5-2014

Balking Incidence and Behavioral Responses during Handling in Fed Cattle with Genetic and Carcass Implications

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Animal Science

by

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May 2014
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This dissertation is approved for recommendation to the Graduate Council.

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Abstract

Temperament in cattle is often defined as the reactivity to human handling or novel environments. Temperament differences have been shown among breed-type categories, within breed types, among crossbreds, and between sexes. Temperament tests are typically completed at weaning time on beef cattle, and rarely on fed Holstein steers for beef production. Balking behavior, or cease in forward motion, in the cattle working facility can pose welfare issues as the electric prod use to coerce movement is implemented. Three observational field projects were designed to evaluate balking behavior incidence in unknown breed-types at the processing plant, Holstein steers in the feedlot and processing plant, and Angus and Hereford-Angus crossbred steers over their lifetime. Objectives of the processing plant study were to determine if cattle of certain coat colors or characteristic markings, or sex, had an effect on balking behavior, and if balking behavior had carcass implications. In 6,510 observations at a slaughter plant, Holstein steers balked more ($P < 0.05$) at entry to the restrainer than all other colors, which balked similarly. Heifers balked more ($P < 0.05$) than steers, while mixed pens of heifers and steers balk intermediately. Neither the presence of horns nor *Bos indicus* influence affected balking behavior. The feedlot source affected ($P < 0.05$) balking score, pen weight, and dressing percentage. In the fed Holstein steer project, responses to handling in the feedlot and at the plant showed no association of balking at the plant to individual hot carcass weight; therefore, no negative carcass economic effects. Project three allowed assessment of behavior over time in Angus and Hereford-Angus crossbred steers, and also to determine if genetic polymorphisms affected behavior or carcass weight. In Angus and Hereford-Angus crossbred steers, the Hereford-Angus crossbred steers balked more ($P < 0.05$) than Angus steers, yet Angus steers reacted more ($P < 0.05$) to restraint in the chute than Hereford-Angus steers. Exit velocity did not

differ by breed-type. Genetically, polymorphisms in the heat shock protein 70 gene promoter region of those same steers affected behavioral responses to handling, specifically in balking and behavior in the chute.

Acknowledgements

No project of this magnitude can be undertaken alone. I am honored to have a diverse committee, representing both academia and industry. Their guidance, cooperation, and support of ideas and possibilities early in this process allowed for a broad investigation of novel research. They provided opportunities that allowed me to network, share our research efforts with collaborators, and have experiences with multiple species to learn of farm animal welfare. Their dedication to helping me succeed is humbling. I will always be indebted to each and every one of them. My committee includes:

- Dr. Yvonne Vizzier Thaxton, my co-major professor, who introduced me to the poultry industry in addition to providing this remarkable opportunity
- Dr. A. Hayden Brown, Jr., my co-major professor, who initiated my acceptance into the field of animal science many years ago and has advanced my knowledge and love of cattle
- Dr. Charles F. Rosenkrans, with whom I have enjoyed much learning and discussion
- Dr. Kellye E. Pfalzgraf, who provided the industry perspective and opportunity
- Dr. Karen D. Christensen, who let me trail along during her work with poultry on numerous occasions

One of the most rewarding aspects of this experience is developing friendships with fellow graduate students. I was warmly accepted despite the age disparity. W. Brandon Smith is a true dear friend and colleague. I would welcome working with him again in the future. Dr. Angie Mays and I share an outside interest that blended our professional and personal relationship deeply. My long hours in the study group with Malea Frank, Amanda Davis, and Megan Hancock will be forever remembered and appreciated. Christina Turner guided me with patience in the laboratory, and I so appreciate her intelligence and friendship. Elizabeth Backes ("E.B.") has included me in her projects concerning animal behavior for which I am grateful, and I sure enjoy spending time with her. There are others, too numerous to mention. I am honored to know great people with great minds.

This project required two consistent observers for animal behavioral scoring. I could not have asked for a better "trained observer", co-worker, and long-time friend than Karen Anschutz. Our endless hours of travel and discussion allowed retrospect, scrutiny, and planning for the next phase of the project. Karen's experience and insight greatly improved upon what I could have done alone. Thank you, Karen.

Dedication

This culmination of work is dedicated twofold. My husband, David, has been my best friend much of my life. The first step in my education as a non-traditional student was only possible due to his support. Our now grown children, Katelyn and Cole, have learned independence from my absences, but I hope they know of my endless love for them. My entire family has continuous encouragement that I fall short of deserving. I will spend my remaining time trying to repay them, and I look forward to the opportunity and time to give. Thank you, Mom, Kelly, Jeny, and their children Madison and Spencer. I hope to thank Dad again after passing from this life.

This effort is also dedicated to Dr. Temple Grandin. I wonder if she realizes how her work in animal science has affected so many people and animals, in so many ways. I can only hope, in some small effort, to continue her work. Thank you, Temple Grandin, for your support and suggestions—for your life long work.

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Chapter 1: Introduction

Review of Literature

Humans have been assessing animal behavior since the beginning of the domestication process. Those species of animals that were amenable to being in close proximity to humans, would tolerate some type of enclosed environment, and provided a benefit to humans were selected for various purposes. Price (1984) defined domestication as "a process by which a population of animals becomes adapted to man and the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events recurring during each generation." Domestic food animals provide meat, milk, eggs, leather, wool, pharmaceuticals, and other byproducts. For beef production, cattle breed types have been selected and developed with the ability to be safely handled in various settings.

Through domestication, genetic adaptation has occurred. Artificial selection for desired traits, improved diet, early detection, treatment and prevention of disease, housing, and other technologies have attributed to the efficiency and production of food animals. Research continues to further improve all aspects of animal production. Intense, often confined, mass food animal production requires husbandry practices that are objectionable to some members of society. Animal welfare, including welfare for food animals, has become a societal concern.

The Brambell Report (1965) from England asserted that farm animals can suffer and have needs to perform normal behavior within the environment provided by humans. The report has evolved through the Farm Animal Welfare Council in Great Britain and now lists the five freedoms as follows:

1. Freedom from Hunger and Thirst - by ready access to fresh water and a diet to maintain full health and vigor.
2. Freedom from Discomfort - by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from Pain, Injury or Disease - by prevention or rapid diagnosis and treatment.
4. Freedom to Express Normal Behavior - by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from Fear and Distress - by ensuring conditions and treatment which avoid mental suffering.

Number 4 describes the freedom to express normal behavior. The definition of normal is subjective. In a review of behavior of cattle on pasture by Kilgour (2012), he states that for domesticated cattle, no such wild ancestor exists, so the best that can be done is to study domesticated cattle in environments with little human interference. Regardless of the environment, we must know what behaviors are normal for that location and situation before abnormal can be determined.

This study focuses on observing cattle behavior in a production environment. Assessing behavior is one component in determining animal welfare. In addition to behavior, animal performance, physiology, anatomy, and health are some of the scientific disciplines necessary to assess animal welfare (Gonyou, 1994). A centennial paper by Johnson (2009) concludes with questions that face researchers of animal welfare science: “How are the animals coping, how should we care for them, and how should we house them?” Additionally, what can we do to assure humane handling, as a component of animal welfare, continues to progress throughout the industry, from farm to plate?

The behavioral response of animals to human handling or novel environments provided by humans is often described as temperament (Burrow, 1997). Changes in behavior, such as their fear response to humans or to novel environments has also been defined as temperament (Fordyce et al., 1988). The degree of skittishness, excitability, apprehension, or calmness of an animal are other terminologies that have been used to describe cattle temperament (Stricklin and Kautz-Scanavy, 1984). Animals that are difficult to handle may be those that are aggressive and potentially cause harm to humans, cohorts, or themselves. Equipment and facilities may also suffer damage or destruction by livestock that are trying to escape an enclosure. Those that are deemed ‘temperamental’ show decreased production traits such as growth, fertility, and carcass and meat quality (Burrow and Dillon, 1997; Vann et al., 2008; and Cooke et al., 2012). Animals that are repeatedly difficult to handle may illicit negative human behaviors that can range from mild frustration to abuse. Managing cattle can be dangerous, create conflict among workers, and is often completed by novices only once or twice a year at cow/calf operations. Even with experienced, trained handlers using equipment designed to facilitate safe, humane, and efficient handling, unforeseen problems can happen. Animals have a temperament, as humans have personality, and react to situations differently. In other words, animals are unpredictable at times.

A review by Burrow (1997) of measurements of temperament and relationship to performance traits of beef cattle addresses animal welfare aspects associated with poor temperament. He offers that modification of management and handling practices can reduce stress on farm animals and is a way to improve welfare. Another method to improve welfare is to select against poor welfare in breeding stock (genetics) or by training animals (non-genetic approach). In order to define the temperament of animals, methods to measure behavior have been devised. These methods then categorize temperament, so that selection or training decisions can be made to enable safe, lower-stress handling.

For example, tests have been designed in attempt to score the animals' ease of handling. Managing cattle often requires moving them from the range or pasture into pens, driven into an enclosure, further driven into a narrowed alley with continued forward motion, and into a restraining device (Burrow, 1997).

Many tests to assess temperament have been reported. Burrow (1988) categorized these tests into restrained tests and non-restrained tests. In non-restrained tests, the animals are free to move within a defined testing area either with or without the presence of a human. These tests include the approachability tests (Murphey et al., 1981; Fordyce et al., 1982; Kabuga and Appiah, 1992) and may be referred to as the pen test, flight distance test, docility tests, or approach/avoidance tests (Murphey et al., 1981; Tillbrook et al., 1989). These tests assess the reactivity to the presence of a human in varying degrees. The human may approach or remain stable and assess time and/or distance the animal approaches the human. Tests also measure the response of the animal to a novel stimulus, or speed at which to move through an open yard or pen system. These tests are often termed open-field tests. Burrow (1997) offers that these tests are likely to be measuring a fear or exploratory response to a novel stimulus or situation, in addition to a handler, usually when the human is within sight of the animal. Another unrestrained temperament test is the flight speed test (Burrow et al., 1988) that measures the time taken for the animal to move a set distance after exiting a scale or chute into an open yard or pen. Authors assert that this test measures the fear response of the animal to being handled by humans, rather than a fear response specifically to the handler, which is the case in the approachability and flight distances tests previously described (Burrow, 1997).

Restrained tests include those in which the animal's movement is physically restricted. The behavior in that particular restraint device or situation is assessed. The bail test of Fordyce et al. (1982) assesses reaction of the animal while the head is restrained in a bail, stanchion, or head gate (Tulloh, 1961b; Hearnshaw et al., 1979; Grandin, 1993). Terminologies differ depending on region. The animal's head is pinned around the sides of the neck limiting forward or backward movement. Typically the reaction is given a subjective score by the observer. If the restraint device is a squeeze chute, the head is captured in the head gate, the sides are then closed and the animal is squeezed or restrained to limit lateral motion. The method of Fordyce et al. (1982) and Grandin (1993) assess the amount of reaction just after the head gate is closed and prior to the sides of the chute squeezing inward. Even with the sides of the chute closed, the amount of movement, vocalizations, tail swishing, kicking, audible respiration, and attempts to escape can be noted and scored as a chute score.

Burrow (1997) further described the ease of movement tests used as a measure of temperament. This aspect of temperament testing may be reflective of balking, or cessation of forward movement. Burrow noted that determining the actual meaning of these measurements is difficult. One aspect could be that fast movement through the facilities might indicate that animals are docile and lack fear of human handling (Burrow, 1997). Conversely, rapid movement could reflect a very high level of fear of humans. Animals that have slow movement through the facilities may be those that are difficult to handle due to balking, or by attempting to escape, or that they may be docile and unafraid of the humans handling them, by simply strolling through the testing facilities (Burrow, 1997). Clearly, temperament scoring cannot fully explain the motivation for the behaviors.

Tulloh (1961a) reported the weighing order and behavior of male and female horned Hereford calves prior to and after weaning. Behavior of animals immediately before entering the scales was scored on a 1 to 4 scale, with 1 being without hesitation up to 4 which was considered difficult to get into the scale. The handling tool mentioned at a score of 3 was the cane, in which the calf had to be urged, but then entered easily. This ease of entry behavior scale was recorded on eight occasions during the trial. A chi square analysis showed that the mean score for behavior before entering the scale decreased over time, but was not significantly different. The overall mean score was 1.4 (Tulloh, 1961a). The conclusion was that ease of entry was not an effective measure of temperament because some docile animals scored high or low, and some calves showed consistent behavior. Tulloh (1961a) also suggested the behavior at entrance to the scale may be related to the design and installation of the scale. This may be the first report of assessing behavior entering a specific portion of the working facility.

In a study to evaluate cattle behavior when entering various parts of cattle handling facilities, Tulloh (1961b) used the 1 to 4 scale previously discussed. This study evaluated Hereford, Shorthorn, and Angus breeds in both steers and heifers after weaning with one-year duration of evaluations. Behavior in the chute was evaluated and scored and this was termed the temperament score. Some animals were described as "stubborn" when they opposed attempts to move them forward, but no attempt was made to score degrees of stubbornness. Tulloh (1961b) concluded that stubbornness is a poor indicator of temperament because both docile and aggressive animals were stubborn at times. The mean score for all breeds and sexes entering the crush was 1.8. Hereford scores were higher ($P < 0.01$) than Angus and Shorthorn, which were similar. Sex differences were not significant. Behavior in the chute showed that Herefords had lower ($P < 0.05$) scores than Shorthorns, but were similar to Angus. Again, no differences were shown between sexes. The author then further determined, using chi square tests, whether the behavior in the chute compared with the score for entering the chute, and there was no relationship between them (Tulloh, 1961b).

Hinch and Lynch (1987) evaluated the ease of movement of cattle through various portions of the yards and through the handling facilities in Hereford bulls and steers. Authors found no differences between bulls and steers in movement through the alley, at entrance to the chute. Tillbrook et al. (1989) wanted to quantify the response of individual cattle to humans and measured the ease with which the animals could be moved and handled. Authors used Holstein-Friesian, Jersey x Holstein-Friesian, or Hereford x Holstein-Friesian bulls or steers. The ease of movement test involved a novel alley and yard system. Time to move through the alley, the number of times physical interaction of a negative nature from the experimenter to the animal, and the number of times the animal balked was evaluated. Balking was defined as when the animal stopped and turned 180°, which is typically not possible in most modernly designed alley systems. Authors concluded no differences between bull and steers in time required to move through the alley, number of negative physical interactions by the handler, or number of balks during the movement test. In evaluating all aspects of the study, the authors suggested that animals which were more fearful of the novel human moved through the yard system more quickly than those animals that were less fearful. Therefore, animals that are highly fearful of humans may respond more to the handler than the novel environment (Tillbrook et al., 1989).

Kabuga and Appiah (1992) used the *Bos indicus* breed of N'dama and the *Bos taurus* breeds of Holstein and West African Shorthorn cows and calves, along with crosses of the breed-types to evaluate ease of handling. Ease of handling was defined as time to enter and exit scales, and authors also assigned a temperament score based on behavior in the weighing scale. Time was recorded with a stop watch. There were no differences in time to enter the scale between breeds, but temperament score differed by breed. N'dama x Holstein crossbreds and Holsteins had the highest ($P < 0.05$) temperament scores compared to the N'dama. Although balking was not measured, the time required to enter the scales was similar between breed-types implying that one breed was not more difficult to handle or get into the working facility.

Grandin (1993) evaluated balking behavior at entrance to both the scale and the chute with bulls and steers of different breeds. Breeds represented were Gelbvieh, Charolais and Simmental crosses raised under extensive pasture conditions. These animals were evaluated every 30 days for five handling sessions, and both balking behavior and chute behavior were analyzed. Balking ratings were rated at entrance to the squeeze chute and scale by the same observer. Cattle were classified into balkers and non-balkers. Non-balkers entered voluntarily or required a light tap on the rump to encourage entry. Balkers required a hard slap on the rump or tail twisting to induce entry to the scale or squeeze chute. Chute scoring was on a 1 to 5 scale, with 1 being calm and no movement in the squeeze chute, up to 5, with rearing, twisting of the body and struggling violently while the head was restrained in the head gate. Results showed that a group of animals remained calm during all restraint sessions, another group became behaviorally agitated during all sessions, and a large group had mixed temperament ratings. In the bulls, behaviorally agitated animals balked less ($\chi^2 < 0.05$) than animals with a calm score. In steers, there were no differences in balking behavior. When these animals were in pens at the feedlot, cattle which had been consistently rated as agitated could not be distinguished from other cattle. Behavioral differences were also not evident during handling in the high-speed slaughter plant as they entered at walking speed into the double rail restrainer system (Grandin, 1988, 1991). Grandin suggested that the inability to detect differences in temperament at the slaughter plant may be explained by the two testing environments. In the handling facility, the stressful effects of being separated from the herd during restraint in the chute were being tested.

In the plant, animals maintain visual and physical contact with each other during handling and stunning (Grandin, 1993).

Baszczak et al. (2006) evaluated the entry force required to move steers into the chute for various breeds of cattle. Breeds included British (Angus, composites with predominance of Red Angus, Angus, Hereford, and Angus x Hereford crossbreds), Continental crossbred (Charolais x British composites, Limousin x Angus), and Brahman crossbred (Beefmaster) that originated from 5 ranches. The level of force required to move the animal into the chute ranged from 1 to 4. A score of 1 was given to steers that entered voluntarily or after encouragement from the handler without physical contact. If the handler had to tap the animal on the rump with his/her hand, the animal received a score of 2. If the electric prod was used once, the animal received a score of 3. If more than one electrical impulse from the prod was required, the animal received a score of 4. Behavior in the chute was also scored, ranging from 1 to 3. Calm behavior in the chute resulted in a score of 1, while a 3 represented moderate struggling. Exit scoring was done visually after the animal was released from the squeeze chute. Those animals that walked out of the chute received a score of 1, while those that ran or galloped out of the chute received a 3 score. Scores were recorded on one occasion near time of finish, just prior to slaughter. Results indicated that entry force score was higher ($P < 0.05$) for Continental crossbred steers compared to Brahman crossbred or British cattle, which were similar. Chute behavior scores did not differ. Exit speed score was slowest ($P < 0.05$) for British cattle compared to Brahman crossbred and Continental crossbred steers, which were similar.

The reaction of the animal toward being placed into the squeeze chute may be motivated by several factors: isolation from herd mates, close human contact, or physical restraint (Kilgour et al., 2006) or a combination of factors. Several scoring systems have been used over time

(Hearnshaw et al., 1979; Grandin, 1993; Kilgour et al., 2006) and recently the most commonly used is on a scale of 1 through 5 according to the method of Grandin (1993).

Exit velocity is an objective measurement defined as the rate (m/s) at which an animal traverses a defined distance after exiting the squeeze chute (Burrow et al., 1988). Curley, Jr., et al. (2008, 2010) stated that the measure of exit velocity specifically quantifies the relative degree of fear response generated by human handling of cattle as suggested by Burrow (1997) and Kilgour et al., (2006). The distance for the animal to transverse can vary by researcher or facility limitations, but is defined as 1.83 m in the method of Burrow et al., 1988. Burrow (1991) further defined cattle with an exit velocity of ≤ 1.9 m/s as calm, and those with an exit velocity of ≥ 2.4 m/s as temperamental. Café et al. (2011) measured flight time over a distance of 1.7 m and then converted to flight speed (FS) as m/s. They determined that flight speeds of 1 to 1.5m/s were equal to cattle walking out of the crush or chute, FS of 2 to 2.5 m/s was equal to cattle leaving the chute at a trot, and a FS of 3 to 3.5 m/s equated to cattle leaving the chute at a run. Lanier and Grandin (2002) noted that when timing lights or stop watches are not available for recording time, a visual, but subjective measure of speed leaving the chute can be determined and used for on-farm temperament scoring.

Many factors contribute to temperament: breed, gender, age, previous handling, and genetics (Burdick et al., 2011). Temperament differences have been proven between breed classifications (Hohenboken, 1987; Tulloh, 1961a; Voisinet, 1997; Grandin, 1980a), within breed classification, crossbreeds (Murphey, et al., 1980, 1981; Stricklin, et al., 1980), and within gender (Voisinet, et al., 1997). Temperament differences among breeds of cattle have been extensively researched. *Bos indicus* breeds are generally considered more temperamental compared to *Bos taurus* breeds (Hohenboken, 1987, Tulloh, 1961b; Voisinet, 1997; Grandin, 1980). Within *Bos taurus* breeds, differences in flight zone, tendency to approach novel objects (Murphey, et al., 1980, 1981), excitability (Stricklin, et al., 1980), and social ranking (Stricklin et al., 1980; Wagnon, et al., 1966) are evident among different breeds. British breeds are more docile than European continental breeds, and have a significantly lower flight-speed while leaving the crush (Hoppe, 2008). Tulloh (1961a) further evaluated differences between the European breeds Angus, Hereford, and Shorthorn as compared to the dairy breed Holstein and discovered that Angus are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move, while Holsteins tend to move more slowly. Temperament is a moderately heritable trait in beef cattle (Stricklin, et al., 1980). While breed temperament generalizations can be made, individual behavioral differences within breeds of cattle must be considered and have been evaluated by Kilgour, et al. (2006).

