Title:

Collagen Disorders in Livestock Hide

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Biographical Sketch:

Originally from south Florida, Katherine Upshaw grew up with a passion for animal agriculture. She graduated from the University of Florida in 2018 with a bachelor's degree in animal science and a minor in business administration. At the University of Florida, Katherine developed her enthusiasm for animal agriculture through involvement in the Equestrian Team, Block & Bridle, and a livestock production internship in France. Throughout her undergraduate career, Katherine was fortunate enough to enroll in a variety of genetics courses (from livestock genetic improvement to human molecular genetics) and eventually join a research lab that focused on genetic responses to environmental stress. Now at Kansas State University, she is pursuing a master's degree in animal breeding and genetics under Dr. Megan Rolf. Katherine's research interests include the prevalence and possible causal variants of gene disorders in beef cattle.

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I certify that I have read and had sufficient time to provide feedback on the attached literature review.

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INTRODUCTION

Collagen is a protein fiber that is commonly found in bone, muscle, and skin tissues. Collagen provides the primary structural support for these tissues through various combinations of packing density and orientation of fibers. In cattle, hide collagen is found mostly in the corium layer of the skin, just above the inner flesh of the animal. Of particular interest to livestock producers are the collagen fibers composing the muscle and hide as they contribute to skin strength and elasticity, as well as meat quality characteristics such as tenderness.

Collagen disorders that affect skin strength are relevant to a variety of industries. Collagen-based materials are often used in the medical field, including processed pericardium for heart valve repairs, and in the industrial sector where they are used in shoes and upholstery (Sizeland et al., 2013). The soft leather and splitting associated with hide collagen disorders produces unsatisfactory leather and can cause significant financial loss (Amos, 1958).

Collagen is also important for meat quality traits in livestock. Meat palatability for consumer satisfaction is determined through a combination of characteristics but perhaps the most influential of these is tenderness (Bailey, 1972). Collagen structure directly affects meat texture through the packing density of collagen fibers in meat products.

Disorders involving collagen are often genetically controlled at the developmental stage of collagen production. Thus, genomic selection on collagen markers may provide livestock producers the means by which to improve hide strength and carcass quality within their herds.

COLLAGEN AND HIDE STRUCTURE

Collagen provides the skin with an interesting dichotomy of both rigidity and flexibility, which is important for the production of leather and leather-made goods. Hide collagen disorders can negatively impact leather quality, which can be detrimental for livestock producers because leather is one of the most important by-products of the meat industry (Naffa et al., 2019).

An important factor involved in hide durability is the alignment of collagen fibers. Sizeland et al. (2013) measured collagen fiber orientation in seven mammalian species to broadly examine the relationship between collagen strength and fiber orientation. The mammals included in the study were sheep, possum, cattle, goat, water buffalo, deer, and horse. Skin samples were collected from each animal, processed into leather, and tested for tear strength.

Sizeland et al. (2013) found collagen fiber orientation had a very strong correlation with tear strength in all species. It was noted that the strongest leathers had fibers that were parallel to the plane of the hide. However, when fibers were perpendicular to this plane, the leather was highly susceptible to tearing. It was also determined that the tear strength of cattle hide is very similar to that of goat and sheep, indicating that research on collagen mutations of various livestock species may hold clues to the etiology and treatment of collagen disorders in cattle. Ultimately, collagen was deemed crucial for the structural composition of leather and skin and any disorder or disease that affects collagen would have devastating effects on the health of an animal.

Vertical Fiber Hide Defect

Vertical fiber hide defect (VFHD) is a disorder affecting the collagen structure of beef cattle (Amos, 1958; Cundiff et al., 1987). Affected hides are identified by the distinctly vertical orientation of collagen fibers centralized to the hindquarter (Amos, 1958). Affected fibers can comprise up to 75% of the hide, extending as far as the belly and shoulder areas (Tancous, 1966; Cundiff et al., 1987). Normal hide collagen lies nearly parallel to the outer layer of skin and interwoven at an angle of 50-60°, but the vertical orientation associated with VFHD causes collagen fiber bundles to have higher angles of weave and reduced fiber interlacing, which can cause affected hides to fall apart (Cundiff et al., 1987). Hides with the abnormality are considerably less durable than hides with normal horizontal fiber weave and have a predisposition to cracking and breaking.

