Chapter

Effects of Pre-Slaughter Stress on Meat Characteristics and Consumer Experience

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Abstract

The current concern regarding how animals are raised, which kind of feedstuffs were fed, and the management activities employed in the livestock segment system is increasing, primarily due to the public and/or customer opinion. Therefore, a positive pressure is being placed in the industry/production to be more effective in communicating these processes and to explain what indeed occurs during the animal's productive life, from birth to slaughter. Hence, it is imperative to explain what type of situations animals face during their productive lives and how these might impact productive, health, and the quality of the final product sold at the supermarket. Additionally, it is important to understand that technologies have been developed that could mitigate some of these stress-related losses (health and productive), as well as to improve meat quality traits and overall customer eating experience.

Keywords: cattle, customer experience, DFD, nutrition, pre-slaughter stress

1. Introduction

During daily management activities, beef and dairy animals are exposed to several situations that may trigger a stress-induced inflammatory response. This response, in turn, might greatly impact health, performance, and well-being of the herd, which affects the overall profitability of livestock operations. Therefore, it is paramount to understand the mechanisms underlying the occurrence of stress and how we can use technologies to alleviate the negative effects of this response for the herd. Hence, the objective of this review is to provide an overview on stress physiology, immune system, and the interaction among these, as well as its effects on meat characteristics of beef animals and consumer acceptability implications on edible products.

2. Stress definition & physiology

The term stress is classically defined as the reaction of an animal to factors that potentially influence its homeostasis, whereas animals that are unable to cope with these factors are classified as stressed [1]. As aforementioned, ruminants are inevitably exposed to several management situations that expose them to the occurrence of stress, such as vaccination, weaning, transport among farms, transport to the stockyard, feedlot, and slaughter, novel environments, management, restriction of water and feed, among others [2]. These situations, also known as stressors, can be categorized as:

Psychological stressors: Include weaning, arrival at a novel environment, commingling.

Physical stressors: Include castration, bruises resulting from fights within a feedlot pen and/or other animals mounting, vaccination abscesses, dehorning.

Physiological stressors: Include endocrine and metabolic alterations resulting from psychological and physical stressors.

Typically, animals are exposed to various stressors throughout the production cycle that elicit a stress response, which may not involve a viral/bacterial/fungi/protozoa pathogen. Nonetheless, it is important to note that the stress may increase the susceptibility of the animal to an individual or group of pathogen(s). Therefore, it is worth mentioning that these pathogens, in turn, might be already living in the organism of the animal, but after the beginning of a stress-induced inflammatory response, the pathogen(s) is(are) able to act and trigger its effects. Although indispensable and needed for the resumption of homeostasis, stress-induced response, and its upcoming inflammatory cascade may be unnecessary and have negative effects on performance and health of the herd.

From a physiological standpoint, when an animal perceives a stressor, the immediate response is the activation of the hypothalamic–pituitary–adrenal (HPA) axis [3], characterized by the synthesis and release of corticotropin-releasing hormone (CRH) and vasopressin (VP) by their respective neurons located in the hypothalamus into the paraventricular nucleus [2]. In cattle, CRH has more potent stimulatory actions than VP [4] in a manner that upon its binding to membrane receptors in the pituitary gland, protein kinase-A is activated and 3′,5′ cyclic AMP (cAMP) is produced, leading to a calcium influx that will activate the release of adrenocorticotropic hormone (ACTH) by the pituitary gland [5]. Within the anterior pituitary gland, corticotrophs are responsible for the production of ACTH and its main function is to promote cholesterol uptake, as well as synthesis and release of steroids by the adrenal gland [2].