In a review previously discussed by Burrow (1997), he referred to a "freeze" response when animals were restrained, particularly in higher Brahman inheritance (Australian Meat Research Committee, 1988). In assessing anxiety-related behaviors in Hereford x Angus cross cattle, Bristow and Holmes (2007) also mentioned the reaction of some animals "freezing" or resisting entry to the squeeze chute, while others walked calmly into the alley. Genetic selection that has occurred over the past few decades may have had effect on the incidence of balking behavior and may be correlated with temperament.

Genetically, individuals differ in their propensity to learn, and unlearn (Boissy, et al., 2005). Behaviors that promote survival of the species are innate and heritable, which include behavioral defensive responses to fear. 'Flight or fight' are typical fear response behaviors. If flight is not an option, freezing behavior is seen in other animals and has been evaluated extensively in rodents. Freezing has been defined as the absence of any movement except for respiratory-related movements (Panksepp, et al., 2011) and is measured by direct observation. In the review by Panskepp, et al. (2011) the authors assert that freezing is positively correlated with anxiety, particularly when paired with previously aversive stimuli. Learned fear, or aversion, seems related to anxiety. Freezing reaction has been shown and measured in humans with panic disorder in anxious situations (Lopes, et al., 2009). While livestock are generally not referred to as having anxiety episodes or being of 'anxious' temperament, we do accept that factors which increase stress, or possibly anxiety, have a negative impact. Previous aversive handling events may be fixed into memory and thus cannot be forgotten or unlearned (Grandin, 1993). The degree or frequency of the aversion may be a factor in memory and future anticipatory behavior.

Environmental factors that increase the tendency to balk have been thoroughly investigated and researched by Grandin (1980a, 1980b, 1993, Grandin, et al., 1994, 1997). Those factors include sensory stimuli such as lighting, shadows, reflections, flooring, noise, smells, and sounds. Grandin has also evaluated handling aids and techniques, equipment type and design, yard design, and other factors in humane livestock handling. She has designed and modified facilities and educated handlers for effective, safe animal movement and has advanced animal welfare. Even with known environmental factors controlled, balking still occurs.

Temperament and stress responsiveness have been related to meat quality (King, et al., 2006) and show that excitable cattle had less tender meat. Electric prod use in calves versus use of a plastic oar or manual stimulation in moving through a chute system caused animals to stumble and hit the sides of the chute which may increase the incidence of bruising (Croney, et al., 2000). Grandin states that temperament is related to production costs in animal handling and that bruises in cattle costs the industry $26 million annually (1993). Thus, animal temperament, response to stressors, and handling procedures can affect the end product.

The end product of beef is typically thought to originate from beef cattle. Fed dairy steers make up about 15 to 20 percent of all fed cattle sent to market for beef production (Wardynsky, 2012). Temperament scoring for dairy cattle differs from beef cattle. The review by Burrow (1997) discussed temperament scoring in dairy cows. The scoring systems vary greatly and are, for the majority, subjective ratings as related to the ease of milking. Contrasting with beef cattle temperament scoring, dairy cattle scores are usually assessed repeatedly over an undefined period of time. This score is often an adjective to describe temperament specific to the milking event rather than herd behavior. A review of the literature reveals no research reports using fed Holsteins to assess behavior with chute scores or exit velocity that are frequently used for beef cattle.

To summarize, few studies address the ease of movement, or balking behavior, from a temperament perspective. Balking has not been proven to be an indicator of temperament. However, balking can create a welfare issue by instigating human reactions that may be perceived as inhumane treatment, especially in very resistant animals.

Correlations do not imply cause and effect. A correlation is a statistical measurement of the relationship, either positive or negative, between two variables. The coefficient can range from 0 to 1, with a score of 1 being the strongest association. Correlation coefficients may also be negative, illustrating an inverse relationship between variables. As one factor increases, the other decreases. Several researches that have investigated cattle behavior have used correlations between chute scores and exit velocity. Vann and Randel (2003) reported a relatively low correlation between exit velocity and chute score. Burrow and Corbet (2000) concluded that in animals with 50% or more *Bos indicus* breeding influence, the objective measure of flight speed, or exit velocity, was the preferred method of assessing temperament. Café et al. (2011) showed that Brahman cattle had greater individual variation in repeated assessments of flight speed, chute scores, and greater correlations within and between those repeated measures than did Angus cattle. These authors also concluded that correlations for repeated measures of flight speed were stronger than for repeated assessments of chute score. Further, the strength of correlations for both declined over time. Temperament scoring tests and scoring systems appear an accepted method to determine reactivity of cattle to human handling.

In a recent review article by Burdick et al. (2011), the authors attribute stress responsiveness associated with cattle temperament. Cattle with more excitable temperaments have increased basal concentrations of stress hormones, poorer growth performance and carcass characteristics, and weaker immune responses. The stress response to routine handling varies with the individual animal and can have long-lasting deleterious effects as they are handled frequently, often in several different locations throughout their lifetime.

The temperament of the animal affects whether they perceive the situation as stressful or not. Genotype and prior learning experiences influence the reaction to humans and handling (Grandin, 1997; Le Neindre et al., 1996). Animals categorized as calm, intermediate, or temperamental have shown differences in growth and immunity. Studies have determined that cattle with slower exit velocities gain weight more rapidly than those with faster exit velocities (Voisinet et al., 1997; Burrow and Dillon, 1997; Café et al., 2011). Time spent eating was reduced and dry matter intake decreased in cattle with greater exit velocities, and a negative correlation was shown between exit velocity and average daily gain (Hoppe et al., 2010). Dairy cows with poor temperaments are correlated with lower milk yield, milk protein, and milk fat content (Breuer et al., 2000). Meat quality is negatively affected in temperamental cattle by increased bruising and carcass pH, and decreased tenderness (Voisinet et al., 1997; Burrow and

Dillon, 1997; King et al., 2006; Café et al., 2011). Carcass weights and rib fat are decreased in temperamental cattle (Café et al., 2011). More temperamental calves have a reduced response to vaccination compared to calm calves. Cattle with excitable temperaments have increased production costs due to increased risk of injury and decreased carcass value. Additionally, similar to stress, temperament may affect immune function negatively (Burdick et al., 2011).

From an economic standpoint, particularly in the processing plant, the time required for handlers to keep cattle moving forward is a loss. Efficiency and meat quality are priorities. The risk of dark cutters or a decline in the quality of carcass traits, particularly from bruising, has been correlated to poor temperament and stress before harvest (King, et al., 2006; Grandin, 1993). Animals that continually must be coerced, especially by electric prod due to balking, may present a significant increased risk of an unfavorable product. However, electric prod use for a handling aid compared to other aids may be most time efficient for cattle line movement (Croney, et al., 2000).

Animals that have a high incidence of intense balking may illicit human handling procedures that are objectionable and could have negative connotations for animal welfare. Economic losses due to animal welfare and negative public perception can be a concern. When standard stimuli fail, animal welfare issues emerge as more persuasive handling aids such as the electric prod are needed to keep animals moving. The Recommended Animal Handling Guideline & Audit Guide: A Systematic Approach to Animal Welfare (American Meat Institute Foundation, July 2013) Core Criteria 5: Electric Prod Scoring Criteria for Cattle states prodding as acceptable in 25 percent or less animals in welfare audits. For purposes of auditing, touching cattle with an electric prod despite whether it is energized or not counts as a prod.

The Humane Slaughter Act sets requirements for humane slaughter, and livestock must be humanely slaughtered in order to become part of the US food system. The Humane Slaughter Act, 7 U.S.C. §§ 1901-1906, is enforced by the Secretary of Agriculture under provisions of the Federal Meat Inspection Act, 21 U.S.C. §§ 603(b). The provisions of this statute and accompanying regulations outline the methods of slaughter that are deemed to be "humane," and thus appropriate for use in slaughtering livestock (National Agricultural Law Center). The Humane Slaughter Act regulations, codified at 9 C.F.R. §§ 313.1-313.90 and discussing livestock handling, states that "electric prods...employed to drive animals shall be used as little as possible in order to minimize excitement and injury" 9 C.F.R. §313.2(b). Any use of the prod, which in the opinion of the inspector is excessive, is prohibited. Inspectors are employed by the Food Safety and Inspection Service (FSIS), an agency of the United States Department of Agriculture. Inspectors that observe inhumane handling shall inform the establishment operator and require that necessary steps be taken to prevent a recurrence. If no action is taken, or if actions are ineffective, the inspector may attach a "U.S. Rejected Tag" to the alleyways leading to the stunning area if the cause of inhumane treatment is the result of establishment employee actions in the handling or moving of livestock. After the tagging of the alleyway, no additional livestock may be moved into the stunning area until the inspector has been assured that no further egregious situations will occur and has removed the tag 9 C.F.R. §313.50. Until the welfare concern has been resolved, production ceases. Further, a definition of "egregious inhumane handling" has been outlined in a directive by the FSIS Directive as any act or condition that results in severe harm to animals. For example: excessive… prodding of ambulatory or non-ambulatory disabled animals or dragging of conscious animals (Food Safety and Inspection Service Directive 6900.2: Revision 2, 8/15/2011).

Corporations are aware of the regulations and repercussions to improper animal handling. Employees that handle cattle are trained according to the American Meat Institute Guidelines (2013) which are updated regularly. Corporations self-audit to assure animals are being treated humanely and employees are following regulations, termed a 1st party or internal audit. Companies that purchase products supplied by the plant also audit adherence to regulations and is considered a 2nd party or external audit. When a company buying the products hires an independent, outside auditing company to conduct an audit, this is considered a 3rd party audit. Virtually all auditors are certified by the Professional Animal Auditor Certification Organization, Inc. Corporations allow an expense of approximately $2000 for 3rd party audits (personal communication, anonymous). A failed audit results in re-auditing, cost of retraining employees, and all customers may request a copy of the audit before purchasing products. Egregious handling events are also part of the public record through the FSIS website (http://www.fsis.usda.gov/wps/portal/fsis/home). Animal handling is a serious issue in many regards in beef processing plants. Balking scores of 4 or 5 represent behavior that may have required the use of the electric prod to continue line movement.

Anecdotal reports from experienced handlers (personal communication, anonymous) suggest certain breed-types cause more difficulties to maintain forward movement than other breed-types. Breed-types can only be speculated by coat color and other characteristic markings when received at the processing plant. The genetic inheritance of coat color spotting patterns is complex. Guidelines for producers to use for selection and marketing are readily available (Kirkpatrick, 2004; Evans, OSU ANSI-3154). Cattle presented to the commercial processing plant arrive from various feedlots and sources. Unless indicated, the history of breeding is unknown.

Holstein breeding, however, is known at commercial processing plants due to feedlot source and is noted on the form for incoming cattle. Balking behavior in dairy cattle and beef cattle differ at entrance to the restrainer in the beef processing plant. Holsteins have been selected for their milk production and docile behavior for ease in human handling during milking. Dairy calves are typically removed from their dam shortly after birth and have exposure to humans throughout their lifetime. Duff and McMurphy (2007) describe typical Holstein steer production in the southwestern part of the United States. Calves are raised in hutches approximately 60 days before weaning. After weaning, calves are managed to reach approximately 125 kg before shipping to feedlots. Finishing Holstein steers to an end weight of 590 kg can require 12 months or more in a somewhat consistent environment (Duff and McMurphy, 2007). This may lead to decreased fear of humans and decreased flight zone. Additionally, this management may impose fewer handling events and less exposure to novel handling events than occurs with typical beef cattle production. Behaviorally, Holstein steers differ from traditional beef breeds (Duff and McMurphy, 2007). Tulloh (1961a) reported that Holsteins tend to move more slowly compared to Angus, Hereford, and Shorthorn cattle. Holsteins have a gentle temperament, are playful, easily bored, may sort through feed, and are difficult to move because they have a tendency to follow humans (Duff and McMurphy, 2007). Difficulty moving may equate with a tendency to balk. An antonym for “bored” is “interested” (http://thesaurus.com/browse/bored). These behavioral generalities may explain that Holstein steers are “curious” or “interested” in inspecting their novel surroundings at entrance to the restrainer, thus increasing balking tendencies. These balking tendencies may be more of a slowing to investigate surroundings.

Tillbrook et al. (1989) investigated the social behavior of 14 mo old cattle, including Holstein, Jersey x Holstein, or Hereford x Holstein crossbred bulls and steers, in an ease of movement test. No differences were shown between the bulls and steers in this test, but authors suggested that animals that are highly fearful of humans may respond more to the handler than the novel environment. The opposite perspective may hold true; animals with low fear of humans may respond more to a novel environment than to a handler.

Behavioral observations and scoring systems have been paralleled physiologically with cortisol measurements. Zavy, et al. (1992) reported that Brahman cross calves have higher cortisol levels than crosses of Angus and Hereford, and Stricklin, et al. (1980) found that Angus have higher cortisol levels and heart rates than Hereford cattle. However, limited studies have evaluated polymorphisms of the heat shock protein 70 gene in cattle related to behavior.

Virtually all organisms respond to non-lethal increases in environmental temperatures (heat shock) by synthesizing a set of proteins called heat shock proteins (HSPs). Many types of environmental stresses in addition to ambient temperature can induce the production of these proteins, including heavy metals, ethanol, amino acid analogues, free radical attack, UV light, ozone, or fever and are also present in unstressed cells (Welch, 1992). Thus, these proteins are often referred to generally as stress proteins.

There is constant interaction between life and the environment, so adaptation to change is essential for animal survival. These heat shock or stress proteins allow cells to adapt to gradual changes in their environment. Thermotolerance to ambient temperatures changes with time, through this adaptation process. It is thought that the production of these proteins due to increased temperatures allow reprogramming of cellular activities to insure survival during stress periods and to protect essential cell components against heat damage. During the recovery period after an elevated heat or stress exposure, normal cellular functions resume rapidly after the initial heat shock event (Burdon, 1986). The HSPs are induced by moderate stresses, which are not necessarily lethal, and help protect the organism from even more severe stress.

This response is the most evolutionarily, highly conserved genetic system known, existing in bacteria, plants, and animals. Additionally, HSPs are present in all organisms at normal temperatures and play vital roles in normal cell function. In all organisms, the induction of HSPs is remarkably rapid and intense, possibly as an emergency response (Lindquist and Craig, 1988).

After heat shock, HSP 70 was found to concentrate mainly within the nucleus and secondarily at cell membranes. This translocation is not completely dependent upon the temperature, because concentration in the nucleus is also observed after exposure to a hypoxic or oxygen deprived environment. During recovery from heat shock, HSP 70 leaves the nucleus and is found mainly in the cytoplasm (Lindquist and Craig, 1988).

These stress proteins belong to a multi-gene family and range in size from 8 to 150 kDa (kilodalton). HSPs are classified according to their molecular weight and the 70 kDa protein is named HSP 70. The most widely studied of all of the heat shock proteins is HSP70 (Agnew and Colditz, 2008). Breed differences in cattle have been reported in lymphocyte responses to increased temperature in HSP 70 (Kamwanja et al., 1994; Lacetera et al., 2006).

Agnew and Colditz (2008) looked at patterns of HSP 70 expression in leukocyte subpopulations from cattle and sheep to determine the optimal experimental conditions for the measurement of leukocyte HSP 70 and for induction of HSP 70 in response to heat shock. They determined that the optimum *in vitro* stress treatment temperature for heat shock induction of hsp70 in leukocytes is 43.5°C in cattle and sheep. They also determined that best results are obtained from fresh blood samples. Even in blood samples stored at room temperature for 24 h, the patterns of HSP 70 expression after heat shock were not consistent with results from fresh blood samples. *In vivo* studies with rodent and human lymphocytes have demonstrated HSP 70 expression in response to many stressors, including increases in body temperature (> 1.5°C), surgery, water immersion stress, trauma, exercise, and restraint stress (reviewed in Rokutan et al., 1998). Leukocyte HSP 70 expression may be a useful indicator of adaptation to environmental or physiological stresses (Agnew and Colditz, 2008).

Al-Aquil et al., 2013 conducted an experiment to determine the effects of combining both pleasant and unpleasant contacts with human beings of physiology and behavior of broiler chickens. Authors found that subjecting birds to pleasant human contact reduced stress and fear reactions to transportation by enhancing the ability to express HSP 70 in the brain. Irrespective of human contact treatment, 3 h of road transportation significantly increased HSP 70 expression. Manipulating the expression of HSP 70 offers a potential for improving nonthermal and transportation stress tolerance in chickens. Thus, handling of livestock may induce HSP70.

Most heat shock proteins (HSPs) have a molecular chaperone activity. A molecular chaperone is a protein that binds to and stabilizes an otherwise unstable version of another protein. By controlling the binding and release of the substrate protein, the chaperone facilitates correct protein function (Ohtsuka and Hata, 2000). The chaperones bind to hydrophobic amino acid residues exposed on the outside of the unfolded polypeptides, then release substrates in a controlled manner, which prevent unproductive aggregation and promotes proper folding. The molecular chaperone system is thought to be a defense mechanism against proteitoxic stresses such as heat and chemicals at the cellular level. Molecular chaperones may suppress the accumulation of damaged proteins, in turn increasing total life span (Ohtsuka and Hata, 2000).

In a review by Collier et al, 2007, authors describe the gene expression in the bovine heat response is under heat shock transcription factor (HSF1) regulation. This transcription factor family is important as the ‘first responders’ during the onset of elevated cell temperature. These transcription factors coordinate the cellular response to thermal stress and affect expression of a wide variety of genes, including heat shock proteins. The model of transcriptional activity indicates that nonstressed cells contain folded HSF1 monomers bound to HSP within the cytoplasm. After heat stimulus, the HSP dissociate from the HSF1 monomers, which then unfold and bind to two other monomers before entering the nucleus. Once inside the nucleus, the HSF1 binds promoters containing heat shock elements (HSE) to activate heat stress target gene transcription. The HSF1 gene has been mapped to chromosome 14 in cattle (Collier et al, 2007).

Enhancers are DNA sequences that stimulate transcription but are located further away from the start site. Enhancers work by binding specific protein factors, called activators. When an activator binds to an enhancer, structural changes in the DNA template allow interaction of the activator with other factors or with RNA polymerase. Transcription factors must bind to DNA sequences and recruit RNA polymerases to the promoter region for a gene to be active. RNA polymerases then synthesize RNA in the 5’ to 3’ direction using the DNA template. RNA polymerases initiate polymerization at promoter sequences, which specifies the start site for transcription (Devlin, 2011).

Banks et al., 2007, determined genetic diversity in a promoter segment of the bovine HSP70 gene and if the identified single nucleotide polymorphisms (SNPs) were related to pregnancy rates in *Bos taurus x Bos indicus* crossbred cows. Results indicated that the promoter region of the HSP 70 gene in cattle is polymorphic and may be a useful in selecting cows with potential higher calving rates (Banks et al., 2007). The relationship between genotypic variation of the bovine HSP 70 promoter area and bull calf weaning weights and serum concentrations of HSP 70 at weaning were determined (Starkey et al., 2007). Serum concentrations were not affected by the ten SNP genotypes, but weaning weight was affected by two of the genotypes. Authors concluded that polymorphisms within the promoter region of the HSP 70 gene are associated with weaning weights. Decreased calving percentages and later calving dates in Brahman cows were shown by Rosenkrans et al. (2010) in HSP 70 SNPs in the promoter area. Turner et al., 2013, illustrated a tendency for increased horn fly density on beef cattle in those animals with polymorphisms in the promoter area. Basiricò et al. (2011) investigated genetic mechanisms associated with individual cellular response to heat shock in Italian Holstein cows. SNPs in the promoter region of the HSP 70 gene were associated with upregulation of gene expression and HSP synthesis, in addition to an increase in response to heat shock in terms of viability. The authors suggest the presence of promoter variants improved binding of corresponding transcription factors and the activation of cellular protective mechanisms associated with increases in cell viability. The HSP 70 promoter area alone has shown associations with cattle fertility in calving rate and calving interval, weaning weight, and susceptibility to horn fly infestation. These measured traits all have economic impact. Biologically, changes in nucleotides in the promoter region have also shown biological importance in increased gene expression and protein synthesis, and protection from future heat shock. Although the exact physiological mechanisms of these polymorphisms have not been identified, the HSP70 gene and effects warrant further study.

While circulating levels of heat shock proteins were not measured in this study, the polymorphisms themselves may be an indicator of altered gene function. Consideration should be given to the possibility that these polymorphisms may change HSP 70 gene function or production of stress proteins, and result in decreased reactivity to stressors such as handling.

Polymorphisms in the HSP 70 gene promoter region may provide insight to behavior and performance. While we cannot attribute behavioral differences solely to genotype, it is a factor to be considered in combination with environment as related to phenotype. The question arises if these polymorphisms increase HSP70 protein production which may allow for easier adaptation, and therefore, less stress, to a new environment with decreased response to handling.

