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Review Article

Tenderness, histomorphology of muscle fiber and gene expression in primal cuts of cattle-A Review

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Abstract

This review focuses on the relationship between tenderness, the structure of muscle fibers (histomorphology) and gene expression in the primal cuts of beef with an emphasis on understanding how these factors contribute to meat quality. This review describes the biochemical and molecular findings in connection with this proteolytic system and their relation with meat tenderness in bovine. The findings of DNA polymorphisms and mRNA and protein expression are described as tools to predict meat tenderness. Tenderness, a key determinant of consumer satisfaction, is influenced by the structural and biochemical properties of muscle fibers, which vary across different primal cuts. The paper synthesizes existing literature on the morphological characteristics of muscle fibers, including fiber type, size, and distribution, and their relationship to meat tenderness. Additionally, it explores the role of gene expression in muscle development and postmortem changes which further impact meat texture and quality. The review discusses key research findings on how genes control muscle growth, the breakdown of proteins, and the structure of muscle fibers, all of which play a role in tenderness. It highlights areas where more research is needed, especially regarding how genes (Calpain, Myostatin, Calpastatin) and muscle structure work together to affect tenderness. Finally, this paper suggests future research directions aimed at improving meat quality through a better understanding of the genetic and structural basis of tenderness in cattle. Research on the molecular basis of meat tenderization may be fruitful particularly to the meat industry and may allow amendment of handling practices of preslaughter andpostmortem treatments that improves meat quality.

Introduction

The demand of beef is increasing due to religious and consumer preference and the demand of crossbred cattle are increasing day by day due to the breeding policy in dairy sector in Bangladesh (Siddiqua et al., 2018; Haque et al., 2017). As a result, more crossbred male calves are being fattened for producing beef. All primal cuts of beef provide important nutrients for human diets. The type of cut has significant effects on the main parameters considered to be important quality indicators (color, intramuscular fat, cooking loses and tenderness) and which have an impact on consumer acceptability (Galvez et al., 2019; Liza et al., 2024). Proper feeding strategies that enhance intramuscular fat deposition and muscle hypertrophy contribute to tenderer and juicier meat by influencing fiber type composition, fiber diameter, and collagen formation. On the other hand, inadequate feeding can lead to leaner meat, tougher texture, and reduced fat marbling. Managing nutrition to balance protein and energy intake, along with a focus on gene expression regulation related to fat deposition, plays a critical role in improving the carcass quality of fattened cattle, leading to better tenderness and overall meat quality (Hashem et al., 1999). Meat cut is the most important factor when choosing bovine meat followed by quality certification (origin) production technique, the type of breed and price (Scozzafava et al., 2016). Meat quality is a major criterion which influencing consumer purchase. According to OECD-FAO estimates, the global meat production in 2020 was 328 million tons or 35 kg per person annually. Between 2020 and 2029, meat consumption is expected to increase by 12% (De Devitiis et al., 2023). The most crucial factor influencing customer pleasure is the tenderness of the meat (Kamruzzaman, 2023). The improvement of meat tenderness can be accomplished by several methods such as ageing, electrical stimulation, electric pulses, exogenous and endogenous proteases, ultrasonic, tender bound vacuum packaging, mechanical or chemical infusion methods (Elzalaky, 2024). To increase beef tenderness, a number of post-mortem procedures have been used in recent years and plant proteases is a new, sustainable method for improving the tenderness of meat (De Devitiis et al., 2023). The tenderness of meat decreases immediately after slaughter, then it increases gradually during post-mortem ageing. Tenderness is measured mechanically by 'shear force' using a Warner–Bratzler shear device (WBS), where higher shear force value indicates low tenderness of meat (Listyarini et al., 2023). Muscle fibers, adipocytes, and connective tissues are the major structural elements of meat that determine its texture and tenderness (Roy and Bruce, 2023).