The adrenal gland is divided into the cortex and medulla, whereas the cortex is responsible for the synthesis and release of 3 hormone types: mineralocorticoids, androgens, and glucocorticoids. In humans and most mammalian species, cortisol is the primary glucocorticoid [2] and generally classified as the "stress hormone". In the metabolism, cortisol elicits several important functions, such as (i) breakdown of glycogen, muscle, and adipose tissues as a mechanism to provide energy to the host during an immunological challenge, (ii) hepatic production of acute-phase proteins (APP), (iii) regulation of stress response, (iv) greater synthesis and release of catecholamines, and (v) suppression of the inflammatory and immune systems to prevent autoimmune disorders. Hence, in situations where chronic diseases or stress are observed, cortisol remains elevated for a prolonged period of time and promotes an anti-inflammatory and immunosuppressive response by decreasing the synthesis and release of pro-inflammatory cytokines [6, 7]. Conversely, acute increases in cortisol concentrations have been reported to trigger a transient and temporary inflammatory response. In ruminants, greater cortisol concentrations have been associated with reduced growth rates and reproductive performance in beef females [8–10].

3. How does the immune system react to the stress?

Before entering into the specifics of the link between immunity and stress, it is worth mentioning how the immune system is structured and its main features.

The immune system is divided into innate and acquired immunity. The acquired immunity is responsible for adapting and building an immune response for each antigen the body encounters, characterized by the production of antibodies and the development of immunological memory [11]. Therefore, it does not come as a surprise that specificity is one of the main features of this branch of the immune system. On the other hand, the innate immunity is characterized by a lack of specificity, given that a similar response is observed when caused by a bacteria, protozoa, fungi, virus, or stress [2]. The barriers created by the body include (i) physical: skin, tears, and mucosal secretions, (ii) chemical: antimicrobial peptides, superoxide anion, and nitric oxide, and (iii) complement system [11]. The primary goal of the innate immune system is to provide enough time for the acquired immunity to develop a strong and effective response against any specific pathogen. The group of cells that comprise the innate immune system includes phagocytic cells (neutrophils, monocytes, macrophages, and dendritic cells), natural killer cells, and cells that release inflammatory mediators, such as mast cells, basophils, and eosinophils.

The innate immunity recognizes certain structures present in different microorganisms, known as pathogen-associated molecular patterns (PAMPs). This is the reason that lipopolysaccharide, a component of the cell wall of gram-negative bacteria, causes an immune reaction in several species, leading to an increase in cortisol concentrations and an acute-phase reaction in cattle [12]. When PAMPs interact with toll-like receptors (TLR) inside or on the surface of phagocytic cells, a cytokine response is initiated in neutrophils and macrophages by the activation of a transcription factor called nuclear factor kappa beta (NFκB), which induces the expression of genes part of the innate immunity, such as cytokines, chemokines, and co-stimulatory molecules [11].

Cytokines are chemical messengers released by phagocytic cells during an immune response and act as mediators of intermediary metabolism in immune-challenged animals [13]. The major pro-inflammatory cytokines released by cells and recognized as important players in an immune response include interleukin-6 (IL-6), IL-1 and tumor necrosis factor-α (TNF-α) [14], which their plasmatic concentrations increase following an acute bacterial challenge (LPS) [12]. Following the injury caused by a pathogenic challenge, the body builds a specific and complex set of reactions aiming to destroy the infectious pathogen, to prevent further tissue damage, and to restore homeostasis. These early and immediate set of responses are called 'inflammatory responses' and are part of the acute-phase response (APR) [15]. Tissue macrophages and blood monocytes are the major cell components involved in the APR and after activation, these cells release IL-1 and TNF-α in the circulation [16]. During an APR, several metabolic changes are observed, but the main physiological responses are increased body temperature (febrile response) and alterations in liver metabolism. These responses are primarily regulated by the aforementioned pro-inflammatory cytokines (IL-1, IL-6, and TNF-α).

Febrile response: a defense mechanism developed to control replication, growth, and kill different pathogens by preventing the formation of bacterial coats. It is mainly induced when the eicosanoid prostaglandin-E2 (PGE2) is produced from 20 carbon omega-6-derived fatty acids. Hence, it is imperative to mention that this response may be modulated by the fatty acid profile in the diets of the animals. In other words, diets containing a greater concentration of omega-6 fatty acids (i.e., linoleic and its derivatives) will lead to a greater pro-inflammatory state, whereas diets containing greater amounts of omega-3 fatty acids (i.e., linolenic and its derivatives) will promote an anti-inflammatory response [17, 18]. Although imperative for controlling an infectious challenge, the increase in body temperature does not come without a significant effect on nutrient requirements of the animals. More specifically, for every 1 °C increase in body temperature associated with an immune response, it is estimated that energy requirements are increased by 10–13% [19].