Rationale

The projects presented in this dissertation are intrinsically linked in nature and focus. In Chapter 2, data will be presented that illustrate balking behavior in the processing plant, in which little is known about the breeding of the animals, other than the Holstein breed. Supporting information provided by the processing plant is utilized, but the genetics of breed-type is unknown.

Chapter 3 focuses on the known Holstein breed and analyzes behavior at the feedlot. Animals from this feedlot are then processed at the plant highlighted in Chapter 2. Behavior of the steers at both locations are assessed and analyzed.

To gain knowledge of animals with known breeding and genetics, Chapter 4 focuses on the breeds Angus and Hereford-Angus crossbred steers, and their behavioral response to handling in multiple locations throughout the lifetime. This chapter delves more deeply into repeated observations over time and looks at trends.

Finally, Chapter 5 views the genotypes of the steers presented in Chapter 4, as related to heat shock protein 70 genetics. This provides a molecular genetic insight to behavior versus a breed-type as discussed in Chapter 4. The progression from observing behavior of cattle based on simply coat color and any presence of characteristic markings to viewing animals with genetic polymorphisms of a particular gene offers a thorough analysis of the topic in this dissertation.

References

Al-Aquil, A., I. Zulkifli, M. Hair Bejo, A.Q. Salili, M.A., Rajion, and M.N. Somchit. 2013. Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. J. Poult. Sci. 92:33-40.

American Meat Institute Foundation. 2013. Recommended animal handling guideline & audit guide: A systematic approach to animal welfare. Grandin, T. and American Meat Institute Animal Welfare Committee. Core Criteria 5: Electric prod scoring criteria for cattle. p51. www.meatami.com/. Accessed 11 October 2013.

Australian Meat Research Committee Final Report UNQ7. 1988. Genetic and environmental methods of improving the temperament of *Bos indicus* and crossbred cattle. James Cook University of North Queensland.

Agnew, L.L., and I.G. Colditz. 2008. Development of a method for measuring cellular stress in cattle and sheep. Vet. Immunol. Immunopathol. 123:197-204.

Banks A., Looper, M.L., Reiter, S., Starkey, L., Flores, R., Hallford, D., Rosenkrans, C., Jr. 2007. Identification of single nucleotide polymorphisms within the promoter region of the bovine heat shock protein 70 gene and associations with pregnancy. Am. Soc. Anim. Sci. South Sect Meet. 2007;85(Suppl. 2):30.

Basiricò, L.,P. Morera, V. Primi, N. Lacetera, A. Nardone, and U.Bernabucci. 2011. Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. Cell Stress & Chaperones.16:441–448

Baszczak, J.A., T. Grandin, S.L. Gruber, T.E. Engle, W.J. Platter, S.B. Laudert, A.L. Schroeder, and J.D. Tatum. 2006. Effects of ractopamine supplementation on behavior of British, Continental, and Brahman crossbred steers during routine handling. J. Anim. Sci. 84:3410-3414.

Boissy, A, A.D. Fisher, J. Bouix, G.N. Hinch, P. Le Neindre. 2005. Genetics of fear in ruminant livestock. Livestock Prod. Sci. 93:23-32

Bourdon, R.H. 1986. Heat shock and the heat shock proteins. Biochem. J. 240:313-324.

Brambell, F.W.R., 1965. Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems. Her Majesty's Stationery Office, London.

Breuer, K., P.H. Hemsworth, J.L. Barnett, L.R. Matthews and G.J. Coleman. 2000. Behavioral response to humans and the productivity of commercial dairy cows. Appl. Anim. Behav. Sci. 66:273-288.

Bristow, Daniel J. and David S. Holmes. 2007. Cortisol levels and anxiety-related behaviors in cattle. Phys. and Behav. 90:626-628.

Burdick, N.C., R.D. Randel, J.A. Carroll, and T.H. Welsh Jr. 2011. Interactions between temperament, stress, and immune function in cattle. [electronic resource]. International Journal Of Zoology. Accessed 16 March 2012.

Burrow, H.M. 1991. Effect of intensive handling of zebu crossbred weaner calves on temperament. Proc. Aust.Asso. Anim. Breed. and Genet. 9:208–211.

Burrow, H.M. 1997. Measurement of temperament and their relationships with performance traits of beef cattle. Anim. Breed. Abstr. 65:477-495.

Burrow, H.M., and N.J. Corbet. 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pastures in the tropics. Aust. J. Exp. Agric. 51:155-162.

Burrow, H.M., and R.D. Dillon. 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreds. Aust. J. Exp. Agric. 37:407-411.

Burrow, H.M., G.W. Seifert, and N.J. Corbet. 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17:154-157.

Café, L.M., D.L. Robinson, D.M. Ferguson, B.L. McIntyre, G.H. Geesink, and P.L. Greenwood. 2011. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. J. Anim. Sci. 89:1452-1465.

Collier, R.J., J.L. Collier, R.P. Rhodes, and L.H. Baumgard. 2007. Invited Review: Genes involved in the bovine heat stress response. J.Dairy Sci. 91:445-454.

Cooke, R.F., D.W. Bohnert, B.I. Cappellozza, C.J. Mueller, and T. DelCurto. 2012. Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females. J. Anim. Sci. 90:3547-3555.

Croney, Candace C., L.L. Wilson, S.E. Curtis, and E.H.Cash. 2000. Effects of handling aids on calf behavior Appl. Anim. Behav. Sci. 69:1-13.

Curley, Jr., K.O., D.A. Neuendorff, A.W. Lewis, J.J. Cleere, T.H. Welsh, Jr., and R.D. Randel. 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm. and Behav. 53:20-27.

Curley, Jr., K.O., D.A. Neuendorff, A.W. Lewis, F.M. Rouquette, Jr., R.D. Randel, and T.H.

Welsh, Jr. 2010. The effectiveness of vasopressin as an ACTH secretagogue in cattle differs with temperament. Phys. and Behav. 101:699-704.

Devlin, T.M. (ed.) 2011. Textbook of biochemistry with clinical correlations. 7ed. John Wiley & Sons, Inc. Hoboken, NJ.

Duff, G.C., and C.P. McMurphy. 2007. Feeding Holstein steers from start to finish. Vet. Clin. Food Anim. 23:281-297.

Evans, J. Oklahoma Cooperative Extension Fact Sheets, ANSI-3154 http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2031/ANSI-3154web.pdf., Accessed 10 July 2013.

Federal Meat Inspection Act, 21 U.S.C. §§ 603(b).

Food Safety and Inspection Service Directives 6900.2; Revision 2, 8/15/2011.

Fordyce, G., M.E. Goddard, and G.W. Seifert. 1982. The measurement of temperament in cattle and the effect of experience and genotype. Proc. Aust. Soc. Anim. Prod. 14:329-332.

Fordyce, G., R.M. Dodt, and J.R. Wythes. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. Aust. J. Exper. Ag. 28:683-687.

Gonyou, H. W. 1994. Why the study of animal behavior is associated with the animal welfare issue. J. Anim. Sci. 72:2171–2177.

Grandin, T. 1980a. Livestock behaviors related to handling facilities design. Int. J. Stud. Anim. Prob. 1:33-52.

Grandin, T. 1980b. Observations of cattle behavior applied to the design of cattle handling facilities. Appl. Anim. Ethol. 6:19-33.

Grandin. T. 1988. Double rail restrainer for livestock handling. Int. J. Agric. Eng., 41:327-338.

Grandin, T. 1991. Double rail restrainer system for beef cattle. Am. Soc. Agric. Eng., Technical Paper 915004. St. Joseph, MI.

Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Grandin, T., K.G. Odde, D.N. Schutz, and L.M. Beherns. 1994. The reluctance of cattle to change a learned choice may confound preference tests. Appl. Anim. Behav. Sci. 39:21.

Grandin, T. 1996. Factors that impede animal movement at slaughter plants. J. Amer.Vet. Med. Assoc. 209:757-759.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.

Hearnshaw, H., R. Barlow, and G. Want. 1979. Development of a "temperament" or "handling difficulty" score for cattle. Proc. Inaug. Conf, Aust. Assoc. Anim. Breeding and Genetics.

Hinch, G.N., and J.J. Lynch. 1987. A note on the effect of castration on the ease of movement and handling of young cattle in yards. Anim. Prod. 45:317-320.

Hoppe, S. 2008. Genetic and environmental factors affecting beef cattle temperament. 15th World Hereford Conference, Copenhagen.

Hoppe, S., H.R. Brandt, S. König, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationship to performance. J. Anim. Sci. 88:1982-1989.

Hohenboken, W.D., 1987. Behavioral genetics. Vet. Clin. North Am.: Food Anim. Prac. 3:217-229.

Humane Slaughter Act, 7 U.S.C. §§ 1901-1906.

Humane Slaughter Act Regulations, 9 C.F.R. §§313.1-313.90, §313.2(b), Handling of Livestock. Tagging § 313.50.

Johnson, A.K. 2009. ASAS Centennial Paper: Farm animal welfare science in the United States. J. Anim. Sci. 87:2179-2179.

Kabuga, J.D., and P. Appiah. 1992. A note on the ease of handling and flight distance of *Bos indicus, Bos taurus* and their crossbreds. Anim. Prod. 54:309-311.

Kamwanja, L.A., C.C. Chase, J.A. Gutierrez, J.A., V. Guerriero, T.C. Hammond, and P.J. Hansen. 1994. Responses of bovine lymphocytes to heat shock as modified by breed and antioxidant status. J. Anim. Sci. 72:438-444.

Kilgour, R.J. 2012. In pursuit of "normal": A review of the behaviour of cattle at pasture. Appl. Anim. Behav. Sci. 138:1-11.

Kilgour, Robert J., Gavin J. Melville, and Paul L. Greenwood. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity to humans, restraint, and novelty. Appl. Anim. Behav. Sci. 99:21-40.

King, D.A., C.E. Schuehle Pfeiffer, R.D. Randel. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74:546-556.

Kirkpatrick, F.D. 2004. Color patterns in beef cattle. Univ. of TN Ag. Ext. Serv. http://animalscience.ag.utk.edu/beef/pdf/ColorPatternsInBeefCattle-FDK-2004.pdf. Accessed 17 April 2012.

Lactera, N., U. Bernabucci, D. Scalia, L. Basiricò, P. Morera, and A. Nardone. 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. J. Dairy Sci. 89:4604-4612.

Le Neindre, P., X. Boivin, and A. Boissy. 1996. Handling of extensively kept animals. Appl. Anim. Behav. Sci. 49:73-81.

Lanier, J.L, and T. Grandin. 2002. The relationship between *Bos taurus* feedlot cattle temperament and foreleg bone measurements. Proc. Western Section Am. Soc. Anim. Sci. 53:97-98.

Lanier, J.L., T. Grandin, R.D. Green, D. Avery and K. McGee. 2000. The relationship between reaction to sudden intermittent sounds and movement and temperament. J. Anim. Sci. 78:1467-1474.

Lindquist, S., and E.A. Craig. 1988. The heat-shock proteins. Annu. Rev. Genet. 22:631-677.

Lopes, Fabiana L., T.M. Azevedo, L.A. Imbiriba, R.C. Freire, A.M. Valenca, D. Caldirola, G. Perna, E. Volchan, and A.E. Nardi. 2009. Freezing reaction in panic disorder patients associated with anticipatory anxiety. Dep. And Anxiety. 26:917-921.

Murphey, R.M., F.A.M. Duarte, M.C.T. Penedo. 1980. Approachability of bovine cattle in pastures: Breed comparisons and a breedxtreatment analysis. Behav. Genet. 10:171-181.

Murphey, R.M., F.A. Moura Duarte, and M.C. Torres Pendo. 1981. Responses of cattle to humans in open spaces: Breed comparisons and approach-avoidance relationships. Behav. Genet. 11:37-48.

National Agricultural Law Center, Overview of Animal Welfare Laws, http://nationalaglawcenter.org/overview/animalwelfare/. Accessed 1 March 2014.

Panksepp, J., T. Fuchs, and P. Iacobucci. 2011. The basic neuroscience of emotional experiences in mammals: The case of subcoritical FEAR circuitry and implications for clinical anxiety. Appl. Anim. Behav. Sci. 129:1-17.

Professional Animal Auditor Certification Organization, Inc. http://www.animalauditor.org/paaco/. Accessed 7 March 2014.

Ohtsuka, K., and M. Hata. 2000. Molecular chaperone function of mammalian Hsp70 and Hsp40-a review. Int. J. Hyperthermia. 16:231-245.

Panksepp, Jaak, Thomas Fuchs, Paolo Iacobucci. 2011. The basic neuroscience of emotional experiences in mammals: The case of subcortical FEAR circuitry and implications for clinical anxiety. Appl. Anim. Behav. Sci. 129; 1-17.

Price, E.O., 1984. Behavioral aspects of animal domestication. Q. Rev. Biol. 59, 1–32.

Rosenkrans, Jr., C., A. Banks, S. Reiter, and M. Looper. 2010. Calving traits of crossbred Brahman cows are associated with Heat Shock Protein 70 genetic polymorphisms. Anim. Repro. Sci. 119:178-182.

Starkey L., Looper M.L., Banks A., Reiter S., Rosenkrans, C., Jr. 2007. Identification of polymorphisms in the promoter region of the bovine heat shock protein gene and associations with bull calf weaning weight. Am. Soc. Anim. Sci. South. Sect. Meet. 85(Suppl. 2):125.

Stricklin, W.R., C.E. Heisler, and L.L. Wilson. 1980. Heritability of temperament in beef cattle. J. Anim. Sci. 57:Suppl 1, 109.

Stricklin, W.R., and C.C. Kautz-Scanavy. 1984. The role of behavior in cattle production: a review of research. Appl. Anim. Ethol. 11:359-390.

Tillbrook, A.J., P.H. Hemsworth, J.L. Barnett, and A. Skinner. 1989. An investigation of the social behavior and response to humans of young cattle. Appl. Anim. Behav. Sci. 23:107-116.

Tulloh, N.M. 1961a. Behaviour of cattle in yards: I. Weighing order and behaviour before entering scales. Anim. Behav. 9:20-24.

Tulloh, N.M. 1961b. Behaviour of cattle in yards: II. A study in temperament. Anim. Behav. 9:25-30.

Turner, C.M., A.H. Brown, Jr., C.D. Steelman, and C.F. Rosenkrans, Jr. 2013. Associations among heat shock protein 70 genotype, forage system, and horn-fly infestation of beef cattle. Prof. Anim. Sci. 29:237-241.

Vann, R.C., and R.D. Randel. 2003. Breed of sire and gender effects on chute exit velocity and chute temperament score in beef calves. J. Anim. Sci. 81(Suppl. 1):121. (Abstr.)

Vann, R.C., J.A. Parish, and W.B. McKinley. 2008. Case Study: Mississippi cattle producers gain insight into temperament effects on feedlot performance and subsequent meat quality. Prof. Anim. Sci. 24:628-633.

Voisinet, B.D., T. Grandin., J.D. Tatum, S.F. O'Connor and J.J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

Wagnon, K.A., R.G. Coy, W.C. Rollins and F.D. Carroll. 1966. Social dominance in a herd of Angus, Hereford and Shorthorn cows. Anim. Behav. 14:474-479.

Wardynsky, F. 2012. Raising dairy steer calves for profitable beef production. Michigan State University Extension. http://msue.anr.msu.edu/news/raising_dairy_steer_calves_for_profitable_beef_production. Accessed 23 March 2014.

Welch, W.J. 1992. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol. Rev. 72:1063-1081.

Zavy, M.T., P.E. Juniewicz., W.A. Phillips, and D.L. VonTungeln,.1992. Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes.1992. Am. J. Vet. Res.53:551-557

Chapter 2: Balking Behavior Incidence in Fed Cattle at the Processing Plant and Carcass Implications

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Abstract

Balking behavior in the cattle processing line can pose welfare issues as electric prod use to coerce forward movement is implemented. Temperament differences have been shown among breed-type categories, within breed-type categories, among crossbreds, and between sexes. Objectives in this study were to determine if breed-type predominance, based on coat color or gender, had an effect on balking behavior, and if that behavior affects carcass economics. A total of 6,510 balking observations over 7 random dates in one year were recorded at the entrance to the restrainer in a high-capacity processing plant. Balking scores were assigned on a scale of 1 to 5 by a trained observer. Twelve color combinations and 15 feedlot sources were represented at random collection dates and times. Holstein cattle balked more (P < 0.0001) than all other colors which were similar. Sex differed in balking incidence with heifers balking more (P = 0.05) than steers, and pens containing both steers and heifers balked intermediately. The feedlot source affected (P < 0.0001) balking behavior, with balking score means varying from the lowest at 1.1 to 2.3 as the highest. Balking behavior was negatively correlated (r = -0.18, P < 0.0001) with

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dressing percentage. Mean pen weight and dressing percentage were also affected (P < 0.0001) by feedlot source. Mean pen weight was affected by color. Holstein cattle had greater (P < 0.0001) mean pen weights than all other colors which were similar. Steers had heavier (P < 0.0001) mean pen weights than mixed pens, with heifers having the lowest mean pen weight (602.8 ± 15.4 kg, 546.1 ± 0.59 kg, and 541.1 ± 0.36kg, respectively). Dressing percentage was affected (P < 0.0001) by coat color and gender (P = 0.01). Steers had the greatest (P = 0.01) mean dressing percentage at 64.8 ± 0.1 versus heifers at 64.3 ± 0.3 with mixed pens being intermediate at 63.4 ± 0.5. Our results suggest an association with dressing percentage and balking behavior, and dressing percentage is affected by coat color and gender.

Introduction

Handling of cattle throughout their lifetime has an impact on learning. Despite their innate gregarious behavior, some learn to avoid places and people which may lead to resistance in moving forward in the working facility. This balking behavior requires stimulus from the handler to coerce the animal and may present a challenge. When standard stimuli fail, animal welfare issues emerge as more persuasive handling aids such as the electric prod are needed to keep animals moving. The Recommended Animal Handling Guideline & Audit Guide: A Systematic Approach to Animal Welfare (American Meat Institute Foundation, July 2013) Core Criteria 5: Electric Prod Scoring Criteria for Cattle states prodding as acceptable in 25 percent or less animals in welfare audits. For purposes of auditing, touching cattle with an electric prod despite whether it is energized or not counts as a prod.

Anecdotal reports from experienced handlers suggest certain breed-types cause more difficulties to maintain forward movement than other breed-types. Breed-types can only be speculated by coat color and other characteristic markings when received at the processing plant.

Behaviors that make handling more difficult, take more time than the average, or present danger to humans are often associated with animals described of as poor temperament. Temperament in cattle is often defined by observed behavioral responses to humans or human handling procedures. Temperament differences have been proven between breed classifications (Hohenboken, 1987, Tulloh, 1961; Voisinet, 1997; Grandin, 1980), within breed classification, crossbreeds (Murphey, et al., 1980, 1981, Stricklin et al., 1980), and within sex (Voisinet et al., 1997).

Temperament differences among breeds of cattle have been extensively researched. In cattle, *Bos indicus* breeds are generally considered more temperamental compared to *Bos taurus* breeds (Hohenboken, 1987, Tulloh, 1961; Voisinet, 1997; Grandin, 1980). Within *Bos taurus* breeds, differences in flight zone, tendency to approach novel objects (Murphey et al,. 1980, 1981), excitability (Stricklin et al., 1980), and social ranking (Stricklin et al., 1980; Wagnon et al., 1966) are evident among different breeds. British breeds are more docile than European continental breeds, and have a significantly lower flight-speed while leaving the crush (Hoppe, 2008). Tulloh (1961) evaluated differences between European breeds of Angus, Hereford, and Shorthorn compared to Holstein and discovered that Angus are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move, while Holsteins tend to move more slowly. Temperament is a moderately heritable trait in beef cattle (Stricklin, et al., 1980). While breed temperament generalizations can be made, individual behavioral differences within breeds of cattle must be considered and have been evaluated by Kilgour et al. (2006).

In a review by Burrow (1997), measurements of temperament included balking rating (Grandin, 1993b) and ease of movement tests (Hinch and Lynch, 1987; Tilbrook et al., 1989; and Kabuga and Appiah, 1992). Burrow (1997) also referred to a “freeze” response in the Australian

Meat Research Committee (AMRC) study (1988) when animals were restrained, particularly cattle with higher Brahman inheritance. In assessing anxiety-related behaviors in Hereford x Angus cross cattle, Bristow and Holmes (2007) mentioned the reaction of some animals "freezing" or resisting entry to the squeeze chute, while others walked calmly into the alley. Grandin (1993b) assessed the relationship between temperament and balking behavior of bulls and steers under consecutive restraint sessions. She concluded that behaviorally agitated animals balked significantly less than animals with a calm score. Tulloh (1961) found no relationship between temperament score and balking in cattle movements entering the scale or crush and concluded that cattle that are difficult to handle do not necessarily have a bad temperament. Genetic selection that has occurred over the past few decades may have had effect on the incidence of balking behavior and may or may not be associated with temperament.