The primary structural and functional unit of muscle fibers are myofibrils, and each muscle fiber has about 1000 myofibrils in total. The primary structural, functional, and contractile unit of myofibrils is the sarcomere (Ivanov, 2023). Oxidative stress can influence muscle fiber structure, tenderness, and biochemical changes in meat (Islam et al., 2018). Heat stress in all species upregulates heat shock proteins, but prolonged exposure can impair muscle protein synthesis and affect meat tenderness, particularly by increasing collagen cross-linking. Managing these genetic and environmental factors is essential for optimizing meat quality, tenderness, and overall carcass yield in cattle (Rana et al., 2014). Meat production worldwide overcome 337 million tons in 2020 and this growth was accompanied by an increasing interest in higher quality products and it is, therefore, necessary to execute work on candidate genes, which can be an important tool in Shaping breeding programs (Kostusiak et al., 2023). The expression levels of Calpastatin (CAST), Calpain (CAPN1, CAPN3), Myostatin (MSTN) were assessed to determine their correlation with meat tenderness. The literature review focuses on the most frequently addressed issues concerning these genes in primal cut tenderness. There is lack of scientific literature on primal cuts in relation to tenderness, muscle structure and gene expression in cattle. This literature review focuses on the establishment a scientific explanation about tenderness of different primal cuts of cattle which can be beneficial to consumers and meat industry

Tenderness of primal cuts

Tenderness is considered the most important meat quality trait regarding its eating quality. It is defined as the quality of meat that determines how easily it can be chewed or cut. Tenderness is a muscle-specific characteristics that has significant impact on customers' acceptance of beef and level of eating satisfaction (Nair, 2019). Tenderness is influenced by various factors, including muscle fiber histomorphology, post-mortem proteolysis, and genetic regulation. While younger cattle tend to offer more tender meat with less marbling, older animals (cattle at 24 months) produce tougher meat with more fat and collagen. The key is to find an optimal slaughter age that balances muscle fiber development, fat deposition, and collagen accumulation to ensure the best quality meat (Hossain et al., 2021). Beef tends to have more marbling and fat content, contributing to a tenderer texture and richer flavor, while goat meat remains leaner and firmer, and sheep meat falls somewhere in between. Understanding these differences is crucial in meat production, processing, and consumer preferences (Ali et al., 2022; Murshed et al., 2014). Moreover, cattle are raised without synthetic hormones, antibiotics, or growth promoters, which can result in slower growth rates and leaner muscle development compared to conventional systems. This can affect tenderness, as higher intramuscular fat (marbling) is generally associated with softer, tenderer meat (Hossain et al., 2016; Sarker et al., 2017; Hashem et al., 2020). The structural composition of muscle fibers, connective tissue, and enzymatic activity determines the textural properties of beef, which vary among different primal cuts (Sadakuzzaman et al., 2024). Muscle fiber composition, collagen cross-linking, and post-mortem proteolysis play vital roles in tenderness variation among different primal cuts and at different cooking temperatures (Torun et al., 2023). In the first 24 hours of post-mortem, metabolic changes have an impact on meat tenderness (McSharry et al., 2021). Sarcomere length, protein denaturation, myofibrillar integrity, connective tissue and cross-links and intramuscular fat are the main factors that determine meat tenderness (Warner et al., 2022). Myofibrillar protein degradation, collagen content, mature collagen crosslinks, and lipid content were playing vital role in predicting tenderness in both the overall tenderness and WBSF models. About 40% of sample variation can be described by the multivariate equation but only 34% of such variation can be explained by the WBSF model and WBSF's inability to identify the tenderness perception from fat is probably the main reason why biochemical components model perform marginally better than WBSF's in the overall tenderness (Hammond et al., 2022). Biomarkers have been used to create instruments that can give objective information on the tenderness potential of live animals and certain beef cuts (Berri et al., 2019). According to the recent proteomic analysis a list of proteins thought to be prospective biomarkers of beef tenderness (Picard et al., 2018). Since flavor and other components of palatability are hard for replicating in a laboratory setting, research has primarily focused on instrumental tenderness (Hocquette et al., 2020). Higher moisture retention is associated with better WHC and lower drip loss, resulting in juicier and more tender meat (Murshed et al., 2014). In accordance with AMSA (2016) criteria, the Warner-Bratzler shear force equipment was used to assess the instrumental tenderness of beef. Following the slaughter, the carcass was prepared and samples of the main primal cuts were gathered in order to assess the Warner-Bratzler Shear Force (WBSF) (Shentema et al., 2023). A 2.5 cm-thick steak was wrapped individually in a polyurethane bag, heated for 50 minutes at 72 degrees Celsius in a thermostatic water bath, and then chilled for at least 40 minutes under running tap water in order to calculate cooking losses and following chilling, eight cylindrical subsamples were removed from the cooked steak using a steel borer in a direction parallel to the meat fibers in order to measure the instrumental tenderness using a dynamometer TA-HDi texture analyzer fitted with a V-slot Warner-Bratzler blade (Khazzar et al., 2023). The maximum shear force (WBSF) that the instrument used to cut the meat sample was used to express the tenderness of the meat. Cuts from support muscles like the ribs and loin can be tenderer than locomotive muscles like the chuck and round because round muscles are found to contain less fat and more connective tissue than support muscles (Egolf, 2021). It was possible for us to identify more tender and less tender muscle by determining the shear force values (Gálvez et al., 2019). Heat stress leads to altered muscle fiber composition, increased fast-twitch fibers, and decreased slow-twitch fibers, which contribute to tougher meat (Rana et al., 2014). Traditional meat quality evaluation primarily focuses on proximate composition, color, and microbial safety. However, modern consumers demand insights into functional properties, secondary metabolites, and enzymatic activities that influence meat texture, post-mortem proteolysis, and overall palatability. Recent advancements in molecular biology and histological techniques allow for a deeper understanding of muscle structure, fiber type distribution, collagen integrity, and gene expression patterns that regulate meat tenderness (Alam et al., 2024).