The abnormality has a detrimental effect on the leather industry and its occurrence can have long-lasting economic impacts (Cundiff et al., 1988). Currently, the only way to confirm the presence of VFHD is through histological examination of the hide, which is not cost-effective due to the high level of skill, financial expense, and lengthy amount of time involved for accurate diagnoses (Cundiff et al., 1987). Unfortunately, this makes identifying the condition before the hide falls apart difficult, because by this point, tanneries have already invested both time and money in the tanning of affected hides.

Amos (1958) was the first to note an incidence of the condition and hypothesized that VFHD might be under genetic control. Everett et al. (1971) presented the first concrete data that VFHD is a hereditary condition when the authors reported higher rates of VFHD in some sire lines, but not all. More recently, Cundiff et al. (1987) offered compelling evidence that VFHD is controlled by an autosomal recessive mutation present uniquely in Hereford and Hereford-crosses, but this has not been confirmed and both the genomic position and causal variant are unknown.

Vertical fiber hide defect has been studied intermittently since its identification in 1958, but without further data on its current prevalence, it is unknown if VFHD is proliferating within the population and causing associated financial losses. Additional research is necessary to better understand VFHD and its prevalence, etiology, diagnosis, and prevention.

Dermatosparaxis

Dermatosparaxis is a genetic connective tissue disorder that commonly affects sheep and cattle and is often characterized by easily torn skin and joint hypermotility (Holbrook et al., 1980). The skin is fragile enough that the abnormality is easily detected at parturition or soon after. Affected animals tend to have limited life spans as a result of accompanying lesions and septicemia (Hanset and Lapiere, 1974). Simar and Betz (1971) used electron microscopy to view dermatosparaxis skin samples from affected calves and discovered the normally horizontal collagen fiber orientation was compromised and fibers tended to orient more vertically than expected.

Dermatosparaxis is attributed to malfunctioning procollagen precursors that assist in assembling collagen fibers. This mutation prevents normal collagen fibers from forming, thereby reducing the skin's tensile strength. The mutation has been traced to a disintegrin and metalloproteinase with the thrombospondin type 1 motif 2 (*ADAMTS2*) gene in Dorper sheep (Zhou et al., 2011), which may prove to be a useful candidate gene for collagen abnormalities in other species, like beef cattle.

Hereditary Equine Regional Dermal Asthenia

Dermatosparaxis is similar to a connective tissue disorder found in horses, called hereditary equine regional dermal asthenia (HERDA). HERDA is a genetic disorder that primarily affects Quarter Horses and is characterized by extremely fragile skin. The animal's skin sloughs off upon minimal physical trauma (Grady et al., 2009). Tryon et al. (2007) used homozygosity mapping of 68 HERDA-affected horses and found that 64 of these animals had a common haplotype on *Equus caballus* chromosome 1 (*ECA1*). A missense mutation in cyclophilin B (PPIB), a chaperone protein responsible for proper collagen protein folding, was uniquely identified in horses with HERDA and proposed as a likely causal candidate mutation. Interestingly, HERDA and VFHD appear to be localized to similarly specific areas on affected animals. Vertical fiber hide defect is found most often on the rumps of cattle, between the hook and pin bones, and HERDA is typically exhibited along the dorsal skin surface of affected horses (Grady et al., 2009).

COLLAGEN AND MEAT QUALITY

An important element involved in meat tenderness is intramuscular connective tissue, which composes three distinct layers of muscle and is made up of collagen fibers. The endomysium is the innermost layer of connective tissue that consists of individual myocytes. The perimysium is the middle layer of connective tissue that surrounds groups of myocytes and consists of over 90% of the intramuscular collagen in meat (McCormick, 1994). Those bundles of myocytes are then combined with other bundles and surrounded by the epimysium, or the outermost layer of the muscle. The epimysium is a protective layer, so it is made up of very tough connective tissue that is usually discarded from the muscle during meat processing. Thus, meat quality traits come primarily from the characteristics of the endomysium and perimysium.