Economically, the time required for handlers to keep cattle moving forward is a loss. Hundreds of workers are placed throughout the processing line and disruption due to animal handling difficulties creates slowed production. Additionally, the risk of decline in the quality of carcass traits has been correlated to poor temperament and stress before harvest (King et al., 2006; Grandin, 1993a). Animals that continually must be coerced may present an increased risk of an unfavorable product. However, electric prod use for a handling aid compared to other aids may be most time efficient for cattle line movement (Croney et al., 2000).

Coat color, characteristic markings, and phenotype may be a suggestion of breed-type classification which is confounded with crossbreeding. Identifying balking tendencies based on breed-type and gender is important for animal wellbeing and industry economics. Using scientific method procedures, objectives were to discover if balking behavior has a breed-type predominance based on coat color, differs between sexes, or affects carcass economics.

Materials and Methods

All experimental procedures performed in this study were reviewed and approved by the University of Arkansas Animal Care and Use Committee (IACUC, protocol number 10013).

Subjective observations (n = 6,510) were taken at a large-capacity federally inspected beef processing plant in Texas. Physical environmental distractions were removed, handlers trained according to American Meat Institute Guidelines and corporate protocol, and facility designed to decrease the incidence of balking. The facility was designed by the method of Grandin (2008), with curved alleys consisting of solid concrete side walls with a gradual incline into the building and entrance to the center track, double rail restrainer (Grandin, 1988, 1991). Line speed was 390 animals/hour. Two consistent trained observers recorded data. Observers stood near the restrainer entrance, out of direct vision and behind the point of balance so as not to cause balking or distraction as animals progressed into the restrainer. One observer recorded coat color and characteristic markings as animals entered the opening to the indoor working facility. Characteristic markings included facial hair color markings, the presence of outward protruding horns measuring at least 14 cm visually, or the obvious presence of a shoulder hump indicative of *Bos indicus*-breeding influence. These notations were based on the previous National Beef Quality Audits, 2000, 2005, and 2011 (McKenna et al., 2000; Garcia et al., 2008; and McKeith et al., 2012). A colored marking was placed on the last animal of the lot to denote different lots.

The other observer recorded balking behavior just prior to the center track, double rail restrainer designed by Grandin (1988, 1991) using the following developed Balking Score Criteria: 1 = none; willing forward movement; 2 = stops; then proceeds on own; 3 = persuasion needed, shake of paddle/handling aid or manual tap on rump/tail area; 4 = persistent balk, 2+ persuasion efforts needed to continue forward motion or 1 use of electric prod; 5 = intense balk;

electric prod 2 ± times required for continued forward motion. Supporting information included lot numbers, indicative of day of week and order of entry for processing, average live pen weight, sex of pen which was identified as steers (1), heifers (2), or mixed pens containing both steers and heifers (3), number of animals per lot, and feedlot source (assigned alphabetical notation, A through O). Not all colors, combinations of colors or characteristic markings, or sex categories were represented on every collection date. Data were recorded on seven different dates from 16 May 2012 through 11 May 2013. Collection dates were random. Two dates included observations from both “A” and “B” shifts under at least two different handlers. All other observation dates had observations from either “A” shift or “B” shift, but not both. Cattle were received from 15 different feedlots and presented 12 color/marking combinations. Dressing percentage (DP) was calculated from mean hot carcass weight divided by mean live pen weight for each lot.

Statistical Analyses

Data were analyzed with SAS (SAS Inst., Inc., Cary, NC) using PROC MIXED, PROC CORR and PROC FREQ. For frequencies, significance was determined using chi-square. For PROC MIXED, the initial model contained fixed effects for color, sex, and presence of horns or hump. Random effect was time within date on the subject of lot within feedlot. A separate analyses using PROC MIXED contained the fixed effect of feedlot. Random effect was lot. Means were reported as least squares using *F*-protected *t*-tests.

Results

Black cattle represented the majority of the colors at 45.7% (*n* = 2,975), followed by Holstein steers at 15.9% (*n* = 1,037), black-white face at 7.3% (*n* = 475), red at 7.3% (*n* = 472), white at 6.5% (*n* = 425), yellow at 4.6% (*n* = 297), red-white face at 4.2% (*n* = 273), brindle at

2.3% (*n* = 148), brown at 1.8% (*n* = 120), gray at 1.6% (*n* = 107), spotted (excluding Holstein) at 1.6% (*n* = 101), and yellow-white face at 1.2% (*n* = 80) (Table 1). The majority of the cattle were steers (*n* = 5,269) at 80.9% while heifers (*n* = 1,097) represented 16.9% of the animals, and the mixed pens containing both steers and heifers (n = 144) were 2.2% of the total (Table 1).

Means (Table 2) illustrate balking score at 1.6 ± 1.1 with all possible balking scores presented from the low of 1 to a high of 5. Mean live pen weight was 596.4 ± 43.8 kg with a low of 479.4 kg to a high of 655.4 kg. Mean number of animals per lot was 124 ± 90.1 with a low of 20 and high of 376. Dressing percentage mean was 64.5 ± 1.5 with the lowest percentage at 61.4 and the highest at 67.6. Only 20 carcasses out of 6,510 were deemed dark cutters (data not shown) as reported by the plant over all observation dates.

Coat color affected balking behavior scores. Holstein steers balked more ($P < 0.0001$) than all other colors which were similar (Table 3). Mean balking score for Holsteins was 2.1 ± 0.1 while all other coat colors varied from a mean of 1.5 ± 0.1 to 1.7 ± 0.1. Sex differed in balking incidence with heifers balking more ($P = 0.05$) than steers, and pens containing both steers and heifers balked intermediately (Table 3). Mean balking score for heifers was 1.73 ± 0.1 while steers mean score was 1.48 ± 0.04, and pens containing both heifers and steers balked intermediately at 1.67 ± 0.2.

Influence of *Bos indicus* breeding was assessed visually on the animal upon entrance into the building, just prior to the restrainer. A *Bos indicus*-type animal had a dorsal thoracic hump > 10.2 cm (Garcia et al., 2008). The visual presence of horns > 12.7 cm was assigned according to the method of McKeith et al., 2012. Neither *Bos indicus*-type breeding nor the presence of horns had effects ($P = 0.4$) on the incidence of balking behavior in these observations (Table 3).

The majority of animals (77.2%, *n* = 5,028) received a balking score of 1 over all dates, reflecting no hesitation to enter the restrainer. Of animals that received a balking score of 4 or 5 (13.1%, *n* = 852 and 1.5%, *n* = 97, respectively), the combined percentage receiving these scores was 14.6%. These animals showed moderate to extreme balking behavior and required action from handlers to continue line speed (Table 4).

In a separate analysis, the feedlot source affected ($P < 0.0001$) balking behavior, with balking score means varying from the lowest at 1.1 ± 0.3 to 2.3 ± 0.7 as the highest mean (Table 5). Feedlots B, J, F, M, H and C had higher ($P < 0.0001$) mean balking score than feedlots G, D, O, E, N, L, I and A, with feedlot K being intermediate.

Mean live pen weight was affected by color (Table 6). Holstein steers had greater ($P < 0.0001$) pen weights than all other colors which were similar. Steers had heavier ($P < 0.0001$) pen weights than mixed pens, with heifers having the lowest pen weight (602.8 ± 15.4 kg, 546.1 ± 0.59 kg, and 541.1 ± 0.36 kg, respectively).

Mean live pen weight was affected ($P < 0$ .0001) by feedlot source (Table 7). Mean live pen weights varied from the highest at 639.3 ± 4.3 kg to the lowest at 479.4 ± 20.6 kg. Feedlots J and N had the highest live pen weights which were similar, and greater than all others except feedlot G which was intermediate. Feedlots B and F were similar with the lowest mean live pen weight, with feedlot A being intermediate.

Dressing percentage was calculated by dividing mean pen hot carcass weight by mean live pen weight and was reported by the plant. Dressing percentage was affected ($P < 0.0001$) by coat color and sex ($P = 0.01$) (Table 8). Data were tested using a contrast statement with mean dressing percentages of each color of beef cattle compared to Holstein cattle. Mean DP for the beef cattle was 64.4 ± 0.21 percent vs. 61.6 ± 0.21 percent for Holstein steers. Steers had the greatest ($P = 0.01$) dressing percentage at 64.8 ± 0.1 versus heifers at 64.3 ± 0.3 with mixed pens being intermediate at 63.4 ± 0.5 (Table 8).Using Pearson product moment correlations, balking behavior was negatively correlated ($r = -0.18$, $P < .0001$) with dressing percentage (Table 9). Mean live pen weight showed no significant correlation with balking behavior.

Dressing percentage (DP) differed by feedlot ($P < 0$ .0001) but not in the same pattern as means for live pen weight. Percentages ranged from 66.3 ± 0.8 at the highest to 62.0 ± 0.8 at the lowest. Table 10 reflects ranking of feedlots from the highest to lowest DP. Feedlots L, H, K and G had the highest DP and differed ($P < 0.0001$) from feedlots I, E, M, A, J, B and F, while feedlots D, O, N and C were intermediate.

Discussion

The Humane Slaughter Act sets requirements for humane slaughter, and livestock must be humanely slaughtered in order to become part of the US food system. The Humane Slaughter Act, 7 U.S.C. §§ 1901-1906, is enforced by the Secretary of Agriculture under provisions of the Federal Meat Inspection Act, 21 U.S.C. §§ 603(b). The provisions of this statute and accompanying regulations outline the methods of slaughter that are deemed to be “humane,” and thus appropriate for use in slaughtering livestock (National Agricultural Law Center). The Humane Slaughter Act regulations, codified at 9 C.F.R. §§ 313.1-313.90 and discussing livestock handling, states that “electric prods...employed to drive animals shall be used as little as possible in order to minimize excitement and injury” 9 C.F.R. §313.2(b). Any use of the prod, which in the opinion of the inspector is excessive, is prohibited. Inspectors are employed by the Food Safety and Inspection Service, an agency of the United States Department of Agriculture. Inspectors that observe inhumane handling shall inform the establishment operator and require that necessary steps be taken to prevent a recurrence. If no action is taken, or if actions are ineffective, the inspector may attach a “U.S. Rejected Tag” to the alleyways leading to the stunning area if the cause of inhumane treatment is the result of establishment employee actions in the handling or moving of livestock. After the tagging of the alleyway, no additional livestock may be moved into the stunning area until the inspector has been assured that no further egregious situations will occur and has removed the tag 9 C.F.R. §313.50. Until the welfare concern has been resolved, production ceases. Further, a definition of “egregious inhumane handling” has been outlined in a directive by the Food Safety Inspection Service Directive as any act or condition that results in severe harm to animals. For example: excessive… prodding of ambulatory or non-ambulatory disabled animals or dragging of conscious animals (Food Safety and Inspection Service Directive 6900.2: Revision 2, 8/15/2011).

Corporations are aware of the regulations and repercussions to improper animal handling. Employees that handle cattle are trained according to the American Meat Institute Guidelines (2013) which are updated regularly. Corporations self-audit to assure animals are being treated humanely and employees are following regulations, termed a 1st party or internal audit. Companies that purchase products supplied by the plant also audit adherence to regulations and is considered a 2nd party or external audit. When a company buying the products hires an independent, outside auditing company to conduct an audit, this is considered a 3rd party audit. Virtually all auditors are certified by the Professional Animal Auditor Certification Organization, Inc. Corporations allow an expense of approximately $2000 for 3rd party audits (personal communication, anonymous). A failed audit results in re-auditing, cost of retraining employees, and all customers may request a copy of the audit before purchasing products. Egregious handling events are also part of the public record through the Food Safety and Inspection Service website. Animal handling is a serious issue in many regards in beef processing plants. Balking scores of 4 or 5 represent behavior that may have required the use of the electric prod to continue line movement. The total percentage of animals observed in this study that received a 4 or 5 balking score was 14.6%, well below the acceptable level of 25% by the American Meat Institute Animal Handling Guidelines and Audit Guide (2013).

An advantage to observing cattle in a high-capacity beef processing plant was that it was possible to view balking behavior in large numbers of animals in a short period of time. However, limitations to the observations were that cattle were sourced from several feedlots located at varying distances, with unknown breeding of animals except for Holsteins. Cattle mostly consisted of *Bos taurus* beef cattle breeding, with *Bos indicus* breeding being represented, as well as fed Holstein steers. This is consistent with cattle selected for production in the southern United States.

Consistent with National Beef Quality Audits (McKenna et al., 2002, Garcia et al., 2008, and McKeith et al., 2012), the number of black-colored cattle have increased over time and present the most predominant coat color (45.7%) in our study. No differences were seen among balking behavior of beef cattle, despite coat color or *Bos indicus* breeding. These findings are not in agreement with Tulloh (1961) who reported that Angus cattle are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move. Our findings suggest no difference in balking among different beef breed-type animals, based on coat color and unknown breeding.

Heifers balked more than steers, with pens containing both sexes balking intermediately. Pens of heifers presented at three out of the seven collection dates, and mixed pens were represented in four of the seven dates. There is a sex effect in the tendency to balk at entry to the restrainer.

Balking behavior in dairy cattle and beef cattle differ at entrance to the restrainer in the beef processing plant. Holsteins have been selected for their milk production and docile behavior for ease in human handling during milking. Dairy calves are typically removed from their dams shortly after birth and have exposure to humans throughout their lifetime. Duff and McMurphy (2007) describe typical Holstein steer production in the southwestern part of the United States. Calves are raised in hutches approximately 60 days before weaning. After weaning, calves are managed to reach approximately 125 kg before shipping to feedlots. Finishing Holstein steers to an end weight of 590 kg can require 12 months in a somewhat consistent environment (Duff and McMurphy, 2007). This may lead to decreased fear of humans and decreased flight zone. Additionally, this management may impose fewer handling events and less exposure to novel handling events than occurs with typical beef cattle production. Behaviorally, Holstein steers differ from traditional beef breeds (Duff and McMurphy, 2007). Tulloh (1961) reported that Holsteins tend to move more slowly compared to Angus, Hereford, and Shorthorn cattle. Holsteins have a gentle temperament and are playful, easily bored and may sort through feed, and are difficult to move because they have a tendency to follow humans (Duff and McMurphy, 2007). Difficulty moving may equate with a tendency to balk. An antonym for “bored” is “interested” (http://thesaurus.com/browse/bored). These behavioral generalities may help qualify the author’s conclusion that Holstein steers are “curious” or “interested” in inspecting their novel surroundings at entrance to the restrainer, thus increasing balking tendencies. These balking tendencies may be more of a slowing to investigate surroundings.

Tillbrook et al. (1989) investigated the social behavior of 14 mo old cattle, including Holstein, Jersey x Holstein, or Hereford x Holstein crossbred bulls and steers, in an ease of movement test. No differences were shown between the bulls and steers in this test, but authors suggested that animals that are highly fearful of humans may respond more to the handler than the novel environment. The opposite perspective may hold true; animals with low fear of humans may respond more to a novel environment than to a handler.

Baszczak et al. (2006) evaluated the entry force required to move steers into the chute for various breeds of cattle. Breeds included British (Angus, composites with predominance of Red Angus, Angus, Hereford, and Angus x Hereford crossbreds), Continental crossbred (Charolais x British composites, Limousin x Angus), and Brahman crossbred (Beefmaster) that originated from 5 ranches. Scores ranged from 1 to 4, with 1 requiring no assistance and 4 requiring two or more electrical prods. Results indicated that entry force score was higher ($P < 0.05$) for Continental crossbred steers compared to Brahman crossbred or British cattle, which were similar. Our results showed no difference in balking in beef cattle breeds, regardless of breeding.

Balking scores differed ($P < 0.05$) depending on feedlot (Table 5). Data collection dates and times were random, so represented feedlots were random as well. Not all feedlots were represented on all collection dates. Further investigation revealed that the mean mileage from the feedlots to the processing plant was an estimated 116 km, ranging from 58 to 394 km. Six of the feedlots were approximately 80 km from the processing plant. With the one feedlot at 394 km removed from simple means, the mean distance of feedlots from the processing plant was 97 km. Thus, location differences should be minimal in affecting balking behavior differences per plant. Consideration should be given to differences in management practices among feedlots that may affect balking behavior.

Mean live pen weight was greatest for Holstein cattle, with all other colors being similar. Mean live pen weight differed among feedlots. Gut fill, shrink, breed, feed ration, health, gender, age, season, location, and frame score are some factors affecting mean live pen weight. Cattle were weighed on trucks prior to unloading. Additionally, mean dressing percentages differed ($P < 0.05$) by feedlot source.

Our results suggest an association with DP and balking behavior in fed Holstein steers. Dressing percentage is lower in Holsteins compared to beef cattle breeds. Dressing percentage is affected by sex in that heifers have lower DP than steers. Other speculative factors that may cause differences in balking behavior at the processing plant are difference in management at the feedlot source, but require further investigation to confirm or deny.

Conclusion

Animal welfare, handler safety, beef quality, and economy of maintenance of line speed are all considerations in balking behavior of cattle at the processing plant. Our data suggest a breed-type predominance in the incidence of balking at the beef processing plant in that fed Holstein steers balk more than beef cattle. There also appears to be a sex effect in balking incidence in that heifers balk more frequently than steers. Feedlot source may be a source of variation in balking behavior of cattle at the processing plant.

References

American Meat Institute Foundation. 2013. Recommended animal handling guideline & audit guide: A systematic approach to animal welfare. Grandin, T. and American Meat Institute Animal Welfare Committee. Core Criteria 5: Electric prod scoring criteria for cattle. 51. www.meatami.com/. Accessed 11 October 2013.

Australian Meat Research Committee Final Report UNQ7. 1988. Genetic and environmental methods of improving the temperament of *Bos indicus* and crossbred cattle. James Cook University of North Queensland.

Baszczak, J.A., T. Grandin, S.L. Gruber, T.E. Engle, W.J. Platter, S.B. Laudert, A.L. Schroeder, and J.D. Tatum. 2006. Effects of ractopamine supplementation on behavior of British, Continental, and Brahman crossbred steers during routine handling. J. Anim. Sci. 84:3410-3414.

Croney, Candace C., L.L. Wilson, S.E. Curtis, and E.H.Cash. 2000. Effects of handling aids on calf behavior Appl. Anim. Behav. Sci. 69:1-13.

Duff, G.C., and C.P. McMurphy. 2007. Feeding Holstein steers from start to finish. Vet. Clin. Food Anim. 23:281-297.

Grandin, T.1993. Behavioral principles of cattle handling under extensive conditions. In Livestock Handling and Transport (T. Grandin, ed.) pp 43-57. CAB International, Wallingford, UK.

King, D.A., C.E. Schuehle Pfeiffer, R.D. Randel, T.H. Welsh Jr., R.A. Oliphint, B.E. Baird, K.O. Curley Jr., R.C. Vann, D.S. Hale, J.W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74; 546-556.

Bristow, Daniel J. and David S. Holmes. 2007. Cortisol levels and anxiety-related behaviors in cattle. Phys. and Behav. 90:626-628.

Federal Meat Inspection Act, 21 U.S.C. §§ 603(b).

Burrow, H.M., 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abst., 65(7):477-495.

Croney, Candace C., L.L. Wilson, S.E. Curtis, and E.H.Cash. 2000. Effects of handling aids on calf behavior Appl. Anim. Behav. Sci. 69:1-13.

Garcia, L.G.; Field, T.G.; Belk, K.E.; Morgan, J.B.; Smith, G.C.; Tatum, J.D.; Scanga, J.A.; VanOverbeke, D.L.; Lawrence, T.E.; Hoffman, T.W.; Nicholson, K.L.; Savell, J.W.; Griffin, D.B.; Hale, D.S. 2008. National Beef Quality Audit-2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. J. Anim.Sci. 86:3533-3543.

Grandin, T. 1980. Livestock behaviors related to handling facilities design. Int. J. Stud. Anim. Prob. 1:33-52.

Grandin, T. 1988. Double rail restrainer for livestock handling. Int. J. Agric. Eng. 41:327-338.

Grandin, T. 1991. Double rail restrainer system for beef cattle. Am. Soc. Aric. Eng., Technical Paper 915004. St. Joseph, MI.

Grandin, T. 1993a. Behavioral principles of cattle handling under extensive conditions. In Livestock Handling and Transport (T. Grandin, ed.) pp 43-57. CAB International, Wallingford, UK.

Grandin, T. 1993b. Behavioral agitation during handling is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Grandin, T. 2008. Humane Livestock Handling. Storey Publishing, MA.

Hinch, G.N., J.J. Lynch. 1987. A note on the effect of castration on the ease of movement and handling of young cattle in yards. Anim. Prod. 45:317-320.

Hohenboken, W.D., 1987. Behavioral genetics. Vet. Clin. North Am.: Food Anim. Prac. 3:217-229.

