J. Dairy Sci. 97:2969–2973
http://dx.doi.org/10.3168/jds.2013-7208

Technical note: Effects of frozen storage on the mechanical properties of the suspensory tissue in the bovine claw

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ABSTRACT

It is proposed that a softening of the suspensory tissue in the claw is involved in the development of lameness and claw lesions in cattle. A relatively small amount of research has been carried out to verify this theory. Research in this area would be simplified if mechanical testing of the suspensory tissue could be performed on frozen and stored specimens. The current study tested whether freezing of the specimens changes the suspensory tissues' mechanical properties. Limbs from 3 freshly slaughtered Danish Holstein dairy cows and 6 nonpregnant Angus heifers, without clinical signs of lameness, were allocated to 1 of 2 treatments (frozen or nonfrozen) in such a way that each cow was represented in each treatment group with a frozen limb and a corresponding nonfrozen limb (i.e., frozen left front, fresh right front, and so on). The frozen limbs were kept at −18°C for a week before processing and the nonfrozen limbs were processed within 2 h of slaughter. Two samples measuring 8 × 8 mm were cut from the abaxial side of each claw in such a way that the sample included the horn of the abaxial wall, pedal bone, and the interposed corium. The samples were kept on ice until being mounted in a large deformation rheometer with an extension testing frame, fixed by the horn and the pedal bone, and loaded to failure. During deformation force and displacement data were recorded, from which corresponding stress and strain were calculated. Young's modulus (a measure of tissue elasticity or stiffness) and a measure of physiological support (PS; force needed to displace the sample 1 mm) were calculated from the data. The response variables, Young's modulus and PS, were analyzed separately by a mixed model. The explanatory variables were treatment (frozen or nonfrozen), limb (front or back), claw (medial or lateral), position of the sample (dorsal or palmar-plantar), and group (Angus or Holstein). Interactions between group and treatment and between limb, claw, and sample position were included in the model. Cow identity was included as a random effect. Model reduction was performed by stepwise backward elimination, until all remaining terms were significant at the 5% level or less. Freezing had no effect on the elasticity of the suspensory apparatus or on PS. However, PS was affected by limb (hind legs had higher PS values than front) and the position of the sample (palmar-plantar samples had higher PS values than dorsal). The Angus group had higher PS values than the Holstein group, but the groups differed in age, parity, body weight, lactation, housing, and management, as well as in breed; therefore, further studies are needed to investigate these effects. The results indicate that mechanical testing of bovine claw suspensory tissue can be performed on specimens that have been frozen, thus aiding research in the mechanical aspect of bovine lameness and claw lesions.

Key words: biomechanics, claw suspensory tissue, Young's modulus, bovine lameness

Technical Note

Lameness has a large effect on economy (Esslemont and Kossaibati, 1997; Vatandoost et al., 2009) and animal welfare in dairy production (Esslemont and Kossaibati, 1997). However, because the pathogenesis behind development of lameness is not completely understood, methods for treatment and prevention are still not optimal. Many theories have been put forth; one well-established supposition proposes that a central element in the pathogenesis is the weakening and subsequent failure of the tissue supporting the third phalanx within the claw capsule. This may induce trauma in the internal tissue in the claw leading to necrosis and lameness (Ossent and Lischer, 1996, 1998; Pollitt, 2010).

If frozen storage of claws before mechanical testing were possible, more precise epidemiological investigations of possible relationships between herd factors, occurrence of lameness, and claw tissue strength would be

