsciencepublications.org/publications/tas/articles/0/0/tas2017.0052. [Accessed 2 6 2018].

[4] J. F. Tarlton, D. Holah, K. Evans, S. Jones, G. Pearson and A. Webster, "Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving.," *Veterinary Journal,* vol. 163, no. 2, pp. 196-204, 2002.

[5] A. Danscher, T. Toelboell and O. Wattle, "Biomechanics and histology of bovine claw suspensory tissue in early acute laminitis," *Journal of Dairy Science,* vol. 93, no. 1, pp. 53-62, 2010.