Hoppe, S. 2008. Genetic and environmental factors affecting beef cattle temperament. 15th World Hereford Conference, Copenhagen.

Humane Slaughter Act, 7 U.S.C. §§ 1901-1906.

Humane Slaughter Act Regulations, 9 C.F.R. §§313.1-313.90, §313.2(b), Handling of Livestock. Tagging § 313.50.

Kabuga, J.D., P. Appiah. 1992. A note on the ease of handling and flight distance of *Bos indicus, Bos taurus* and their crossbreds. Anim. Prod. 54:309-311

Kilgour, Robert J., Gavin J. Melville, and Paul L. Greenwood. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity to humans, restraint, and novelty. Appl. Anim. Behav. Sci. 99:21-40.

King, D.A., C.E. Schuehle Pfeiffer, R.D. Randel, T.H. Welsh, Jr, R.A. Oliphint, B.E. Baird, K.O. Curley, Jr., R.C. Vann, D.S. Hale, J.W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74; 546-556.

McKeith, R. O.,Delmore, R. J.,Jr., L. M.,Lawrence, T. E.,Hale, D. S.,Gray, G. D.,Jr., L. M., Meadows, M. E.,Lawrence, T.E.,Belk, K. E. 2012**.** National Beef Quality Audit 2011: Harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and byproducts. J. Anim. Sci. 90:5135-5142.

McKenna, D.R, D.L. Roebert, P.K. Bates, T.B. Schmidt, D.S. Hale, D.B. Griffin, J.W. Savell, J.C. Brooks, J.B. Morgan, T.H. Montgomery, K.E. Belk, and G.C. Smith. 2002. National Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. J. Anim. Sci. 80:1212-1222

Murphey, R.M., F.A.M. Duarte, M.C.T. Penedo. 1980. Approachability of bovine cattle in pastures: Breed comparisons and a breedxtreatment analysis. Behav. Genet. 10:171-181.

Murphey, R.M., F. A. Moura Duarte, and M.C. Torres Pendo. 1981. Responses of cattle to humans in open spaces: Breed comparisons and approach-avoidance relationships. Behav. Genet. 11:37-48.

National Agricultural Law Center, Overview of Animal Welfare Laws, http://nationalaglawcenter.org/overview/animalwelfare/. Accessed 1 March 2014.

Professional Animal Auditor Certification Organization, Inc. http://www.animalauditor.org/paaco/. Accessed 7 March 2014.

Stricklin, W.R., C.E. Heisler, and L.L. Wilson. 1980. Heritability of temperament in beef cattle. J. Anim. Sci. 57:Suppl 1, 109.

Tillbrook, A.J., P.H. Hemsworth, J.L. Barnett, and A. Skinner. 1989. An investigation of the social behavior and response to humans of young cattle. Appl. Anim. Behav. Sci. 23:107-116.

Tulloh, N.M., 1961. Behavior of cattle in yards. II. A Study of temperament. Anim. Behav. 9:25-30.

USDA-FSIS, Directive 6900.2, Revision 2-- Humane Handling and Slaughter of Livestock, 8/15/2011.

Voisinet, B.D., T. Grandin, J.D. Tatum, S.F. O'Conner, and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

Wagnon, K.A., R.G. Coy, W.C. Rollins and F.D. Carroll. 1966. Social dominance in a herd of Angus, Hereford and Shorthorn cows. Anim. Behav. 14:474-479.

Table 1. Distribution of colors, sex, and presence of horns or *Bos indicus* influence over all dates.

Color		*n*	Percentage
Black		2,975	45.7
Black-white face		475	7.3
Brindle		148	2.3
Brown		120	1.8
Gray		107	1.6
Holstein		1,037	15.9
Red		472	7.3
Red-white face		273	4.2
Spotted (excluding Holstein)		101	1.6
White		425	6.5
Yellow		297	4.6
Yellow-white face		80	1.2
	Total	6,510	
Sex			
Steers		5,269	80.9
Heifers		1,097	16.9
Mixed pens of steers and heifers		144	2.2
Presence of horns or *Bos indicus*-type breeding			
Horns		260	4.0
Bos indicus influence		532	8.2

Table 2. Simple means of balking score, pen weight, animals/lot, and dressing percentage.

Variable	Means	N	SD	Extremes Low	Extremes High
Balking Score[1]	1.6	6510	1.1	1	5
Pen Weight, kg	596.4	6510	43.8	479.4	655.4
Animals/lot	124	6406	90.1	20	376
Dressing Percentage[2]	64.5	6406	1.5	61.4	67.6

[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.
[2]Dressing percentage: Hot carcass weight/live weight.

Table 3. Color, sex, presence of horns, and *Bos indicus* effects on balking score.

Color	Balking Score[1]	SE
Black	1.6 [b]	0.07
Black-white face	1.6 [b]	0.08
Brindle	1.5 [b]	0.11
Brown	1.5 [b]	0.12
Gray	1.5 [b]	0.13
Holstein	2.1[a]	0.10
Red	1.5 [b]	0.08
Red-white face	1.7 [b]	0.09
Spotted	1.7[b]	0.13
White	1.5 [b]	0.08
Yellow	1.6 [b]	0.09
Yellow-white face	1.6 [b]	0.14
Sex		
Steer	1.5[b]	0.04
Heifer	1.7[a]	0.10
Mixed pen	1.6[ab]	0.17
Horns		
Present	1.6	0.08
Not present	1.6	0.05
Bos indicus influence, thoracic hump		
Present	1.6	0.08
Not present	1.6	0.05

[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[ab]Within a column, means without a common superscript differ ($P < 0.05$).

Table 4. Distribution of balking scores[1] over all dates.

Balk Score	1	2	3	4	5	Total
n	5,028	313	220	852	97	6,510
Percent	77.2	4.8	3.4	13.1	1.5	100

[1]Balk score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

Table 5. Feedlot effect on balking score.

Feedlot	N	Percent	Balking Score[1]	SE
A	47	0.72	1.1[b]	0.28
B	39	0.6	2.3[a]	0.29
C	24	0.4	1.9[a]	0.32
D	316	4.9	1.5[b]	0.13
E	113	1.7	1.4[b]	0.25
F	263	4.0	2.0[a]	0.24
G	1275	20.0	1.6[b]	0.07
H	200	3.1	1.9[a]	0.25
I	305	4.7	1.3[b]	0.12
J	981	15.1	2.0[a]	0.06
K	71	1.1	1.6[ab]	0.21
L	90	1.4	1.3[b]	0.26
M	303	4.7	1.9[a]	0.11
N	1662	25.6	1.3[b]	0.07
O	818	12.6	1.4[b]	0.10

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.
[ab]Within a column, means without a common superscript differ ($P < 0.05$).

Table 6. Color effect on mean live pen weight.

Color	Pen weight, kg	SE
Black	560.3[b]	0.52
Black-white face	560.3[b]	0.52
Brindle	560.3[b]	0.52
Brown	560.3[b]	0.52
Gray	560.3[b]	0.52
Holstein	599.2[a]	0.77
Red	560.3[b]	0.52
Red-white face	560.3[b]	0.52
Spotted	560.3[b]	0.52
White	560.3[b]	0.52
Yellow	560.3[b]	0.52
Yellow-white face	560.3[b]	0.52

[ab]Within a column, means without a common superscript differ ($P < 0.05$).

Table 7. Feedlot effect on mean pen weight.

Feedlot	Pen weight, kg	SE
A	499.0[de]	20.62
B	487.6[e]	20.62
C	578.3[bc]	20.64
D	584.0[bc]	10.30
E	550.2[cd]	20.60
F	479.4[e]	20.59
G	598.0[ab]	21.07
H	544.3[cd]	20.59
I	544.3[cd]	8.42
J	639.3[a]	4.30
K	537.5[d]	14.58
L	594.2[bc]	20.60
M	575.4[c]	7.79
N	626.0[a]	5.32
O	597.8[b]	7.79

[abcde]Within a column, means without a common superscript differ ($P < 0.05$).

Table 8. Breed-type and sex effect on dressing percentage.

Breed-type	Dressing Percentage[1]	SE
Beef breeds	64.4[a]	0.21
Holstein	61.6[b]	0.21
$P < 0.0001$		
Sex		
Steers	64.8[a]	0.14
Heifers	64.3[ab]	0.32
Mixed pens	63.4[b]	0.52
$P = 0.01$		

[1]Dressing Percentage: Hot carcass weight/mean live weight.

[ab]Within a column, means without a common superscript differ ($P < 0.05$).

Table 9. Pearson correlation coefficients (*r*) with balking score, live pen weight, and dressing percentage.

Balking score[1] mean	Mean live pen weight	Mean pen dressing percentage[2]
r	-0.02	-0.18
P-value	0.18	<.0001
n	6,510	6,406

[1]Balk score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.
[2]Dressing percentage: Hot carcass weight/live weight.

Table 10. Feedlot effect on dressing percentage.

Feedlot	Dressing Percentage[1]	SEM
A	62.7 [b]	0.79
B	62.1 [b]	0.78
C	64.9 [ab]	0.78
D	65.3 [ab]	0.39
E	63.3 [b]	0.78
F	62.0 [b]	0.78
G	65.5 [a]	0.21
H	66.2 [a]	0.78
I	64.6[b]	0.32
J	62.3 [b]	0.16
K	66.0 [a]	0.55
L	66.3[a]	0.78
M	62.9 [b]	0.29
N	65.1 [ab]	0.20
O	65.2 [ab]	0.29

[2]Dressing percentage: Hot carcass weight/live weight.

[ab]Within a column, means without a common superscript differ ($P < 0.05$).

Acknowledgements

I offer my sincere gratitude to the corporation and personnel at the processing plant that allowed privileged access for this study.

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MEMORANDUM

TO: Hayden Brown

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 9, 2009

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : November 8, 2012

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#10013- "GENETIC CONSIDERATIONS FOR BEEF CATTLE PRODUCTION IN CHALLENGING ENVIRONMENTS** ". You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **11-08-2012,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

UofA
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RESEARCH & EXTENSION
University of Arkansas System

Center for Food Animal Wellbeing

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April 11, 2014

To Whom It May Concern:

I am writing this letter to certify that Michelle Lynn Thomas conducted the work and wrote in excess of 51% of chapter 2 of her dissertation entitled *"Balking Incidence and Behavioral Responses During Handling in Fed Cattle with Genetic and Carcass Implications"*.

Please contact me if you have further questions or concerns.

Sincerely,

YvonneVizzier Thaxton, Ph.D.
Professor and Director, Center for Food Animal Wellbeing

Chapter 3: Behavioral Responses during Handling of Fed Holstein Steers at the Feedlot and Balking Behavior at the Processing Plant

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Abstract

Balking behavior in the cattle processing line can pose welfare issues when electric prod use to coerce forward movement is implemented. Tests have been devised to assess temperament, with each test measuring different aspects of behavior. The objective of this field study was to discover any association of balking behavior with chute behavior or chute exit velocity in fed Holstein steers at the feedlot. These animals were subsequently followed to the beef processing plant to gain a balking score at entrance to the restrainer to discover if balking behavior between the feedlot and the plant was associated. Two groups of fed Holstein steers differed by age, weight, and scheduled treatment at time of behavioral observation in one feedlot. Balking scores and chute scores were assigned in addition to exit speed leaving the chute. Balking scores were on a scale of 1 through 5 taken at entry to the scale or chute. Balking scores including degree of balking: 1 = None; willing forward movement; 2 = Stops; then proceeds on own; 3 = Persuasion needed; shake of paddle or tap on rump/tail area; 4 = Persistent balk; two or

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more persuasion efforts needed to continue forward motion, or 1 use of electric prod; 5 = Intense balk; multiple persuasion efforts or electric prod two or more times required for continue forward motion. In Group 1 steers at the feedlot, there was a negative relationship between balking score and chute score (r = -0.21, P = 0.0003), a positive association between balking score and exit velocity (r = 0.20, $P < 0.0001$), and an inverse association between chute score and exit velocity (r =-0.96, $P < 0.0001$). No significant ($P > 0.05$) coefficients were shown on Group 1 steers between plant balking, feedlot balking, feedlot chute score, feedlot exit velocity or hot carcass weight. In Group 2 steers at the feedlot, balking behavior was inversely associated (r = -0.13, P = 0.03) with live weight. Chute scores were negatively associated (r = -0.90, $P < 0.05$) with exit velocity. At the feedlot, chute score was greater ($P < 0.05$) for Group 2 than Group 1 (2.2, 1.6, respectively). Group 1 steers had faster ($P < 0.05$) exit velocity compared to Group 2 steers (1.4, 1.0, respectively). At the plant, balking score was higher ($P < 0.05$) for Group 2 than for Group 1 (2.4, 1.6, respectively), even at similar finished weights. Balking behavior at the feedlot, in addition to other temperament indicator scores, do not necessarily forecast balking behavior at the processing plant. In this study, balking behavior at neither the feedlot nor the plant appears to be associated with hot carcass weight, and therefore, carcass economics.

Introduction

Balking behavior in the cattle processing line can pose welfare issues as electric prod use to coerce forward movement is implemented. This balking behavior requires stimulus from the handler to coerce the animal and may present a challenge. When standard stimuli fail, animal welfare issues emerge as more persuasive handling aids such as the electric prod are needed to keep animals moving.

Anecdotal reports from experienced handlers suggest certain breed-types cause more difficulties to maintain forward movement than other breed-types. Animals described as having poor temperament are usually those who exhibit behaviors that make handling more difficult, take more time than average to perform management procedures, or present danger to humans. In studies evaluating temperament in cattle it is often defined by observed behavioral responses to humans or human handling procedures. Tulloh (1961) evaluated differences between European breeds of Angus, Hereford, and Shorthorn compared to Holstein and discovered that Angus are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move, while Holsteins tend to move more slowly.

Chute scores and exit velocity are accepted tests to assess temperament. Chutes scores assign a subjective score with varying degrees for the behavior when cattle are restrained (Grandin, 1993). In contrast, an objective measure of exit velocity can be a calculation based on time required for the animal to traverse a set distance after leaving the chute (Burrow, 1988; Curley et al., 2006). Flight speed can also be scored as a visual scoring of the animal leaving the chute; although a subjective method, it is also valid. The behavior this test is measuring is debatable, in that it could be measuring the level of fear of humans the animal feels while receiving treatment, or the degree of aversiveness of the treatment, as it affects the speed at which the animal exits to escape the human and/or treatment. Another philosophy is that it measures the degree of gregariousness, or the speed at which the animal uses to join herd mates. The actual motivation of the animal is still under question.

The objective of this observational field study was to evaluate behavioral responses to handling fed Holstein steers in two environments; the feedlot and the processing plant. The incidence of balking and balking behavior scores have not been used in the past as a temperament indicator test. This study was developed to discover any association of balking behavior with chute behavior or exit velocity in fed Holstein fed steers. Further, these animals were followed to the beef processing plant to gain a balking score at entrance to the restrainer to discover if balking behavior between the two locations was associated, and if other behavioral scoring systems were related to balking behavior at the feedlot.

Materials and Methods

All experimental procedures performed in this study were reviewed and approved by the University of Arkansas Animal Care and Use Committee (IACUC, protocol number 10013).

Two lots of fed Holstein steers differed by age, weight, and scheduled treatment at time of behavioral observation. The feedlot was located in west Texas. Steers were fed a corn-based, step-up ration. Behavior scoring coincided with a scheduled application of growth implant on July 30, 2012. Group 1 steers (GRP1, n = 290) was approximately 8.6 mo of age, and the other group, Group 2 (GRP2, n = 269) were older, at approximately 11.6 mo of age. Steers originated from two dairy calf ranches in California which were located within 65 km of each other. Each group had been placed at the feedlot at approximately 4 mo of age, and entered the feedlot weighing an average of 136 kg. Each of the two lots was divided into two slaughter groups upon finish, resulting in four slaughter dates. Animals were transported 64 km and observed at the entrance to the restrainer in a federally inspected processing plant.

Individual hot carcass weights were obtained by reading radio frequency electronic identification ear tags that were matched to the carcasses. Pen weights were obtained by weight taken on the truck prior to unloading, so individual weights were not recorded. Mean dressing percentage was calculated using hot carcass weight divided by mean pen weight.

Animals

Behavioral observations for both groups occurred on July 30, 2012. The GRP1 steers presented into the working facility for application of their second growth implant. There were 290 steers in this group. Animals were approximately 260 days of age, or 8.7 months. The steers that were finished first (*n* = 65) were slaughtered on March 29, 2013 at approximately 16.7 mo of age.

The GRP2 steers presented to the working facility for their third and final growth implant. Steers were approximately 350 days of age, or 11.7 months. The top finishing portion of the steers (*n* = 66) were slaughtered on December 6, 2012, at approximately 16 mo. Animals were transported to the same processing plant as the younger steers. Only data on the first finishing animals for each group are presented on balking scores at the plant.

Behavior Scoring

Balking scores and chute scores were assigned in addition to flight speed leaving the chute. Balking scores were determined by one trained observer using a modified scoring system based on the method by Grandin (1993b). Balking scores were on a scale of 1 to 5 taken at entry to the scale or chute. Balking scores including degree of balking: 1 = None; willing forward movement; 2 = Stops; then proceeds on own; 3 = Persuasion needed; shake of paddle or tap on rump/tail area; 4 = Persistent balk; two or more persuasion efforts needed to continue forward motion, or 1 use of electric prod; 5 = Intense balk; multiple persuasion efforts or electric prod two or more times required for continue forward motion. A final balking score was taken at the entry to the center track double rail restrainer in the federally inspected beef processing plant.

Chute scores ranged from 1 to 5 according to the method by Grandin (1993b). Chute scores were determined by one trained observer. Scores were assessed after the head was restrained in the head gate and prior to the sides being closed on the chute. The scoring system included: 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

Flight speed exiting the chute was recorded using infrared sensor timing lights (Farm Tek Inc., North Wylie, TX) to remotely trigger the start and stop of timing. Flight speed was converted to exit velocity (1.83 m (distance)/time (s)) as described by Burrow et al., 1988. To discover if balking behavior was associated with chute behavior and exit velocity leaving the squeeze chute, Pearson correlation coefficients were determined.

Statistical Analyses

Data were analyzed with SAS® (SAS Inst., Inc., Cary, NC) using PROC MIXED and PROC CORR. Simple means were reported for PROC CORR. For PROC MIXED, the initial model contained fixed effect of lot. Random effect was steer individual identification. Means were reported as least squares and were separated using *F*-protected *t*-tests.

Results

For GRP1 steers, balking score mean at entry to the scale was 1.7 ± 1.0, and chute score mean was 1.5 ± 0.7. Exit velocity mean was 1.5 ± 0.5. GRP1 steers weighed 266 ± 26.5 kg at the observation date (Table 1). Mean balking score for GRP2 steers at the feedlot was 1.5 ± 0.1. Mean chute score was 2.0 ± 0.1, and mean exit velocity was 1.2 ± 0.6. Mean weight was 445 ± 32 kg (Table 1).

Pearson correlation coefficients (r), and P-values for feedlot behavioral observations and live weight in both GRP1 and GRP2 steers are reported in Table 2. In GRP1, there was a negative relationship between balking score and chute score (r = -0.21, P = 0.0003). There was a positive association between balking score and exit velocity (r = 0.20, P < 0.0001). There was an inverse association between chute score and exit velocity (*r* =-0.96, $P < 0.0001$). In GRP2 steers, In GRP2 steers, Pearson correlation coefficients reflect balking behavior at the feedlot was inversely associated with live weight (*r* = 0.13, $P = 0.03$). Chute scores had a positive association ($P < 0.05$) with exit velocity at the feedlot (Table 2).

Correlation coefficients for behavior at the feedlot and those at the plant are shown in Table 3 for GRP1 steers. There were no significant ($P > 0.05$) Pearson correlation coefficients shown between plant balking, feedlot balking, feedlot chute score, feedlot exit velocity, feedlot live weight, or hot carcass weight (Table 3).

Table 4 illustrates Pearson correlation coefficients in GRP2 steers. There was a positive association (*r* = 0.25; $P = 0.05$) between balking behavior at the plant and live weight at the feedlot (Table 4). Additionally, mean hot carcass weight is positively associated (*r* = 0.44, $P = 0.0003$) with live weight recorded at the plant months prior to slaughter. All other correlations were non-significant ($P > 0.05$).

Table 5 reflects least squares means for both GRP1 and GRP2 steers that finished earlier than the majority of the group. Mean balking score at the feedlot were similar between GRP1 and GRP2 steers. Mean chute score was greater ($P < 0.05$) for GRP2 steers (2.2) than GRP1 steers (1.6). GRP1 steers had faster (1.4, $P < 0.05$) EV compared to GRP2 (1.0). Mean balking score at the plant was higher ($P < 0.05$) for GRP2 (2.4) than GRP1 (1.6), even at finished weights. Hot carcass weight did not differ between GRP1 and GRP2 steers

Discussion

Gaining access to private feedlots and large commercial beef processing plants is a privilege. Conditions were not consistently available on all dates due to unexpected challenges common in an industry production setting. Therefore, data for only the first finishing animals in each group obtained at the processing plant are presented for comparison. Data for all animals for each group during the feedlot observations are presented.