Histomorphology of muscle fibers

Muscle microstructure can show the quality of the meat. Muscle microstructure features, which are analyzed about meat quality, include the number and diameter of muscle fibers, sarcomere length, collagen content, and muscle fiber type composition (Campbell and Devies, 2012). Muscle microstructure correlates with the proteins involved in the formation of muscle cells (myofibrils). One of these proteins is myostatin. Myostatin is a member of transforming growth factor-β (TGF-β), which is encoded by the myostatin gene (MSTN), which functions as the main regulator of the muscle-building process (myogenesis) and skeletal muscle growth(Ono Y and Sorimachi H,2012).Overexpression of myostatin causes a decrease in muscle mass, muscle fiber area, and muscle protein (Sorimachi H et al.,2011).Muscle fiber is the primary unit of muscle and muscle fiber types are important factor affecting the quality of meat. The chemical and physical makeup of muscle fibers, including their quantity, propensity for hypertrophy, conversion of different types of fibers, and intramuscular fat deposition are important factors that influence meat production (Zeng and Du, 2023). Several types of muscle fibers make up skeletal muscle and there is a strong correlation between the features of muscle fibers and beef quality. An important determinant of muscle fiber type in skeletal muscle is protein (Feng et al., 2020). One technique for determining the specific ingredients of meat products such as the level of autolysis in muscle fiber is histological testing (Mkrtchyan et al., 2020). A quantitative assessment of the fiber number, average fiber diameter, and fiber cross-sectional area is the goal of the histomorphometry analysis (Assunção et al., 2023). Immunohistochemical staining was used to identify the types of muscle fibers, while hematoxylin and eosin staining was used to evaluate the morphological characteristics of the muscle fibers (Weng et al., 2022). Using immunohistochemical techniques and an electrophoretic separation method, four fiber types (I, IIA, IIB, and IIX) can be categorized based on the identification of myosin heavy chain isoforms and lower tenderness (Lang et al., 2020). Meat produced by muscles that include lots of type II fibers, especially IIB produce meat with a low level of tenderness (Listrat et al., 2020). Small mammals frequently have type IIB and IIX skeletal muscles, whereas larger species- including humans, cattle, goats, and horses have nearly no skeletal muscle in any of these regions (Purslow, 2023). The type of muscle fiber has an opposite effect on the background and rate of tenderization. Smaller diameter fibers are particularly preferred since they improve meat quality and are thought to be a sign of its tenderness (Kasprzyk and Bogucka, 2020). Through genetic engineering or breeding management modification, the composition of muscle fiber types can be engineered to produce more optimal meat production with preferred tenderness (Sofyan et al., 2021). A better understanding of muscle characteristics is important to produce meat with maximum quantity and quality. Muscle mass can be maximized through the number and size of muscle fibers, as well as muscle fiber transformation.

