Exploring Factors that Contribute to Beef Tenderness

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Introduction

Consumers consistently rank beef tenderness among the most important factors that determine eating satisfaction. Although industry efforts have improved the consistency and tenderness of beef, Brahman and other *Bos indicus* breeds have garnered a reputation for lower marbling, tougher beef, and greater variation in eating quality. This reputation puts *Bos indicus* cattle and their crosses at a distinct marketing disadvantage: value based marketing systems reward higher marbling, and many U.S. branded beef programs have restrictions on hump height. Yet, consumers are willing to pay $1-2 per pound premium for a product they know will be tender (Igo et al., 2013; Platter et al., 2013). Thus, there is considerable economic incentive to continue improving tenderness and consistency, particularly for *Bos indicus* influenced beef.

Beef tenderness is a function of connective tissue (Purslow, 2014), marbling or intramuscular fat (Platter et al., 2003), and postmortem protein degradation (Huff-Lonergan et al., 2010). Age of animal, as well as location of the meat cut, explain a large proportion of connective tissue-related differences in beef tenderness. In contrast, when considering the same cut from carcasses within an age (maturity) group, marbling and protein degradation are key contributors to variation in eating quality. While marbling remains a challenge in *Bos indicus* influenced beef, toughness is largely attributed to altered activity of protein degradation systems during meat aging.

The primary factor governing protein breakdown in postmortem muscle is the calpain-calpastatin system (Koohmaraie, 1992; Geesink et al., 2006). Calpain cuts proteins into fragments, which disrupts the structure and integrity of muscle cells, and contributes to tenderization of beef. Calpastatin specifically inhibits the degradative action of calpain. *Bos indicus* cattle are well-documented to possess elevated calpastatin content in muscle, which inhibits protein degradation and results in tougher beef (Wheeler et al., 1990; Whipple et al., 1990b; Pringle et al., 1997).

Why is calpastatin higher in muscle from *Bos indicus* cattle?

While elevated calpastatin in *Bos indicus* is a well-documented phenomena, the regulation of its content in muscle is poorly understood. Calpastatin is present in muscle throughout an animal's life, and calpastatin content is established in muscle before the animal is slaughtered. Thus, there is likely an important physiological basis for differences in calpastatin among breed types, and it is logical to link this with heat tolerance. Thermotolerance may result from reduced heat production, increased capacity for heat loss, or a combination. While slick hair and sweat gland properties improve heat dissipation in *Bos indicus* cattle, there is also evidence to support that metabolism is altered to reduce heat production. While limiting metabolism is beneficial for thermoregulation, this would also be expected to negatively impact production parameters, such as milk production or muscle growth.

Muscle growth is an energetically demanding process that contributes to metabolic heat production. In order to increase muscle mass, proteins must be generated as well as degraded, which is also referred to as protein turnover. The net balance of synthesis and degradation dictates the gain in muscle mass. In living muscle, the calpain-calpastatin system, along with several other systems, contribute to protein degradation. While calpain affects numerous proteins, calpastatin is the only known inhibitor for calpain (Goll et al., 2003). In this manner, calpastatin alone has broad capacity for inhibiting protein degradation. Thus, greater calpastatin content observed in Brahman and *Bos indicus* breeds may be a mechanism for restricting protein turnover in the live animal, in order to restrain metabolic rate and heat production.

Postmortem protein degradation and beef tenderness

In living muscle, calpain and calpastatin action are tightly regulated to have precise control over protein degradation. While the content of calpain and calpastatin is important in dictating the breakdown of muscle, the function or activity is also significant. These mechanisms are studied intensively in living muscle with regard to agricultural production and human health, but understanding regulation of activity in the changing environment of postmortem muscle is quite complex.

At slaughter, muscle does not immediately become meat; rather, a number of changes occur during the "conversion of muscle to meat." The physical, biochemical, and energetic changes that ensue in muscle after slaughter are critical for determining the development of meat quality attributes. For instance, the loss of oxygen supply leads to a shift in metabolism and a gradual depletion of available energy resources. In turn, the loss of energy results in rigor, or the stiffness of death. Meanwhile, the pH of muscle declines from approximately 7.4 to 5.6, and carcasses slowly cool from body temperature (101°F) to <40°F at 24 hours postmortem. The rate and extent by which these changes occur influence development of beef tenderness.

Although refrigerated storage of meat for several weeks after slaughter (aging) improves beef tenderness, the majority of tenderization caused by protein degradation occurs in the first 24-72 hours postmortem. During this time, changes in pH, temperature, and calcium, affect calpain activity. In particular, increases in calcium within muscle cells trigger activation of calpain. Once activated, calpain begins breaking down structural and contractile proteins within muscle cells. Calpain also clips calpastatin; these calpastatin fragments retain inhibitory activity, which declines during the subsequent aging period. Classically, the ratio of calpain: calpastatin activity is considered a predictor of tenderness, and this ratio is also generally less favorable in *Bos indicus* cattle.

Are other muscle attributes different in *Bos indicus* influenced cattle?

Considering muscle represents the largest proportion of the body on a weight basis, shifting muscle characteristics could be important to regulating overall body metabolism and heat tolerance. Thus, it raises the question: what else might be different in *Bos indicus* influenced muscle? Muscle is a heterogeneous tissue, and the properties of individual cells vary in order meet specific functional demands. Muscle fibers (cells) are classified according to their contractile and metabolic properties, which is dictated by functional demands. There is an association between fiber type characteristics and meat tenderness. Further, certain fiber types tend to have greater calpastatin content, which is linked to growth rate and function of specific muscles within the animal. However, when comparing the same muscle, the type of contractile proteins expressed in *Bos taurus* and *Bos indicus* is not significantly different (Seideman, 1985; Whipple et al., 1990a; Wright, 2016), and thus likely does not explain variation in either calpastatin content or tenderness.

Curiously, metabolic characteristics and regulation of metabolism may be important for the adaptability of *Bos indicus* muscle. Recent evidence supports that mitochondria content may be greater in Brahman (Wright, 2016), and functional aspects of mitochondria may also differ. Considering mitochondria are the energy "powerhouses" of the cell, this has potential ramifications for metabolic heat production and thermotolerance, as well as postmortem tenderization. From a postmortem perspective, there is a couple possibilities for how mitochondria may influence tenderization. First, mitochondria can sequester calcium; in turn, this could prevent increases in calcium within the cell, thereby delaying the activation of calpain. Moreover, mitochondria participate in pathways that initiate cell death, which are expected to hasten postmortem metabolism and instigate protein breakdown. The expectation is that Brahman muscle may be more resistant to cell death. In general, these ideas reflect that *Bos indicus* muscle possesses additional mechanisms to maintain or protect the cell in the face of adverse physiological circumstances.

Conclusions

Beef tenderness is a complex trait influenced by inherent muscle properties, as well as the conditions that exist in muscle after slaughter and processing. It is well-established that calpastatin content is generally greater in *Bos indicus* influenced cattle. However, the physiological basis for elevated calpastatin and its significance to heat tolerance and muscle growth is poorly understood. While calpastatin clearly plays a role in tenderization, other differences in muscle metabolism exist in *Bos indicus* muscle, and these also are likely to contribute to impact protein degradation and tenderization. Ultimately, it is important to understand the relationships between muscle characteristics and heat tolerance, in order to develop strategies that optimize growth and tenderness without sacrificing thermoregulatory capacity in *Bos indicus* breeds.

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