Chute scores are an estimated temperament measure of reaction to restraint (Tulloh, 1961; Fordyce et al., 1988; Gonyou et al., 1986). It is a subjective scoring system as the observer must determine the degree of struggle after restraint, if exhibited. It may also measure the degree of fear while humans are within the animal’s flight zone, or learned fear due to previous handling experiences or procedures. Variation in chute scores has been shown between breed types, among breed types, and among individuals within a breed. Consistency of chute scores over consecutive assessment dates has also been evaluated.

Exit velocity is theorized to measure the degree of gregariousness in that the isolated, restrained animal is trying to join herd mates after release. Another theory is that exit velocity is a measure of fear of humans. Handlers are typically located deep into the flight zone of the animal performing necessary management procedures while the animal is in the chute. Therefore, the flight speed may reflect how quickly the animal wants to escape from the presence humans. This temperament measure can be accomplished as an objective score by the use of electronic timing devices that provide a precise unit of measure over a set distance. The data in this study were collected using the method of Burrow et al., 1988. The distance between the first and second infrared barriers was 1.8 m and time (s) to traverse that distance was recorded. To calculate exit velocity, the distance (1.8 m) was divided by the time (s). Exit velocity may be the most accurate method to determine temperament as it is an objective measure (Burrow, 1988; Curley et al., 2006; Müller and von Keyserlingk, 2006). Further, it has been suggested to calculate an overall temperament score using chute score and EV. A combined score may reflect a measure of varying aspects of response to handling, and have greater reliability compared to individual behavior score assessments (Café et al., 2011; Fransciso et al., 2012) However, these scoring systems are rarely performed on fed Holstein steers in the U.S.

While we know correlations do not imply cause and effect, they suggest a relationship between two components under investigation. Table 2 illustrates correlation coefficients between balking scores, chute scores, and EV within each group (GRP1 and GRP2) of steers at the feedlot. Balking scores were negatively associated with chute score in GRP1, showing an inverse relationship. This implies the higher the balking score, the lower the chute score. In GRP1 steers, there was a positive relationship between balking score and exit velocity. This suggests animals that had higher balking scores would also have faster exit velocities from the chute. There was a very strong negative relationship between chute score and EV in GRP1 steers. Animals that were more reactive in the chute had slower exit velocities. In GRP2 steers, balking score at the feedlot was slightly, negatively correlated with live weight (Table 2). Animals that weighed less balked more. Chute score was strongly inversely related to exit velocity. Animals that were agitated in the chute exited the squeeze chute slowly. This same effect was seen in the GRP1 steers.

In Table 3, correlations between feedlot behavioral observations and processing plant balking scores for GRP1 steers are shown. There were no significant ($P > 0.05$) correlations of plant balking behavior with previous balking at the feedlot, chute score, or exit velocity. Additionally, there were no significant ($P > 0.05$) correlations of hot carcass weight with feedlot behavior scores or balking at the plant. This would imply no negative carcass effects of balking behavior. Feedlot live weight, taken several months prior to slaughter, was positively associated with final hot carcass weight.

For GRP2 steers, there was a slight positive association between balking behavior at the processing plant and live weight at the feedlot (Table 4). A positive association implies that animals that had a higher balking score at the plant, or were more resistant to entering the restrainer, were the heavier animals at the time of data collection at the feedlot. Hot carcass weight was also moderately positively associated with heavier weight at the feedlot.

Table 5 shows least squares means between the first finishing animals of both GRP1 and the GRP2. Balking scores at the feedlot between GRP1 and GRP2 were similar. However, chute scores were higher for GRP2 compared to GRP1. The GRP2 steers were heavier, larger, had more time for growth and development, and responded with more agitation to restraint than the GRP1 steers. Conversely, GRP1 steers that were lighter weight, smaller, and faster reacted more calmly in the chute, but exited the chute more quickly than GRP2 steers. Interestingly, balking at the plant differed between the two steer groups, despite being similar in final end weight, as reflected by mean pen weight. The GRP2 steers at the plant had a higher mean balking score than GRP2 steers. These two groups of animals were developed in the same feedlot, under the same diet and management, traveled the same distance to the same processing plant, and both were slaughtered during the morning hours of different dates. Between the two slaughter dates, air temperature varied by 4.1°C, relative humidity by 30%, and barometric pressure was similar. Handlers at the plant were the same. The driver transporting the animals is not known. Animals were, however, sourced from two different calf ranches located within 65 km of each other prior to entering the feedlot.

Behaviorally, Holstein steers differ from traditional beef breeds (Duff and McMurphy, 2007). Holsteins are more sensitive to noise and sudden movements than beef cattle breeds (Lanier et al., 20000. Holsteins have been selected for their milk production and docile behavior for ease in human handling during milking, not for handling as beef cattle. Tulloh (1961) reported that Holsteins tend to move more slowly compared to Angus, Hereford, and Shorthorn cattle. Holsteins have a gentle temperament and are playful, easily bored and may sort through feed, and are difficult to move because they have a tendency to follow humans (Duff and McMurphy, 2007). Difficulty moving may equate with a tendency to balk. Dairy cattle typically have exposure to humans throughout their lifetime. Duff and McMurphy (2007) describe typical Holstein steer production in the southwestern part of the United States. Calves are raised in hutches approximately 60 days before weaning. After weaning, calves are managed to reach approximately 125 kg before shipping to feedlots. Finishing Holstein steers to an end weight of 590 kg can require 12 months in a somewhat consistent environment (Duff and McMurphy, 2007). This may lead to decreased fear of humans and decreased flight zone. Additionally, this management may impose fewer handling events and less exposure to novel handling events than occurs with typical beef cattle production. These behavioral generalities may help qualify the author's conclusion that Holstein steers are "curious" or "interested" in inspecting their novel surroundings at entrance to the restrainer, thus increasing balking tendencies. These balking tendencies may be more of a slowing to investigate surroundings.

Conclusion

Balking behavior at the feedlot, in addition to other temperament indicator scores, do not forecast balking behavior at the processing plant. Balking behavior at neither the feedlot nor the plant appears to be associated with hot carcass weight, and therefore, carcass economics. In fed Holstein steers, balking may result from the novelty of a new environment.

References

Burrow, H.M., Seifert, G.W., Corbet, N.J., 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17, 154-157.

Burrow, H.M., 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abst., 65(7):477-495.

Café, L.M., D.L. Robinson, D.M. Ferguson, B.L. McIntyre, G.H. Geesink, and P.L. Greenwood. 2011. Cattle temperament: persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. J. Anim. Sci. 89:1452-1465.

Curley, Jr., K.O., J.C. Paschal, T.H. Welsh, and R.D. Randel, Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

Duff, G.C., and C.P. McMurphy. 2007. Feeding Holstein steers from start to finish. Vet. Clin. Food Anim. 23:281-297.

Fordyce, G., R.M. Dodt, and J.R. Wythes. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. Aust. J. Exp. Agric. 28:683-687.

Francisco, C.L., R.F. Cooke, R.S. Marques, R.R. Mills, and D.W. Bohnert. 2012. Effects of temperament and acclimation to handling on feedlot performance of *Bos taurus* feeder cattle originated from a rangeland-based cow-calf system. J. Anim. Sci. 90:5067-5077.

Gonyou, H.W., P.H. Hemsworth, and J.L. Barnett. 1986. Effects of frequent interactions with humans on growing pigs. Appl. Anim. Behav. Sci. 16:269-278.

Grandin, T. 1993b. Behavioral agitation during handling is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Lanier, J.L.,T.Grandin, R.D. Green, D. Avery and K. McGee. 2000. The relationship between reaction to sudden intermittent sounds and movement and temperament. J. Anim. Sci. 78:1467-1474.

Müeller, R., M.A.G. von Keyserlingk. 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. Appl. Anim. Behav. Sci. 99:193-204.

Tulloh, N.M., 1961. Behavior of cattle in yards. II. A Study of temperament. Anim. Behav. 9:25-30.

Vann, R.C., J.A. Parish, and W.B. McKinley. 2008. Case study: Mississippi cattle producers gain insight into temperament effects of feedlot performance and subsequent meat quality. Prof. Anim. Sci. 24:628-633.

Table 1. Simple means for balking score[1] and chute score [2], exit velocity [3], and live weight in GRP1 and GRP2 steers at the feedlot.

Variable	GRP1 Mean age: 8.6 mo				GRP2 Mean age: 11.6 mo			
	n	Mean	SE	Range	*n*	Mean	SE	Range
Balking score	289	1.7	1.0	1 to 5	265	1.5	0.1	1 to 5
Chute score	290	1.5	0.7	1 to 4	266	2.0	0.1	1 to 5
Exit velocity	288	1.5	0.5	0.5 to 1.8	263	1.2	0.6	0.4 to 1.8
Weight, kg	290	266.0	26.5	181 to 346	266	445.0	32.0	295 to 538

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[2]Chute score: 1 to 5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

[3]Exit velocity = 1.83 m (distance)/time (s).

Table 2. Pearson correlation coefficients (*r*), and *P*-values for feedlot behavioral observations and live weight in GRP1 (*n* = 289) and GRP2 (*n* = 263) steers.

	GRP1 Mean age: 8.6 mo Mean weight, kg ± SE: 266 ± 27			GRP2 Mean age: 11.6 mo Mean weight, kg± SE : 445 ± 32		
Variable	Chute score	Exit velocity	Weight, kg	Chute score	Exit velocity	Weight, kg
Balking score[1]						
r	-0.21	0.20	0.03	0.02	0.02	-0.13
P-value	0.0003	0.0008	0.61	0.67	0.79	0.03
Chute score[2]						
r		-0.96	-0.06		-0.90	0.09
P-value		< 0.0001	0.27		< 0.0001	0.11

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[2]Chute score: 1 to 5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

Table 3. Pearson correlation coefficients (*r*) with behavior scores at the feedlot and at the plant, and behavior scores with hot carcass weight, in GRP1 steers (*n* = 251).

	Feedlot balking[1] Score	Feedlot chute score[2]	Feedlot exit velocity[3]	Feedlot weight, kg	Plant balk
Plant balking score					
r	0.02	-0.06	0.06	0.04	
P-value	0.7	0.4	0.4	0.54	
Hot carcass weight *r*	0.05	-0.08	0.09	0.19	0.04
P-value	0.4	0.2	0.2	0.003	0.55

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.
[2]Chute score: 1 to 5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.
[3]Exit velocity = 1.83 m (distance)/time (s).

Table 4. Pearson correlation coefficients (*r*) with balking score[1] at the feedlot and at the plant in GRP2 steers (*n* =64).

	Feedlot balking score[1]	Feedlot chute score[2]	Feedlot exit velocity[3]	Feedlot live weight	Plant Balk
Plant balking score[1]					
r	0.02	-0.08	0.15	0.25	
P-value	0.87	0.54	0.24	0.05	
Hot carcass weight					
r	0.01	-0.17	0.06	0.44	-0.007
P-value	0.93	0.17	0.61	0.0003	0.96

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[2]Chute score: 1 to 5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

[3]Exit velocity = 1.83 m (distance)/time (s).

Table 5. Least squares means in first finishing steers of GRP1 and GRP2 (n = 131) for behavior scores, exit velocity, and live weight at the feedlot, and balking behavior, pen weight, HCW, and DP at the plant.

	GRP1			GRP2		
Variable	*n*	Mean	SE	*n*	Mean	SE
Balking score[1] at feedlot	65	1.6	0.1	66	1.5	0.1
Chute score[2]	65	1.6[b]	0.7	66	2.2[a]	1.0
Exit velocity[3]	65	1.4[a]	0.5	65	1.0[b]	0.5
Live weight, kg	65	287[b]	26.5	66	480[a]	16.8
Balking score[1] at plant	65	1.6[b]	1.2	65	2.4[a]	1.4
Pen weight, kg	65	627	0	65	635	0
HCW, kg	65	391	27.3	65	392	25.2
Dressing percentage	65	62.3	0	66	63.0	0

[1]Balking score: 1 to 5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.
[2]Chute score: 1 to 5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.
[3]Exit velocity = 1.83 m (distance)/time (s).
[ab]Within a row, means without a common superscript differ ($P < 0.05$).

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MEMORANDUM

TO: Hayden Brown

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 9, 2009

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : November 8, 2012

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#10013- "GENETIC CONSIDERATIONS FOR BEEF CATTLE PRODUCTION IN CHALLENGING ENVIRONMENTS** ". You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **11-08-2012,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

Center for Food Animal Wellbeing

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE OF AGRICULTURAL, FOOD & LIFE SCIENCES

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April 22, 2014

To Whom It May Concern:

I am writing this letter to certify that Michelle Lynn Thomas conducted the work and wrote in excess of 51% of chapter 3 of her dissertation entitled "Balking Incidence and Behavioral Responses during Handling in Fed Cattle with Genetic and Carcass Implications".

Please contact me if you have further questions or concerns.

Sincerely,

Yvonne Vizzier Thaxton, Ph.D.
Professor and Director, Center for Food Animal Wellbeing

Chapter 4: Behavioral Responses to Handling in Angus and Hereford-Angus Crossbred Steers under Different Environmental Conditions and Carcass Implications

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Abstract

Breed-type effects on cattle temperament are recognized. However, balking behavior has not been studied extensively throughout the lifetime of fed steers in multiple environments. Objectives of this study were to observe potential differences in balking behavior between Angus and Hereford-Angus crossbred steers, evaluate any relationship of balking behavior to other temperament scoring systems, and evaluate any carcass effects related to this behavior. Angus and Hereford sires were utilized on the Angus-based cow herd at the University of Arkansas for fall 2011 calving. At weaning, balking and chute behavior scores were assessed, in addition to exit velocity (velocity = distance (m)/time(s)). Balking scores were on a scale of 1 to5 with 1 indicating no balking and 5 indicating a persistent balk, and chute scores ranged from 1 to 5 with 1 being docile and 5 intense frenzy. Animals were backgrounded and finished at West Texas A&M University and were slaughtered in a federally inspected plant, with 6 data collection times during the entire production. Hereford-Angus crossbred steers balked more (1.6, $P < 0.05$) than Angus steers (1.3, $P < 0.05$). Balking scores changed over the collection dates related to different

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locations and typical phases of beef cattle production. There was an interaction of collection time by breed on chute score, and Angus steers were more ($P < 0.0001$) reactive in the chute than Hereford-Angus steers at collection time 3. Pearson correlation coefficients suggested some association of balking means with other traits, but was only positively associated ($r = 0.30$, $P = 0.02$) with chute score means at collection time 4. Balking scores at collection time 2 showed several negative correlations with weight for collection times 1, 2, 3, and 4 ($r = -0.31$, $P = 0.02$; $r = -0.32$, $P = 0.01$; $r = -0.29$, $P = 0.02$; and $r = -0.26$, $P = 0.04$, respectively). Additionally, balking score at collection time 2 also correlated negatively ($r = -0.27$, $P = 0.04$) with hot carcass weight. While correlations only suggest a possible relationship, these data imply balking behavior is more associated as a function of weight than temperament.

Introduction

Necessary handling of cattle in various locations throughout their lifetime has an impact on learning. Some learn to avoid places and people and this may lead to balking behavior, or cease in forward motion, in the working facility. Cattle frequently show a resistance to continue forward motion, such as from the crowding pen into the alley or from the alley into the chute, scale, or restrainer. Some refuse to continue to move forward with their herd mates despite their innate gregarious behavior. The situation often requires some type of stimulus from the handler to coerce the animal forward. This can be a considerable challenge and animal welfare issues may emerge as more persuasive tools or repeated use of the last resort electric prod as a handling aid are needed to efficiently complete the task.

Temperament in cattle is often defined by observed behavioral responses to humans or human handling procedures. Various scoring systems exist for behavior in the chute, exit velocity from the chute, and to a much lesser extent, pen score and balking (Burrow, 1997). Behaviors that make handling more difficult, take more time than the average, or present danger to humans are often associated with animals described of as poor temperament. Temperament differences have been proven between breed classifications (Hohenboken, 1987, Tulloh, 1961; Voisinet, 1997; Grandin, 1980a), within breed classification, crossbreeds (Murphey et al., 1980, 1981, Stricklin et al., 1980), and within sexes (Voisinet et al., 1997).

Temperament differences among breeds of cattle, sheep, pigs, and other livestock animals have been extensively researched. In cattle, *Bos indicus* breeds are generally considered more temperamental compared to *Bos taurus* breeds (Hohenboken, 1987, Tulloh, 1961; Voisinet, 1997; Grandin, 1980a). Within *Bos taurus* breeds, differences in flight zone, tendency to approach novel objects (Murphey et al., 1980, 1981), excitability (Stricklin et al., 1980), and social ranking (Stricklin et al., 1980; Wagnon et al., 1966) are evident among different breeds. British breeds are more docile than European continental breeds, and have a significantly lower flight-speed while leaving the crush (Hoppe, 2008). Tulloh (1961) further evaluated differences between European breeds of Angus, Hereford, and Shorthorn compared to the dairy breed Holstein and discovered that Angus are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move, while Holsteins tend to move more slowly. Holsteins are also more sound-sensitive and touch sensitive than beef cattle (Lanier et al., 2000).

Whatley et al. (1974) and Hansen et al. (2001) have shown that sheep of different breeds show differences in flocking behavior, and response to aversive stimuli, respectively. Pig breeds show significant differences in temperament scores during loading into the scale, within the scale, and a vocal score which have been related to performance (Yoderet al., 2011). Temperament is a moderately heritable trait in beef cattle (Stricklin et al., 1980). While breed temperament generalizations can be made, individual behavioral differences within breeds of cattle must be considered and have been evaluated by Kilgour et al. (2006).

In a review by Burrow (1997), measurements of temperament included balking rating (Grandin, 1993b) and ease of movement tests (Hinch and Lynch, 1987; Tilbrook et al., 1989; and Kabuga and Appiah, 1992). Burrow (1997) also referred to a “freeze” response in the AMRC study (1988) when animals were restrained, particularly in higher Brahman inheritance. In assessing cortisol levels and anxiety-related behaviors in Hereford x Angus crossbred cattle, Bristow and Holmes (2007) also mentioned the reaction of some animals “freezing” or resisting entry to the squeeze chute, while others walked calmly into the alley. Grandin (1993b) assessed the relationship between temperament and balking behavior of bulls and steers under consecutive restraint sessions. She concluded that behaviorally agitated animals balked significantly less than animals with a calm score. Tulloh (1961) found no relationship between temperament score and balking in and cattle movements entering the scale or crush and concluded that cattle that are difficult to handle do not necessarily have a bad temperament. Genetic selection that has occurred over the past few decades may have had effect on the incidence of balking behavior and may be correlated with temperament.

Genetically, individuals differ in their propensity to learn, and unlearn (Boissy et al., 2005). Behaviors that promote survival of the species are innate and heritable, which include behavioral defensive responses to fear. ‘Flight or fight’ are typical fear response behaviors. If flight is not an option, freezing behavior is seen in other animals and has been evaluated extensively in rodents. Freezing has been defined as the absence of any movement except for respiratory-related movements (Panksepp et al., 2011) and is measured by direct observation. In the review by Panskepp et al. (2011) the authors assert that freezing is positively correlated with anxiety, particularly when paired with previously aversive stimuli. Learned fear, or aversion, seems related to anxiety. Freezing reaction has been shown and measured in humans with panic disorder in anxious situations (Lopes et al., 2009). While livestock are generally not referred to as having anxiety episodes or being of ‘anxious’ temperament, we do accept that factors which increase stress, or possibly anxiety, have a negative impact. Previous aversive handling events may be fixed into memory and thus cannot be forgotten or unlearned (Grandin, 1993a). The degree or frequency of the aversion may be a factor in memory and future anticipatory behavior.

Environmental factors that increase the tendency to balk have been thoroughly investigated by Grandin (1980, 1987, 1993a, 1994, and 1997). Those factors include sensory stimuli such as lighting, shadows, reflections, flooring, noise, smells, and sounds. Grandin has also evaluated handling aids and techniques, equipment type and design, yard design, and other factors in humane livestock handling. She has designed and modified facilities and educated handlers for effective, safe animal movement and has advanced animal welfare. Even with known environmental factors controlled, balking still occurs.

An objective of this study was to observe potential differences in balking behavior between different Angus and Hereford-Angus crossbred steers from the same cow herd. A second objective was to evaluate any relationship of balking behavior to other temperament scoring systems to discover if balking is associated with temperament and has potential to be a temperament indicator.

Materials and Methods

All experimental procedures performed in this study were reviewed and approved by the University of Arkansas Animal Care and Use Committee (IACUC, protocol number 10013).