Hepatic metabolism: under homeostasis, hepatocytes produce several acute-phase proteins (APP) at a relatively steady state, but this manufacturing state dramatically changes when the animal is immunologically challenged. Noteworthy is the fact that not only the amount of APP is significantly changed during an immune response, but also the APP profile is changed, given that some increase and other decrease following an immune challenge [20]. The increase in liver metabolism also leads to dramatic increases in metabolizable protein (MP) requirements in ruminants. Moriel et al. (2015) demonstrated that increasing MP supply from 85 to 115% of daily requirements to newly-weaned beef calves improved performance during a 42-day preconditioning period, without effects on messenger RNA expression of hepatic genes involved in the production of two of the major APP, haptoglobin and ceruloplasmin [21]. Moreover, some APP peak between 1–4 days post-challenge, reducing dry matter intake (DMI) and animal performance, as well as increasing the incidence of morbidity and antimicrobial treatment [22–24]. In ruminants, haptoglobin, ceruloplasmin, serum amyloid-A, and fibrinogen have been the most studied APP [25], with significant attention given to haptoglobin and its effects when evaluating an APR. Haptoglobin has been the most reliable and consistent APP because it is almost undetectable in healthy animals, whereas a significant increase is observed after a disease, injury, or stress response [23, 26, 27].

Nonetheless, PAMP recognition may not apply in stress situations as no pathogen is directly involved at the beginning of the stress-induced inflammatory response and the cortisol is responsible for triggering such response and tissue mobilization [27, 28]. Additionally, in a stressful situation, damage-associated molecular patterns (DAMPs) are host biomolecules that can initiate and perpetuate a non-infectious inflammatory response [29]. Several DAMPs are nuclear or cytosolic proteins with a defined intracellular function that, when released outside the cell after injury and/or mobilization, move from a reducing to an oxidizing milieu resulting in their functional denaturation [30]. Examples of these molecules include DNA, RNA, mono- and polysaccharides, purine metabolites (ATP, ADP, and uric acid), as well as S-100 proteins [31–33]. In a research effort to evaluate the mechanisms underlying the effects of stress on inflammation, Cooke and Bohnert (2011) developed a neuroendocrine stress model using CRH as a non-pathogenic stressor [27]. These authors reported that cortisol peaked 1 h after CRH infusion at a dose of 0.1 μg/kg body weight (BW) followed by increases in plasma concentrations of IL-6 and haptoglobin, demonstrating stress also activates the innate immune response in animals without the presence of a specific pathogen.

4. Effects of stress on health and performance

In beef cattle, weaning is considered one of the most stressful events in the entire life of the animals. In order to evaluate this response, Arthington and colleagues (2005) evaluated the effects of early vs. traditional weaning on stress markers and growth performance of beef animals [34]. These authors reported that at the time traditional weaning occurred (approximately 300 days of age), early-weaned were lighter than traditionally-weaned cohorts (48 kg), whereas ADG (days 0–28 post-weaning = 0.87 vs. 0.40 kg/day; days 29–112 post-weaning = 1.38 vs. 1.18 kg/day) and feed efficiency (days 0–28 post-weaning = 157 vs. 81 g/kg; days 29–112 post-weaning = 159 vs. 136 g/kg) were greater for early-weaned calves. From traditional weaning until slaughter, early-weaned animals gained roughly 30 kg more BW and had greater feed efficiency than traditionally-weaned calves. These results can be explained by the relative steady concentrations of haptoglobin and ceruloplasmin in early-weaned calves at the moment traditional weaning occurred and a lessened APR observed in early-weaned steers receiving a pathogenic challenge, suggesting that the animal might be less susceptible to developing any kind of disease following a period in which its immune system might be suppressed upon facing a stressful situation.