Gene expression related to tenderness in primal cut

The biochemical postmortem processes are key steps in meat tenderization (Herrera-Mendez CH et al.,2006). Tenderization is unanimously regarded as an enzymatic process of proteolytic systems. Many authors suggest that calpains are the only proteases responsible for meat tenderization (Koohmaraie and Geesink, 2006). So, it is necessary to highlight the importance of the use of molecular approaches to unravel the mechanisms behind the variations in meat tenderization. Various methods can be employed to enhance the genetic quality of beef cattle. Those are in chronological order, phenotypic selection (not useful for meat quality traits), selective breeding (heritability of traits related to beef quality varies greatly depending on breed, trait, and conditions), and indirect selection (possibly useful) through NIRS predictions, etc (Bittante, 2023). Quantitative polymerase chain reaction (qPCR) is a widely used method to assess target gene expression in a variety of samples including tissues, blood, and cultured cells from humans, animals, plants, and microorganisms (Kuang et al., 2018). Key genes in cattle have been shown to either directly or indirectly impact meat quality (Pecina and Ivankovic, 2021) and the most significant are *CAPN1* and *CAST*, the calpain and calpastatin genes that regulate tenderness, *MSTN*, the myostatin gene, sometimes known as the "double-muscle gene. CAPN1, CAPN2, CAST genes showed significantly different expression for tough and tender meat (Frezarim et al., 2022). The enzyme calpain, a calcium-dependent cysteine protease that breaks down muscle proteins during the process of meat tenderization following slaughter, is encoded by the CAPN1 gene and the calpastatin protein, which is an endogenous inhibitor of μ-calpain and other calpain enzymes, is encoded by *CAST*. Calpain-1 is highly associated with meat tenderness out of the fifteen members of the calpain family (wright et al., 2018). Calpain-1 was mostly active during the first 14 days while Calpain-2 improved the tenderness of the beef after 14 days of aging (Colle and Doumit, 2017). CAPN1 can function at levels of 10−40 μM calcium ions, but CAPN2 requires 1.2 mM Ca2+ for activation, which is accountable for myofibrillar protein degradation (Węglarz et al., 2020). Calpains *(CAPNs)* and calpastatin *(CAST)* play a variety of roles in physiological processes such as muscle growth, differentiation, postmortem proteolysis and the tenderization of meat in domestic animals. This allows it to modulate the degree of meat tenderization and protein degradation (Tian et al., 2024). All cuts of meat from *MSTN*-null cattle are softer than meat from other cattle, even tougher portions like round and chuck. This is probably because more muscle fiber hyperplasia rather than just hypertrophy is present in the meat (Aiello et al., 2018). By deactivating, the *MSTN* gene can significantly boost beef cattle's muscle yields and tenderness. Primal cut yields which are known to be genetically connected with carcass merit and premium prices have been recommended as a way to describe the quality and quantity of meat in beef cattle and therefore, knowledge of genetic parameters is necessary to determine the weight of primal cuts which may be utilized as the selection criteria for designing future breeding programs (Naserkheil et al., 2021). Various preservation techniques, such as refrigeration, freezing, and irradiation, have been explored to maintain meat quality, safety, and shelf life, yet their effects on muscle fiber integrity and gene expression require further investigation (Rahman et al., 2023). Understanding the histomorphology and gene expression patterns in primal cuts is critical for evaluating meat quality traits such as tenderness, marbling, and muscle fiber composition. By investigating muscle structure and the expression of key genes associated with muscle growth, fat deposition, and extracellular matrix remodeling, this research aims to enhance knowledge on meat quality determinants, particularly in sustainable and organic meat production systems (Hossain et al., 2021; Hashem et al., 2006).

Conclusion

This review article has been designed in three main sections related to: tenderness, histomorphology of muscle fibers and expression of different genes in beef primal cuts. The information will be allowed consumers to make healthful food choices, creating diets aimed at trying to correct deficiencies and proving objective data differently priced cuts of beef. In the livestock business and consumer markets, meat tenderness in cattle has a substantial socio-economic impact on a range of sectors. By producing offspring with improved meat quality attributes, breeding efforts that work to improve the tenderness of the meat in cattle can pay off in the long run. Higher economic rewards for producers can result from cattle with meat that is tenderer. Because tender meat is in high demand, producers can increase profitability by getting more value out of their livestock. The market needs and consumer desire for cattle with tender meat from diverse primal cuts drive genetic improvement projects, export competitiveness, and producer profitability. Investing in methods to improve meat tenderness can have a major positive economic impact as well as support the growth and sustainability of the meat and livestock industries as a whole.

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