Angus and Hereford sires were utilized on the Angus-based cow herd at the University of Arkansas, located in northwest Arkansas. Calves were born fall 2011 and heifers were not analyzed in this study. Calves were either Angus (n = 27) or Hereford-Angus (n = 33) crossbred calves. Animals were weaned at approximately seven mo of age. Angus steers were sired by artificial insemination by one of four sires. Five Hereford bulls were utilized with natural service.

Balking behavior, chute behavior, and flight speed upon exiting the chute were assessed multiple times (six) throughout the lifetime of the steers. All behavioral assessments were not available at all locations. Initial scoring at weaning occurred 7 May 2012.Upon weaning, calves were moved to the university stocker unit approximately 3.2 km south of their birth site. Steers were weaned at approximately 7.3 mo of age.

Animals were reared and backgrounded on endophyte-infected tall fescue (*Fesctuca arundinacea [Schreb.]*Darbysh.) and common bermudagrass (*Cynodon dactylon* [L.] Pers.) in northwest Arkansas prior to shipping to Texas. At approximately 9.4 months of age, animals were transported approximately 837 km to Texas. Steers grazed native pastures, comprised of buffalograss (*Bouteloua dactyloides* (Nutt. J.T. Columbus) and blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), for 42 days. Steers were then grazed on a brown mid-rib (BMR) sorghum-sudangrass (Sorghum X drummondii) hybrid (Sweeter 'N Honey II, Richardson Seeds, Vega, TX) for 66 days. Steers were placed into the research feedlot in pens of seven to eight animals. Diet during the finishing phase consisted of a common corn-based, step-up ration. Slaughter occurred at a federally inspected, modernly designed beef processing plant located approximately 89 km from the feedlot.

Balking scores and chute scores were assigned in addition to flight speed leaving the chute. Balking scores were taken by one consistent observer using a modified scoring system based on the method by Grandin (1993b). Balking scores were on a scale of 1 to 5 taken at entry to the scale or chute. Balking scores including degree of balking: 1 = None; willing forward movement; 2 = Stops; then proceeds on own; 3 = Persuasion needed; shake of paddle or tap on rump/tail area; 4 = Persistent balk; two or more persuasion efforts needed to continue forward motion, or 1 use of electric prod; 5 = Intense balk; multiple persuasion efforts or electric prod two or more times required for continue forward motion. A final balking score was taken at the entry to the center track double rail restrainer in the federally inspected beef processing plant. Behavior scores were taken the morning of slaughter at the feedlot, and later in the afternoon at the plant, which only allowed a balking behavior score.

Chute scores ranged from 1 to 5 according to the method by Grandin (1993b). Considering these scores are subjective, ratings were determined by one trained observer throughout all trials. Ratings were made after the head was restrained in the headgate and prior to the sides being closed on the chute. The score system included: 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

Flight speed exiting the chute was recorded using infrared sensor timing lights (Farm Tek Inc., North Wylie, TX) to remotely trigger the start and stop of timing. Flight speed was converted to exit velocity (EV) (1.83 m (distance)/time (seconds)) as described by Burrow et al., 1988. Flight speed was only able to be recorded on collection times 1 to 3. Steers were evaluated for behaviors six times from weaning until slaughter.

Statistical Analyses

Data were analyzed with SAS© (SAS Inst., Inc., Cary, NC) using PROC MIXED, PROC CORR and PROC FREQ. For PROC FREQ, significance was determined using chi-square. For PROC MIXED, the model contained fixed effects for collection time, breed-type, and collection time by breed-type interactions. Random effects were steer individual identification. Time was analyzed as repeated measure. Means were reported as least squares means and separated using Tukey adjusted LSD.

Results

Table 1 reflects collection dates, times, location, number of animals, and behavior scores for steers throughout their production. Data collection began at weaning which was at a mean age of 7.3 mo. Prior to shipping to Texas, a second behavioral assessment was recorded at approximately 9.4 months of age. A third behavior assessment was recorded after grazing native forages and summer annual forages in Texas, and mean animal age was 12 mo. Upon entry to the research feedlot adjacent to the range where steers grazed, behaviors were recorded at collection time 4. Mean age was 13.2 mo. Approximately 3.5 mo later, steers were handled through the working facility and behavior scores assessed, with mean age 16.7 mo. At this collection date (collection 5), 30 steers were deemed finished and were loaded in the morning for transport to the beef processing plant. Balking behavior at entrance to the restrainer in the processing plant was assessed that same afternoon. This procedure was followed for the remaining steers (30) that were finished at collection date 7, with mean age of 17.6 mo.

Steer live weights progressed over time and differed ($P < 0.05$) each collection time except for time 5 and 7 which were similar (Table 2). Collection times 5 and 7 were just prior to shipping to the processing plant, reflecting finished weights. Live weight varied ($P < 0.05$) by breed. Angus steers weighed more ($P < 0.05$) than Hereford-Angus crossbred steers, at 433.0 ± 6.6 kg vs. 387.5 ± 5.6 kg, respectively (Table 2).

Breed had an effect on balking behavior and mean scores (Figure 1). Hereford-Angus crossbred steers balked more (1.6, $P < 0.05$) than Angus steers (1.3, $P < 0.05$). Mean balking scores changed over the collection dates related to different locations and typical phases of beef cattle production (Figure 2). Balking score means were similar in collection times 1 to 3 (1.5, 1.6, and 1.8, respectively) but differed (*P* = 0.0037) from collection time 4 (1.1), while collection times 5 and 7 were intermediate (1.4 and 1.4, respectively).

There was an interaction of collection time by breed on chute score (Figure 3). Angus steers were more (P < 0.0001) reactive in the chute compared to Hereford-Angus crossbred steers at collection time three. Mean Angus chute score at collection time three was 2.3 compared to Hereford-Angus crossbred steers with mean of 1.7. Chute scores were similar by breed over all other collection times and means varied from 1.0 to 1.3.

Exit velocity increased (P < 0.05) over each consecutive collection time (Table 3). Exit velocities were 1.1 ± 0.56, 1.6 ± 0.89, and 2.1 ± 0.81 for collection times 1, 2, and 3, respectively. While EV was not possible to assess in collection times 4 to 7, animals visually appeared to slow in EV during the remainder of the trial.

Correlations were assessed to discover any associations with balking behavior scores with other temperament scoring systems and live weight or HCW. Pearson correlation coefficients (Table 4) suggested some association of balking with other traits. Balking score at collection time 1, at weaning, was negatively correlated (*r* = -0.33, *P* =0.01) with EV 3, but no other behavioral scores taken at the same date. Balking score at collection time 2 was negatively correlated (*r* = -0.39, *P* =0.03) with balking score at collection time 6. Balking scores at collection time 2 showed several negative correlations with weight for collection times 1, 2, 3, and 4 (*r* = -0.31, *P* =0.02; *r* = -0.32, *P* = 0.01; *r* = -0.29, *P* = 0.02; and *r* = -0.26, *P* =0.04, respectively). Additionally, balking score at collection time 2 also correlated negatively (*r* = -0.27, *P* = 0.04) with HCW. Balking behavior at collection time 3, with numerically the highest balking score mean, was negatively correlated with weights at collection times 1, 2, 3, and 4 (*r* =

-0.26, P =0.05; r = -0.31, P = 0.02; r = -0.37, P = 0.004; and r = -0.32, P =0.01, respectively). At collection time 4, balking was positively correlated (r = 0.57, P = 0.001) with balking at collection time 5 and again with collection time 7(r = 0.40, P = 0.03). Balking at collection time 4 was also positively correlated with chute scores at collection times 4 and 7 (r = 0.30, P =0.02; r = 0.61, P = 0.0005, respectively).

Figure 4 graphically reflects all behavior score means taken at each collection date. There appears to be little parallelism in behavior means over time. This also may be a visual indication of little to slight relationship between balking, chute, and exit velocity behaviors.

Discussion

The cow herd has been artificially selected against temperament problems over several years. Research has shown that temperament is moderately heritable in cattle (Stricklin et al., 1980). Breed differences in temperament have also been researched in cattle. University cattle are often extensively studied, and steers in this study were weighed or handled 5 to 6 times prior to weaning using the same handling facility and personnel. When cattle are managed intensively and consistently, behavior differences among breeds, or among individuals, may be more apparent. The steers appeared acclimated to handling and may be reflected in the low behavior scores and slow exit velocity at weaning. These low scores reflect a docile temperament.

Numerically, balking scores rose steadily during collection times 1 to 3 and dropped at time 4, possibly reflecting adaptation to a new environment or to handling in various facilities with differing personnel. A noticeable increase in chute score was apparent in both breeds at collection time 3. Although balking means were similar in all collection times except time 4, balking behavior means were numerically highest for collection time 3. This was the first collection time after transport to a new environment in Texas from Arkansas. Exit velocities did differ at each of the three collection times. This may be a function of more muscle growth and development, allowing for an accelerated locomotion out of the chute. Exit velocity may assess the degree of gregariousness of the animals as they attempt to join herd mates. Speed exiting the chute was not able to be assessed during the latter behavior scoring sessions due to a limitation in design of the facility. The consistent observer in all of the trials reported that exit velocity decreased over the remainder of the trial, as did other behavioral assessment scoring systems.

While correlation does not imply cause and effect, it does indicate some association or relationship between two variables. To evaluate the potential for balking behavior as an indicator of temperament, Pearson correlation coefficients with chute score means and EV means were evaluated. Chute scoring and EV are accepted methods to assess temperament (Tulloh, 1961; Grandin, 1993; Curley et al., 2006; King et al., 2006; Müller et al., 2006; Hoppe et al., 2010; and Cooke et al., 2012). Recent research combines chute score and exit velocity to assign a general temperament score (Curley et al., 2006; King et al., 2006). Table 5 illustrates Pearson correlation coefficients with respective *P*-values. The only instance in which balking behavior correlates with chute score at the same time is collection time four. A correlation coefficient ® of 0.30 is a classified as a slight association between balking and chute score at collection time 4. In all other instances, no association is apparent with balking behavior and chute score or EV. However, balking behavior at data collection periods 2 and 3 reveal a slight negative association with weight, implying those steers that balk more weighed less. Inversely, those steers that balked less weighed more. This inverse, or negative, relationship was reflected at data collection time two with final HCW. The strongest relationships were exhibited at data collection period 4. Balking behavior at collection time 4was moderately (0.57, P = 0.001) and positively associated with balking at collection time 5. Additionally, balking score mean at collection time 4 was moderately (0.61, $P = 0.0005$) associated with mean chute score 7. As these coefficients are generally categorized as slight to a few moderate associations, and not always at the same data collection times, the conclusion is that balking behavior is not a strong predictor of overall temperament.

Graphically, an overlay of balking behavior means and chute score means over all data collection times, with EV for collection times 1 to 3 do not reflect parallel lines (Fig. 4). A peak at collection time 3 was indicated in chute score behavior, but then flattened. Balking behavior scores steadily rose in collection times 1 through 3, with 3 being the greatest mean, dropped to the lowest mean at collection time 4, and then rose again. Collection time 3 was the first data collection period after relocation to Texas from Arkansas. This relocation was a strong environmental change in landscape, temperature, humidity, and diet. Previously, the steers had been in the Ozark Mountains of northwest Arkansas with hills, trees, and valleys. The plains of Texas offered no shade, windbreaks, or potential hiding from predators compared to their environment in Arkansas. Perhaps this new environment elicited a slightly higher mean balking score, more agitation in the chute, and the fastest EV at the first handling since relocation. Interestingly, behavior at the next collection date reflected the lowest mean balking score and chute score mean returned to previous levels. This may be due to an adaptation to the environment, acclimation to handling, and return to previous overall temperament.

Conclusion

These data have shown that behavior can change in response to a new environment but that animals adapt. Balking behavior is not closely associated with other accepted temperament indicator scores, but was more frequently, slightly, and inversely associated with weight and even hot carcass weight. Angus steers were more reactive in the chute at one particular collection time, and balked less overall, than the Hereford-Angus crossbred steers.

Implications

Balking behavior is not an indicator of temperament but may be reflective of potentially lower weight, lower hot carcass weight, and therefore, carcass economics. Additionally, balking may create handling problems and become an issue of animal welfare for those animals that consistently balk throughout their lifetime of production.

Acknowledgements

Sincere appreciation to the many people involved in the animal care and handling in this project, especially Pete Hornsby, Jana Reynolds, Darren Bignar, and Doug Galloway, University of Arkansas, and staff at West Texas A&M University. Cooperation among universities and industry is beneficial to all of animal agriculture, and privileged access was greatly appreciated.

References

Australian Meat Research Committee (AMRC) Final Report UNQ7. 1988. Genetic and environmental methods of improving the temperament of *Bos indicus* and crossbred cattle. James Cook University of North Queensland.

Boissy, A, A.D. Fisher, J. Bouix, G.N. Hinch, P. Le Neindre. 2005. Genetics of fear in ruminant livestock. Livestock Prod. Sci. 93:23-32.

Bristow, Daniel J. and David S. Holmes. 2007. Cortisol levels and anxiety-related behaviors in cattle. Phys. and Behav. 90:626-628.

Burrow, H.M., Seifert, G.W., Corbet, N.J., 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17, 154-157.

Burrow, H.M., 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abst., 65(7):477-495.

Cooke, R.F., D.W. Bohnert, B.I. Cappellozza, C.J. Mueller, and T. Delcurto. 2012. Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females. J. Anim. Sci. 90:3547-3555.

Curley Jr., K.O., J.C. Paschal, T.H. Welsh, Jr., and R.D. Randel. 2006. Exit velocity as a measurement of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

Grandin, T. 1980. Livestock behaviors related to handling facilities design. Int. J. Stud. Anim. Prob. 1:33-52.

Grandin, T. 1987. Animal handling. In E.O. Price (ed) Vet. Clin. N. Amer. 3:323-338.

Grandin, T. 1993a. Behavioral principles of cattle handling under extensive conditions. In Livestock Handling and Transport (T. Grandin, ed.) pp 43-57. CAB International, Wallingford, UK.

Grandin, T. 1993b. Behavioral agitation during handling is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Grandin, T. 1994. Solving livestock handling problems, Vet. Med., 89: 989-998.

Grandin, T., K.G. Odde, D.N. Schutz, and L.M. Beherns. 1994. The reluctance of cattle to change a learned choice may confound preference tests. Appl. Anim. Behav. Sci. 39:21.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.

Hansen, I.; Braastad, B.; Bakken, M.; Christiansen, F.; Hansen, H.S. 2001. Variation in behavioural responses of ewes towards predator-related stimuli. Appl. Anim. Behav. Sci., 70:227-237.

Hinch, G.N., J.J. Lynch. 1987. A note on the effect of castration on the ease of movement and handling of young cattle in yards. Anim. Prod. 45:317-320.

Hohenboken, W.D., 1987. Behavioral genetics. Vet. Clin. North Am.: Food Anim. Prac. 3:217-229.

Hoppe, S. 2008. Genetic and environmental factors affecting beef cattle temperament. 15th World Hereford Conference, Copenhagen.

Hoppe, S., H.R. Brandt, S. König, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. J. Anim. Sci. 88:1982-1989.

Kabuga, J.D., P. Appiah. 1992. A note on the ease of handling and flight distance of *Bos indicus, Bos taurus* and their crossbreds. Anim. Prod. 54:309-311

Kilgour, R.J., G.J. Melville, and P.L.Greenwood. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity to humans, restraint, and novelty. Appl. Anim. Behav. Sci. 99:21-40.

King, D.A., C.E. Schuehle Pfeiffer, R.D. Randel, T.H. Welsh, Jr., R.A. Oliphint, B.E. Baird, K.O. Curley, Jr., R.C. Vann, D.S. Hale, J.W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74; 546-556.

Lanier, J.L., T. Grandin, R.D. Green, D. Avery, and K. McGee. 2000. The relationship between reaction to sudden, intermittent movements and sounds and temperament. J. Anim. Sci. 78:1467-1474.

Lopes, Fabiana L., T.M. Azevedo, L.A. Imbiriba, R.C. Freire, A.M. Valenca, D. Caldirola, G. Perna, E. Volchan, and A.E. Nardi. 2009. Freezing reaction in panic disorder patients associated with anticipatory anxiety. Dep. And Anxiety. 26:917-921.

Murphey, R.M., F.A.M. Duarte, M.C.T. Penedo. 1980. Approachability of bovine cattle in pastures: Breed comparisons and a breedxtreatment analysis. Behav. Genet. 10:171-181.

Murphey, R.M., F.A. Moura Duarte, and M.C. Torres Pendo. 1981. Responses of cattle to humans in open spaces: Breed comparisons and approach-avoidance relationships. Behav. Genet. 11:37-48.

Müeller, R., M.A.G. von Keyserlingk. 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. Appl. Anim. Behav. Sci. 99:193-204.

Panksepp, J., Thomas Fuchs, Paolo Iacobucci. 2011. The basic neuroscience of emotional experiences in mammals: The case of subcortical FEAR circuitry and implications for clinical anxiety. Appl. Anim. Behav. Sci. 129; 1-17.

Stricklin, W.R., C.E. Heisler, and L.L. Wilson. 1980. Heritability of temperament in beef cattle. J. Anim. Sci. 57:Suppl 1, 109.

Tillbrook, A.J., P.H. Hemsworth, J.L. Barnett, and A. Skinner. 1989. An investigation of the social behavior and response to humans of young cattle. Appl. Anim. Behav. Sci. 23:107-116.

Tulloh, N.M., 1961. Behavior of cattle in yards. II. A Study of temperament. Anim. Behav. 9:25-30.

Voisinet, B.D., T. Grandin, J.D. Tatum, S.F. O'Conner, and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

Wagnon, K.A., R.G. Coy, W.C. Rollins and F.D. Carroll. 1966. Social dominance in a herd of Angus, Hereford and Shorthorn cows. Anim. Behav. 14:474-479.

Whateley, J., R. Kilgore, and D.C. Dalton. 1974. Behavior of hill country sheep breeds during farming routines. Proc. N.Z. Soc Anim. Prod. 34:28-36.

Yoder, C.L., C. Maltecca, J.P. Cassady, W.L. Flowers, S. Price, M.T.See. 2011. Breed differences in pig temperament scores during a performance test and their phenotypic relationship with performance. Lvst. Sci. 136:93-101.

Table 1. Conditions, data collection numerals based on date, times of data collection, location, *n*, and behavior scores.

							Behavior Scores		
Conditions	Collection time	Age, mo	Date	Time	Location	*n*	Balk	Chute	Exit velocity
Weaning	1	7.3	May12	AM	AR	60	✓	✓	✓
Prior to shipping	2	9.4	July 12	AM	AR	60	✓	✓	✓
Native forages 42 d, sorghum-sudan 30d	3	12.0	Sept12	AM	TX range	60	✓	✓	✓
Entry to feedlot	4	13.2	Oct12	AM	TX range	60	✓	✓	
feedlot	5	16.7	Feb13	AM	TX feedlot	30	✓	✓	
Plant	6			PM	TX plant	30	✓		
Feedlot	7	17.6	Mar 13	AM	TX feedlot	30	✓	✓	
plant	8			PM	TX plant	30	✓		

Table 2. Effect of collection time and breed on live weight.

Collection Time	Weight, kg	SE
1	221.0[e]	4.76
2	269.9[d]	4.76
3	353.3[c]	4.76
4	369.5[b]	4.76
5	618.1[a]	5.33
7	629.8[a]	5.51

Breed	Weight, kg	SE
Angus	433.0[a]	6.56
Hereford-Angus cross	387.5[b]	5.93

[abcde]Within a column, means without a common superscript differ ($P < 0.05$).

Table 3. Exit velocity[1] scores over three collection times.

Collection Time	Mean	SD	Min	Max
1	1.1[c]	0.56	0.44	3.03
2	1.6[b]	0.89	0.38	4.32
3	2.1[a]	0.81	0.32	3.89

[1]Exit velocity = 1.83 m (distance) / time (s).

[abc]Within a column, means without a common superscript differ ($P < 0.05$).

Table 4. Pearson correlation coefficients (*r*) and *P*-values.