Another set of stressful events is transport and feedlot entry, which may occur together or in a short period of time. During transport, animals remain without feed and water for a significant period of time, as no rest stops are adopted in traditional beef-producing countries. In an effort to evaluate the metabolic effects that feed and water restriction cause in the ruminant, Marques and colleagues (2012) reported a similar loss in performance, as well as similar concentrations of non-esterified fatty acids (NEFA) and APR of nutrient-restricted vs. transported animals, demonstrating that feed and water deprivation are major contributors to tissue mobilization, APR, and reduced receiving feedlot performance in animals enrolled to long-distance transport [35]. In a subsequent study, the same research group [36] demonstrated that 24-hours feed restriction was considered the major factor causing a neuroendocrine response, explained by the greater cortisol, NEFA, and ceruloplasmin concentrations compared to animals water- and feed + water-restricted [36].

Based on these results, one could speculate that providing water and feed to cattle during the transport to the feedyard would alleviate the stress-induced inflammatory response of the herd. Cooke and colleagues (2013) designed a study to evaluate whether 2-hours rest stops every 8 hours of transport with full access to feed and water would benefit health and, consequently, improve performance of beef animals during feedlot receiving [37]. In partial agreement with the hypothesis, rest stops for feed and water consumption did reduce plasma cortisol, NEFA and haptoglobin concentrations, but did not improve feed intake and performance when compared to animals continuously transported for 24 hours. These results might be explained by the fact that (i) 2 hours rest stops were not enough to improve performance and (ii) unloading and loading the animals during rest stops also cause a stress response, similarly impairing performance of the herd.

Overall, these stressful events are faced by most, if not all, ruminants during their productive lives. Depending on production system and management, these events are faced more than once in their lifetime and which might negatively impact the overall productivity in beef cattle system.

5. Pre-slaughter stress

The stress animals face prior to slaughter has been under public scrutiny for a long period of time in the U.S. and other parts of the world. Public opinion has been forcing the industry to adopt good management and transport practices, demanding information on the origin of the food and how the animals were raised throughout their productive lives, with a special focus on animal welfare [38]. Therefore, it is imperative to understand the factor(s) that may cause a stress-induced response in finishing animals and how these situations impact the quality of the edible product (i.e., meat) and customer acceptability.

Several stressful situations are faced by an animal from the feedlot until slaughter, including management in the working chute, truck loading at the feedlot [37], transport itself plus feed and water deprivation [35, 36], truck unloading at the slaughter plant, weather conditions, novel environment and management, as well as feed deprivation until slaughter [39]. These situations, which may occur together or in a short period of time, also trigger a stress-induced inflammatory response that, ultimately, can impact carcass characteristics and, consequently, visual and qualitative aspects of the meat being offered in the market [40]. More specifically, animals exposed to a greater number and/or magnitude of physical and psychological stressors prior to slaughter are more prone to produce the dark, firm, and dry (DFD) carcass and this process will be covered herein.

All pre-slaughter stressors can, inevitably, alter meat quality and customer acceptance, particularly due to an increase in meat pH and changes in meat tenderness and color [41]. Meat pH is a result of the amount of pre-slaughter glycogen levels present in the muscles, which, in turn, depends greatly on the factors responsible for physical and psychological stress [42]. Exposure to stressors before slaughter results in muscle energy reduction, depletion of glycogen and changes in important meat physical and chemical attributes, such as pH, softness, and color [43]. Meat pH values greater than 6.0 at 12 to 48 h post-mortem results in DFD cuts that are more susceptible to microbial contamination and a shorter shelf-life. Other authors have also considered 5.80 as the threshold for the determination of DFD [44, 45]. High meat pH will lead to a product not appreciated and accepted by customers, as DFD cuts are dark-red to brown-black and have a dry, firm, and sticky consistency [46]. Meat traits that have greater influence on consumer satisfaction are tenderness, juiciness, and flavor of the cooked meat, all of which are severely impacted in DFD meat cuts [47].