The relationship between meat texture and collagen lies mostly in the structure of collagen fibers. McCormick (1994) suggested that meat tenderness changes in animals as they mature because it takes time to develop strong, tightly bound bundles of collagen fiber. These tightly bound collagen fibers are associated with decreased tenderness and palatability in meat. McCormick (1994) noted that collagen fibers can start showing signs of increased rigidity as early as one year of age in beef steers.

Wojtysiak (2013) assessed connective tissue changes in the *longissimus lumborum* muscles of 45 Polish Large White pigs as the animals aged. The pigs were sampled in groups ranging in age from 90 to 210 days and it was discovered that the collagen fiber density in the endomysium increased as the animals got older. The samples taken at 90 days of age were found to have very loose collagen fibers, but the fibers of the 210-day-old samples were bound very closely together. Moreover, the perimysium became thicker with age. Shear force values also increased with age, which suggests the loose arrangement of collagen fibers in younger animals results in more tender meat than the tightly bound fibers of older animals.

Collagen structure also appears to differ by sex. Gerrard et al. (1987) examined the effect of collagen in 32 Charolais-Angus bulls and 32 Charolais-Angus steers. The bulls had more overall intramuscular collagen and were less consistent in tenderness values. The authors suggested that collagen from the bulls matured more quickly than that of the steers, leading to increased collagen fiber cross-linking and subsequently, decreased tenderness. Maiorano et al. (1993) found similar results when the authors evaluated intramuscular collagen in 18 Columbia ram and wether lambs. Intramuscular collagen fibers were much more closely linked in rams than wethers and shear force values were also higher for rams, again indicating meat from intact males is tougher than meat from castrated males.

THE FUTURE OF COLLAGEN DISORDERS AND TREATMENTS

Collagen abnormalities exist in many species, which may help elucidate the underlying mechanisms and genetic markers of livestock collagen disorders, and particularly those that impact beef cattle producers. Research on collagen development has also provided breakthroughs in medical and industrial applications.

Model Organisms

Biochemical processes identified in mice may provide clues toward the development of collagen disorders in other mammals, like beef cattle. Decorin is one of four main small leucine-rich proteoglycans (SLRPs) involved in collagen fiber formation and tissue regulation (Grady et al., 2009). Danielson et al. (1997) interrupted, or knocked-out, the decorin gene in mice and discovered that the resulting knockout mice had easily torn skin with lower tensile strength, much like the skin of cattle and sheep with dermatosparaxis or horses with HERDA. The decorin-deficient animals were found to have loosely arranged collagen fibers, while the wild-type and heterozygous knockouts retained skin structure and elasticity.

Biochemical studies of livestock collagen abnormalities may prove helpful in diagnosing and selecting against them in the future. However, biochemical studies often involve the use of protein assays, which are considerably more difficult to do than genomic testing, especially in the cattle industry. Thus, these types of studies may prove useful in obtaining more information about the disorder and components, like decorin, potentially involved in its expression but would not necessarily be practical for industry-wide implementation.

Humans

Collagen abnormalities have also been known to affect humans, as is the case with Ehlers-Danlos Syndrome and osteogenesis imperfecta. Ehlers-Danlos syndrome type VIIC arises as a result of mutations in the genes in charge of producing normal collagen precursors, which are in turn responsible for producing normal collagen fibers (Nusgens et al., 1992). Nusgens et al. (1992) conducted a case study on a two-year-old girl affected by Ehlers-Danlos syndrome type VIIC. She had delicate skin that tore easily and bruised frequently. Severe wounds could not be treated surgically due to the skin's fragility and associated inability to suture it. The researchers noted the child's skin was comparable to the skin of calves affected by dermatosparaxis.

Osteogenesis imperfecta (OI) is a genetic disorder characterized by collagenous structural defects and often results in brittle bones. The abnormality is caused by mutations of the *COL1A1* or *COL1A2* genes that are responsible for the production of procollagen, a substance that is later processed into collagen (Gajko-Galicka, 2002). Cases of OI range from mild to lethal, but most share traits of bone fragility, osteoporosis, and scoliosis. Mild cases are associated with a null allele, while severe cases are associated with a dominant antimorphic mutation that replaces necessary glycine residues with larger amino acids. Mild expression of OI results in patients that have reduced production of procollagen, while more acute cases have abnormal collagen structure (Gajko-Galicka, 2002). Gene therapy, specifically the use of antisense oligonucleotides to target mRNA, has been shown to mitigate the effects of the severe form of OI by preventing translation of the antimorphic mutation and reducing its expression. Effectively, this converts the antimorphic mutation to a null mutation and reduces the severe form of OI to the milder form of

OI. Innovative gene therapies like this may prove useful for treating other collagenous structural abnormalities in the future.