Received July 1, 2013.
Accepted February 5, 2014.
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possible. One can hypothesize that freezing could have a detrimental effect on the suspensory tissue, which would render any results unreliable, as the suspensory tissue is not a homogenous tissue. The bearing force is created by a dense matrix of corium connective tissue that connects the dermal-epidermal junction to the parietal surface of the distal phalanx and thus suspends the distal phalanx from the inner wall of the claw capsule (Pollitt, 2010). The effect of freezing on ligaments, which is composed of connective tissue, has been investigated with varying results. Dorlot et al. (1980) found that frozen storage of canine anterior cruciate ligaments resulted in decreased elasticity, whereas Matthews and Ellis (1968) found the opposite to be true in a study on cat extensor communis and lateralis tendons. Viidik and Lewin (1966), Van Brocklin and Ellis (1965), and Abreu et al. (2009) found no change in the elastic properties of rabbit anterior cruciate ligaments, human extensor digitorum tendons, and rat tail tendons, respectively, after freezing. Tarlton and Webster (2000) reported that storage at −20°C had no effect on the biomechanical strength of the suspensory tissue in bovine claws, but the details of this study have not been published. Thus, the consequences of freezing are not clear and, therefore, the current study aimed to investigate the effect of frozen storage on biomechanical properties of the suspensory tissue in the bovine claw.

Animals

Limbs from 3 Danish Holstein dairy cows and 6 nonpregnant Angus heifers collected from abattoirs were used in our study. The 3 Holsteins originated from 3 different herds and their background was unknown. The 6 Angus heifers originated from the same herd, were 18 mo old, and had been raised entirely on pasture.

Tissue Samples

Limbs, cut below the hock or carpal joint, were collected after slaughter. Limbs were assigned to 2 treatments (frozen and nonfrozen) in such a way, that each animal's frozen limb had a corresponding nonfrozen leg (i.e., frozen left front, nonfrozen right front and frozen right hind, nonfrozen left hind). Within 2 h of slaughter, the nonfrozen limbs were processed as described herein. The frozen limbs were stored at −18°C for a week before processing.

Tissue samples were obtained by use of a band saw according to Figure 1. The frozen claws were cut directly from the freezer. This produced 4 samples per foot: a palmar-plantar and a dorsal sample from the medial claw and the same from the lateral claw. The dorsal samples were marked and the set of dorsal and palmar-plantar samples were stored in a plastic container with tap water-soaked cotton wool and then placed on ice until mechanical testing.

Mechanical Testing

The dimensions of the samples were measured using digital calipers (Limit, Teng Tools International, Alingås, Sweden) and entered into Excel, calculating the area and volume of the lamellar tissue and corium. Each sample was mounted in an Instron 5564 mechanical testing frame, with a 500-N load cell (Instron, High Wycombe, UK) fixed by the horn and the bone. The frame was loaded to failure at a constant extension rate of 2.0 mm per second, producing data on the uniaxial extension (mm) and force (N). Most samples did not reach the point of failure, as the testing frame's maximum load was 500 N. No more than 2 h passed between cutting the samples until the mechanical testing commenced.

To compare samples of different sizes, the data was converted to standardized values. The displacement (mm) during extension was converted into Hencky strain, which is a measure of the total height upon extension of a sample ($\mathbf{H_t}$) relative to the initial height of the sample ($\mathbf{H_0}$):

$$\text{Hencky strain} = \ln(H_t/H_0).$$

The force (F_t), in newtons, during extension was converted to stress ($Pa = N/m^2$), and thus standardized relative to the cross sectional area of the sample (A_0) and for the change in height during the testing process (H_t /H_0):

$$\text{Stress} = (F_t/A_0) \times (H_t/H_0).$$

The data were plotted with the stress against strain, where the slope is the elastic modulus, also called Young's modulus, and thus representing the tissue's proportionality constant between stress and strain (Figure 2).

To assess a measure of the claw tissue's resistance to physiologic stress levels, the samples' physiological support level (**PS**), here defined as the stress needed to displace the horn 1 mm in relation to the bone, was recorded (Danscher et al., 2010). Measures of physiological support have been used by Danscher et al. (2010) and Tarlton et al. (2002) as a measure of the tissues' strength or elasticity. The PS uses absolute measurements that have not been standardized in relation to the sample height as it changes during the extension,