Evaluating pre-slaughter stress and its effects on carcass characteristics, Carrasco-Garcia and colleagues (2020) reported that 80% of the carcasses analyzed had a pH greater than 5.80 [45]. The measurement of pH is one of the most important quality traits as it is related to depletion of muscle glycogen reserves [45]. After animal death by exsanguination, the muscle tissue becomes anoxic, triggering the anaerobic glycolysis cascade. High levels of stress hormones before or during slaughter decrease muscular glycogen reserves, as glycogen is hydrolyzed to lactic acid. In fact, higher pre-slaughter lactate concentrations are associated with a reduced consumer eating quality score [48]. Therefore, it is paramount that meat pH decreases from approximately 7.0 to 5.5 in order to avoid bacterial growth in the edible product [49]. As cortisol is one of the key players controlling the stress response and meat acidification, it would be reasonable to evaluate its concentration prior to slaughter. In the same study, Carrasco-Garcia et al. (2020) demonstrated that 67% of sampled animals had blood cortisol level greater than 45 ng/mL, which is indicative of excessive stressful conditions prior to slaughter, but no association has been observed among pH and blood cortisol [45].

Alterations in meat color are one of the most pronounced effects of high meat pH and, consequently, DFD occurrence. Carcasses with pH greater than 6.0 are usually classified as darker, less red, and less yellow [50]. During DFD episodes, a muscle absorbs light and meat becomes darker, which can be attributed to a lower amount of free water at its surface and lower oxygenation of myoglobin [51]. Conversely to blood cortisol levels and meat pH, Carrasco-Garcia and colleagues (2020) reported a positive association among meat pH and colorimetric parameters of crossbred beef animals [45]. Color is the most important sensory attribute that influences customer purchasing decisions, as they associate a bright cherry red color with freshness and quality, whereas any deviation from this parameter is perceived as poorer quality. Hence, a darker meat would be less acceptable by the customers and, consequently, cause substantial economical losses to the meat industry worldwide. In the early 2000s, it was estimated that the Australian beef industry lost roughly 38.5 million dollars due to DFD, resulting in an average loss of $ 90/carcass. Nonetheless, these numbers might be even greater in other regions, depending on cattle breeds and production settings in which animals are reared for fattening.

In agreement with the carcass-reduced quality cascade, meat tenderness is often observed in carcasses with greater pH and reduced lightness [52]. The mechanisms underlying a greater shear force in DFD cuts might be related to a reduced sarcomere length [53], which is recognized as an important cause of increased toughness in meat and its length increases as pH decreases below 6.2 [54]. The sarcoplasmic reticulum is a membranous cellular organelle responsible for regulating the amount of calcium ions in the sarcoplasm of the muscle fiber [55]. After slaughter, calcium concentration in the sarcoplasm increases due to loss of the calcium-accumulating ability of the sarcoplasm reticulum [56] accompanied by a gradual leakage of calcium ions into the sarcoplasm. Calcium is paramount in meat tenderization and the calpain system requires the presence of calcium to be activated [57]. Hence, relevant changes in sarcomere length begin to occur early postmortem when sarcomeres contract as the muscle goes into rigor. If calcium is released when ATP is still available, muscle contraction occurs, resulting in shorter sarcomeres and detrimental effects on meat tenderness [55]. Hence, it is possible that the amount of stress during pre-slaughter might affect calcium release and sarcomere length, resulting in a tougher meat.

Stress itself is not the only factor predisposing DFD occurrence in ruminants, in a manner that sex, breed (*Bos taurus* vs. *Bos indicus*), nutrition, animal category (bull vs. steer), temperament, and age are other important factors. In Mexico, Loredo-Osti and colleagues (2018) reported that 13.5% of the *Bos indicus*-crossbred carcasses processed by a slaughter plant were classified as DFD [58], whereas this value approached 2% in a recent survey conducted in the U.S. [59], but no data have been reported for Brazil, where zebu breeds prevail. Although no data have been reported, *Bos indicus* breeds are well-known as more temperamental than *Bos taurus* breeds [60, 61]. Hence, these animals have a heightened stress and APR response, which leads us to speculate that DFD occurrence would be greater in *B. indicus* vs. *B. taurus* herds. Besides variation among breeds, within breeds variation on temperament might also impact the stress response and carcass traits. Francisco and colleagues (2015) demonstrated that more temperamental *Bos indicus* animals had a greater carcass bruise count, reduced color index, fat content, and marbling score [62].