Medical Bioengineering

Bioengineering is the intersection of biological principles and engineering design. It often involves the construction and design of replacement materials to fix or restore ineffective biological components. Bioengineered products, called biomaterials, are typically implemented in medical applications. Collagen is commonly used in the development of biomaterials due to its strength and elasticity.

One source of collagen for biomaterials is bovine pericardium, a by-product of the beef cattle industry, because it is readily available, highly biocompatible, and flexible (Santos et al., 2013). Bovine pericardium offers an inexpensive option for biomedical engineers to extract collagen and develop biomaterials for applications like soft tissue repair.

Bovine pericardium is also useful in the production of heart valve replacements. Valvular heart disease is a global threat to human health. Sellaro et al. (2006) reported a prevalence of over 275,000 cases globally. Bioengineered collagenous tissues are frequently used in heart valve replacement surgery as these provide a strong, yet flexible material that is easily acclimatized to a biological environment (Sellaro et al., 2006). However, the process by which collagen is extracted from bovine pericardium allows for structural damage to the biomaterial over time.

Sellaro et al. (2006) evaluated structural changes in collagen extracted from bovine pericardium tissues and determined that collagen fiber orientation is crucial for the structural support of biomaterials and their response to fatigue over time. When a cyclic loading force was applied to the biomaterial, fiber orientation was very sensitive to direction. Stress applied perpendicular to the preferred collagen fiber orientation resulted in reoriented fibers to support the load. Conversely, when fibers were parallel to the preferred orientation, they did not reorient as much in response to mechanical stress and remained steady. Thus, damage to collagenous biomaterials can likely be mitigated by selecting collagen sources with preferred initial collagen orientations.

Collagenous biomaterials can also be used for chronic wound repair in the form of biological dressings. Collagen dressings are often made from bovine intestinal tissue and are applied directly to a cleaned and debrided wound. When compared to conventional treatments, like povidone iodine and nadifloxacin, collagen dressings were found to more quickly reduce the presence of pathogenic organisms and reduce healing time (Singh et al., 2011). Collagen is very useful for biomedical applications and future research into collagen abnormalities may help to reveal additional breakthroughs in tissue replacement and wound repair practices.

IMPLICATIONS OF COLLAGEN DISORDERS TO FURTHERING THE GENETIC IMPROVEMENT OF BEEF CATTLE

Collagen is a protein fiber that provides structural support for skin and muscle tissues. In hide, loose, vertically oriented collagen fibers are often responsible for fragility and susceptibility to tearing. Although there are many factors that contribute to beef cattle genetic improvement and collagen disorders should not overstate other traits of economic importance, these hide abnormalities are important for beef producers. The fragility associated with hide collagen disorders produces unsatisfactory leather and can cause significant financial loss because leather is considered one of the most profitable by-products of the meat industry.

Fiber structure plays a significant role in muscle development as well. In meat, tenderness is a crucial component of consumer palatability. Tightly packed collagen fibers are associated with decreases in tenderness, which, in turn, decrease profits for beef producers. Collagen disorders not only affect skin strength and meat quality but can also be harmful to the biomedical industry that uses collagen sourced from livestock for biomaterials, like replacement heart valves and wound dressings.

Many collagen disorders, such as VFHD, dermatosparaxis, HERDA, Ehlers-Danlos Syndrome, and OI, appear to be genetically controlled. Some of these disorders, like HERDA and OI, have candidate genes or mutations that have already been identified. Disorders involving collagen seem to be genetically controlled at an early stage of collagen production, which makes research on these abnormalities viable across many species. Thus, further research concerning genomic selection on collagen markers may provide livestock producers the means by which to improve hide strength and carcass quality within their herds.

Ultimately, collagen disorders have significant implications for the beef and leather industries and further research into candidate genes and causal variants of these disorders may prove useful for genomic testing or other strategies required for the practical identification and control of these conditions.

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