In summary, DFD cuts can be recognized by the customers as:

Colorimetric aspects: dark-red to brown-black

Consistency: dry, firm, and sticky

Greater tenderness and reduced juiciness

Reduced visual acceptability, desire to purchase, and customer eating experience

Hence, it is imperative to develop strategies that reduce the magnitude of a stress response and, consequently, improve carcass quality and customer sensory eating quality score. Loudon and colleagues (2019) suggested that 2-weeks resting periods following social/group mixing as an alternative to improve customer eating quality [48]. Recently, Cappellozza and colleagues (2020) reported that the administration of bovine appeasing substance (BAS; 5 mL/head) immediately at loading to slaughter reduced mean carcass pH (5.82 vs. 5.75) and the proportion of carcass classified as having post-mortem pH greater than 5.80 (42.2. vs. 26.2%) [63]. The use of appeasing pheromones has been initially discovered in swine and shown to reduce the agonistic behavior of piglets [64, 65]. Pheromones are species-specific semiochemicals compounds released from one specific individual to induce both a behavioral and physiological response in a conspecific [66]. In cattle, BAS is based on a mixture of fatty acids, reproducing the composition of the natural substance produced by the dam during the parturition [67]. Therefore, BAS is expected to have calming effects that, in turn, will improve health and performance of the herd. As an example, Cooke et al. (2020) demonstrated that BAS administration at weaning reduce mean plasma haptoglobin concentration 15 days post-weaning and improved 45-day post-weaning performance (+ 70 grams/day) [68]. Moreover, Cappellozza and colleagues (2020) reported a similar improvement in performance of pure *Bos indicus* newly-weaned grazing beef animals during a 45-day post-weaning period [63]. The benefits of BAS administration seem to rely on transient temperamental changes [69], stress-induced inflammatory response [68, 69], reduced disease susceptibility due to an increased vaccine efficacy [69], recovery from a pathogen challenge [70], and a subsequent improvement in feed efficiency [71] and BW change and gain [72].

6. DFD cuts and human health

As aforementioned, DFD cuts are undesirable because they are esthetically unpleasant and are more susceptible to microbial growth [73], but the eating quality of these cuts were less defined in beef cuts. As an example, until 1997, the United States Standards for Grades of Carcass Beef stated that "there is little or no evidence which indicates that the dark-cutting condition has any adverse effect on palatability..." [74], contradicting what has been reported for pig cuts and similar conditions of carcass issues [75, 76]. In order to address this matter, Wulf and colleagues evaluated the effects of DFD cuts on carcass traits and beef palatability [77].

These authors demonstrated that several carcass quality attributes were significantly affected in DFD cuts, such as backfat thickness (31% less), USDA quality grade (9.5% less), intramuscular fat (29% less), pH (11% greater), and lower colorimetric readings [77]. Moreover, cooked beef palatability was substantially lower for DFD vs. normal carcasses, whereas tenderness variation and shear force were greater [77]. In the sensory panel, DFD carcasses produced a greater percentage of "tough" Longissimus steaks and a reduced percentage of "very tender" steaks vs. normal carcasses [77]. Additionally, off-flavors classifications were more frequent observed in panelists analyzing DFD vs. normal cuts.

To the best of our knowledge, no other study evaluated chemical composition of DFD vs. normal cuts. It is noteworthy mention that visual parameters are the major driver of a customer's desire to buy a piece of meat and, therefore, these cuts are well desired in the shelf of a store. Nonetheless, it can also be assumed that no harm is observed after a consumption of DFD cuts, given that human sensorial analysis have been reported [77].

7. Conclusions

Stressful situations are often faced by ruminants during their entire productive lives, from birth until slaughter. These situations predispose animals to health and performance losses which, ultimately might impact carcass quality and customer eating experience. Therefore, additional strategies and/or technologies must be developed to reduce the losses caused by stress and improve carcass traits, which will result in greater acceptability of edible products by the food chain and likely reduce the scrutiny of the public opinion regarding animal production.

Conflict of interest

The authors declare no conflict of interest.

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