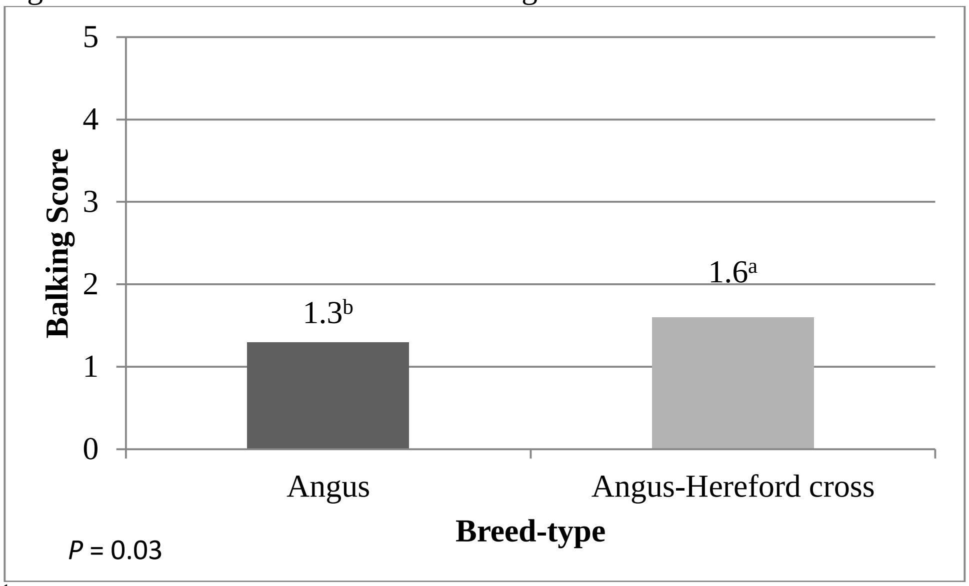
	Balk5	Balk6	Balk7	Chute[2] 4	Chute7	EV[3] 3	Wt1	Wt2	Wt3	Wt4	HCW
Balk[1] 1						-0.33					
						0.01					
Balk 2		-0.39					-0.31	-0.32	-0.29	-0.26	-0.27
		0.03					0.02	0.01	0.02	0.04	0.04
Balk 3				-0.28			-0.26	-0.31	-0.37	-0.32	
				0.04			0.05	0.02	0.004	0.01	
Balk 4	0.57		0.40	0.30	0.61						
	0.001		0.03	0.02	0.0005						

[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[2]Chute score: 1-5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

[3]Exit velocity = 1.83 m (distance) time (s).

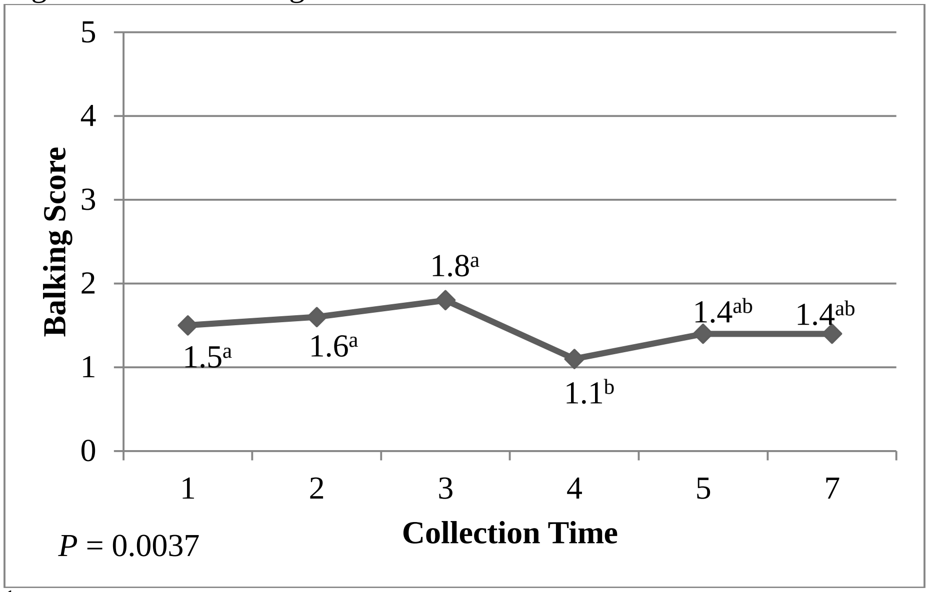
Figure 1. Effect of breed on mean balking scores[1].



[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[ab]Within a column, means without a common superscript differ ($P < 0.05$).

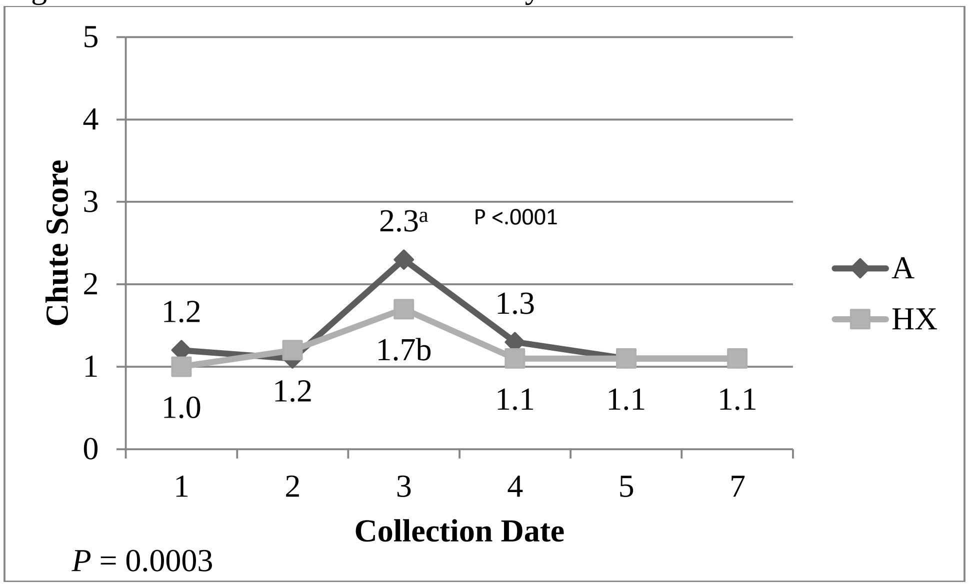
Figure 2. Mean balking scores[1] across collection dates.



[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

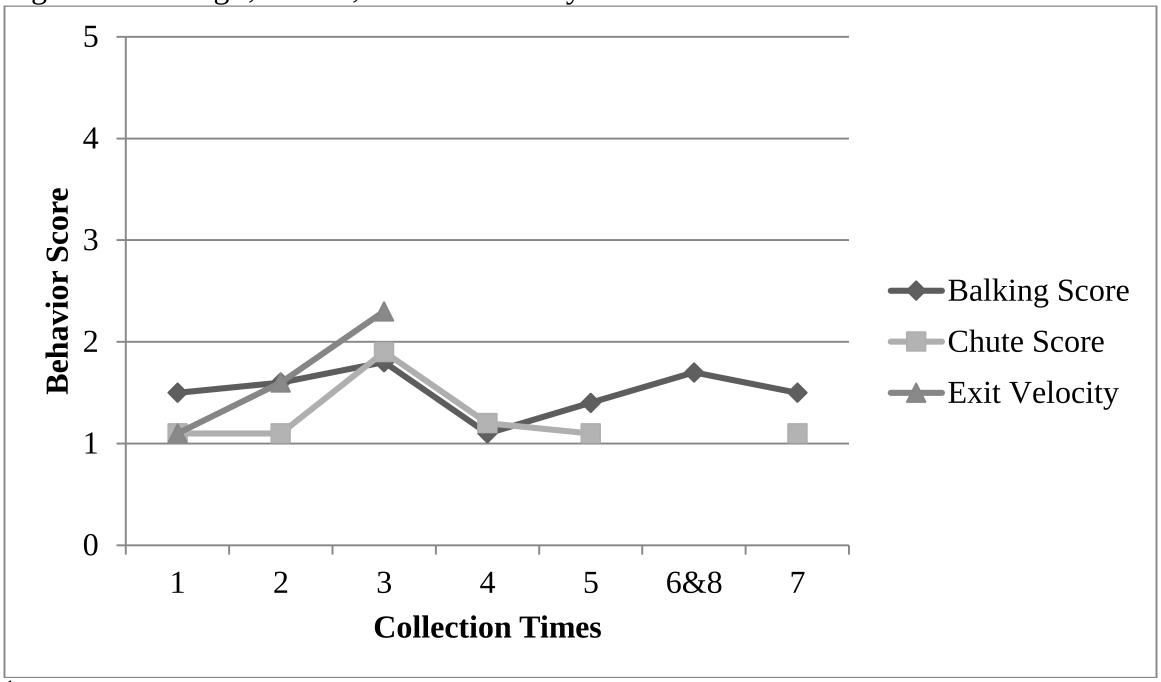
[ab]Data point means without a common superscript differ ($P < 0.05$).

Figure 3. Interaction of collection time by breed on chute score[1].



[1]Chute score: 1-5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.
[ab]Data point means without a common superscript differ ($P < 0.05$).

Figure 4. Balking [1], chute [2], and exit velocity[3] means over all collection dates.



[1]Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2 + efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

[2]Chute score: 1-5; 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

[3]Exit velocity = 1.83 m (distance)/time (s).

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MEMORANDUM

TO: Hayden Brown

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 9, 2009

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : November 8, 2012

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#10013- "GENETIC CONSIDERATIONS FOR BEEF CATTLE PRODUCTION IN CHALLENGING ENVIRONMENTS** ". You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **11-08-2012,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

Center for Food Animal Wellbeing

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April 22, 2014

To Whom It May Concern:

I am writing this letter to certify that Michelle Lynn Thomas conducted the work and wrote in excess of 51% of chapter 4 of her dissertation entitled "Balking Incidence and Behavioral Responses during Handling in Fed Cattle with Genetic and Carcass Implications".

Please contact me if you have further questions or concerns.

Sincerely,

Yvonne Vizzier Thaxton, Ph.D.
Professor and Director, Center for Food Animal Wellbeing

Chapter 5: Behavior and Carcass Weight of Angus and Hereford-Angus Crossbred Steers are associated with Heat Shock Protein 70 Genetic Polymorphisms

M.L. Thomas[8], Y.V. Thaxton[2], A.H. Brown, Jr[1], K.E. Pfalzgraf[3], K.D. Christensen[4], K.S. Anschutz[1], J.G. Powell[1], E.B. Kegley[1], J.T. Richeson[5], and C.F. Rosenkrans, Jr.[1]

Abstract

Breed-type effects on cattle performance and temperament are recognized. However, the impact of SNPs in the promoter region of the bovine heat shock protein 70 gene on behavior and carcass characteristics are not well documented. Angus and Hereford sires were used on the Angus-based cow herd at the University of Arkansas for fall 2011 calving. At weaning, balking and chute score behaviors, and exit velocity were determined, in addition to blood samples for genotyping. Balking scores were on a scale of 1 to 5 with 1 signifying no balking and 5 a persistent balk, and chute scores ranged from 1 to 5 with 1 being docile and 5 violently struggling. Animals were backgrounded and finished at West Texas A&M University and were slaughtered. Two SNPs previously described in the Hsp70 promoter region expressed associations with carcass weight and behavior scores. The A1125C SNP affected ($P < 0.05$) HCW. Steers that were AA at the A1125C SNP had heavier ($P = 0.0037$) HCW than the AC genotype (384 ± 12.9 kg, 361 ± 10.9 kg, respectively). Genotype at the A1125C SNP was also

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associated with breed-type differences ($P < 0.05$) in that 85.7% of the AA genotype (n = 18) were Angus compared to 14.2% of the AC genotype (n = 3) as Hereford-Angus crossbred steers. The T1204C SNP also affected hot carcass weight and differed ($P = 0.0008$) by genotype. Steers that were TT at the T1204C SNP had heavier ($P = 0.0008$) HCW than the CT and CC genotypes, which were similar (390 ± 12.9 kg, 366 ± 11.9 kg, and 359 ± 12.9 kg, respectively). Genotype at the T1204C SNP was also associated with breed-type differences ($P < 0.05$). The majority of the black steers were TT compared to CT and CC genotypes (69.2%, 23.0%, and 7.7%, respectively). Hereford crossbred steers were CC and CT genotypes (50%, 50%, respectively) with no TT genotypes represented. Balking score at the scale entrance was also affected by the T1204C SNP. Steers that were CC (n = 18) balked more ($P = 0.0037$) than TT (n = 22), while CT genotypes (n = 18) balked intermediately (1.76 ± 0.10, 1.23 ± 0.11, 1.50 ± 0.09, respectively). Chute score was also affected by the T1204C SNP. The Hsp70 promoter region may offer partial insight to differences in cattle breed performance and temperament.

Introduction

Heat shock proteins (HSPs), or stress proteins, are present in all cells of the body. HSP 70 (Hsp70) is one of the most abundant members of the heat shock protein family and are increased when animals are subjected to stressors (Lindquist and Craig, 1988). HSPs promote normal cell function acting as chaperones, preventing unsuitable protein folding associations. Although they do not change the final outcome of the protein folding process, they prevent protein aggregation prior to completion of folding and prevent formation of nonproductive protein intermediates (Devlin, 2011).

Expression of the HSP 70 gene is under partial control of elements in the promoter region (Wu, 1984). The promoter is a region of DNA that binds one or more proteins that regulate transcription initiation. The promoter region is immediately adjacent to the genes they regulate and specify where transcription begins, along with direction of transcription (Klug et al., 2013).

Bovine HSP 70 promoter area associations with fertility and production traits have been previously reported. Decreased calving percentages and later calving dates in Brahman cows were shown by Rosenkrans, Jr., 2010. Huang et al., 2002, showed a decreased pregnancy percentage and semen quality in swine. Turner et al., 2013, illustrated a tendency for increased horn fly density on beef cattle in those animals with polymorphisms in the promoter area.

Behavioral responses to humans, or human handling procedures, is often defined as temperament in cattle (Burrow, 1997). Various scoring systems exist for behavior in the chute, exit velocity from the chute, and to a much lesser extent, pen score and balking (Burrow, 1997). Balking is a resistance to, or ceasing, of forward motion in the working facility. Behaviors that make handling more difficult, take more time than the average, or present danger to humans are often associated with animals described of as poor temperament. Temperament differences have previously been proven between breed classifications (Hohenboken, 1987, Tulloh, 1961; Voisinet, 1997; Grandin, 1980a), within breed classification, among crossbreeds (Murphey, et al., 1980, 1981, Stricklin, et al., 1980), and within sex (Voisinet, et al., 1997).

Tulloh (1961) evaluated differences between European breeds of Angus, Hereford, and Shorthorn compared to Holsteins and discovered that Angus are more nervous than Herefords or Shorthorns and tend to be stubborn and refuse to move, while Holsteins tend to move more slowly. Grandin (1993b) assessed the relationship between temperament and balking behavior of bulls and steers under consecutive restraint sessions. She concluded that behaviorally agitated animals balked significantly less than animals with a calm score. However, Tulloh (1961) found no relationship between temperament score and balking in and cattle movements entering the scale or crush and concluded that cattle that are difficult to handle do not necessarily have a bad temperament. Genetic selection that has occurred over the past few decades may have had effect on the incidence of balking behavior and may be associated with temperament.

Objectives for this study were to discover if single nucleotide polymorphisms (SNPs) in the HSP 70 genetic promoter were associated with behavior traits or had carcass implications.

Materials and Methods

All experimental procedures performed in this study were reviewed and approved by the University of Arkansas Animal Care and Use Committee (IACUC, protocol number 10013).

Data collection began May 7, 2012 and continued until slaughter March 25, 2013. Animals were assessed for behavioral scores on six occasions in each location of production. Angus and Hereford sires were used on the Angus-based cow herd at the University of Arkansas for fall 2011 calving. Breed distribution included Angus (n = 26) and Hereford-Angus crossbred (n = 32) steers. Steers were weaned at approximately 7 months of age. Balking scores and chute scores were assigned in addition to flight speed leaving the chute. Balking scores were taken by one consistent observer using a modified scoring system based on the method by Grandin (1993). Balking scores were on a scale of 1 to 5 taken at entry to the scale, chute, or restrainer on eight occasions throughout the lifetime of the steers. Balking scores including degree of balking: 1 = None; willing forward movement; 2 = Stops; then proceeds on own; 3 = Persuasion needed; shake of paddle or tap on rump/tail area; 4 = Persistent balk; two or more persuasion efforts needed to continue forward motion, or 1 use of electric prod; 5 = Intense balk; multiple persuasion efforts or electric prod two or more times required for continue forward motion.

Chute scores ranged from 1 to 5 according to a modified method by Grandin (1993). Considering these scores are subjective, ratings were also determined by one consistent observer throughout the trial. Ratings were made after the head was restrained in the headgate and prior to the sides being closed on the chute. The score system included: 1 = calm, no movement; 2 = slightly restless, 3 = squirming, occasionally shaking the squeeze chute, 4 = continuous, very vigorous movement and shaking of the squeeze chute and 5 = rearing, twisting of the body and struggling violently.

Flight speed exiting the chute was recorded using infrared sensor timing lights (Farm Tek Inc., North Wylie, TX) to remotely trigger the start and stop of timing. Flight speed was converted to exit velocity (1.83 m (distance)/time (s)) as described by Burrow et al.,1988.

Animals were reared and backgrounded on endophyte-infected tall fescue (*Fescuta arundinacea Schreb.*) and common bermudagrass (*Cynodon dactylon* [L.] Pers.) in northwest Arkansas prior to shipping to Texas. After a 837 km transport to Texas, animals grazed native pastures, comprised of buffalograss (*Bouteloua dactyloides* (Nutt. J.T. Columbus) and blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), for 42 days. Steers were then grazed on a brown mid-rib (BMR) sorghum-sudangrass (Sorghum X drummondii) hybrid (Sweeter 'N Honey II, Richardson Seeds, Vega, TX) for 66 days. Steers were placed into the research feedlot in pens of 7 to 8 animals. Diet during the finishing phase consisted of a common corn-based, step-up ration. Slaughter occurred at a federally inspected, modernly designed beef processing plant located approximately 89 km from the feedlot.

Jugular blood samples were collected at weaning and the plasma was harvested. Blood tubes were placed in ice immediately after collection and transported to the laboratory. Tubes containing EDTA (Vacutainer, Becton Dickinson, Inc., Franklin, NJ) treated blood were cooled to 4° C, centrifuged (1500 x g for 25 min), plasma decanted, and buffy coats harvested. Buffy coats were then stored at -80° C for further genomic analysis. Genomic DNA was extracted and purified using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) per manufacturer's instructions. A Qubit 2.0 Fluorometer (Invitrogen, Eugene, OR) was used to quantify DNA after purification. Stock samples were diluted to 20ng before sequencing. Sequencing of HSP 70 genotypes was completed using Sequenom technology (Washington University, St. Louis, MO). Genetic data was successfully collected on 58 steers.

Statistical Analyses

Data for each of the 6 SNPs (C895D, A1125C, G1128T, T1204C, G1851A, and G2033C) were analyzed independently using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Fixed effects were genotype, time, and their interactions. Breed within SNP was designated as random for each analysis, and time was treated as a repeated measurement. Kenward-Rogers'approximation was used to calculate degrees of freedom for the pooled error term. Means were reported as least squares means and were separated using *F*-protected *t*-tests with the Tukey-Kramer adjustment. Genotype distribution and allelic frequencies were tested by chi-square analysis within each SNP.

Results

Experiment design and logistics are described in Table 1. Collection dates are numbered as collection times for ease of discussion. At collection time 5, half of the steers (n = 30) were at finished weights and shipped after behavioral assessments and final live weight was taken in the morning, and then shipped to a federally inspected processing plant. A second balking score was determined at entrance to the center line double rail restrainer in the afternoon. On collection date 6, the second half of the steers (n = 30) were assessed, weighed, and shipped in the same manner as the previous group.

Table 2 illustrates the region on the promoter or coding sequence on the HSP70 gene by sequence position of the SNP. The frequency is the percentage of steers with each particular SNP in our population of 60 steers. Minor allele frequency percentages that were 10% or above included sequence positions 895, 1125, 1128, and 1204 will be further discussed. Breed type by allelic percentage is shown. The HSP70 promoter SNP C895D was found to have a minor allele frequency of 10% and A1125C had a minor allele frequency of 29%. Promoter region SNP G1128T had a minor allele frequency of 18%, and SNP T1204C had the highest minor allele frequency at 50%. SNPs in the coding region had minor allele frequencies less than 10% and will not be discussed.

There was an effect of data collection time on live weight, balking score, chute score, and exit velocity on several genotypes.

Collection Time Effects:

Live Weight

Collection time had an effect on live weight for the following SNPs: 895, 1125, and 1204. For each SNP, weight increased and differed ($P < 0.05$) for collection times 1 to 4 (Table 3) and illustrates the pattern for genotypes 895 and 1204. Weight at collection time 5 and 7 were final finished weights, were similar, and differed from collection times 1 to 4.

Balking Scores

Collection time had an effect on balking score for SNPs 895, 1125, 1128, and 1204. The table for 1125 genotype (Table 4) illustrates how mean balking scores changed over time, and differences ($P < 0.05$). Collection time 3 had the highest mean balking score (1.8), differed ($P < 0.05$) from collection times 1, 4, 5, and 7, and was similar to time 2. Collection time 4 had the lowest mean balking score (1.1) and was similar to times 5 and 7, and differed from collection times 1 through 3. Collection time 2 mean balking score was intermediate to times 1 and 3.

Exit Velocity

Collection time had an effect on exit velocity for all analyzed SNPs. To illustrate, Table 4 shows that exit velocity increased ($P < 0.05$) at each collection time for 1125 genotype as did all others. At collection time 1, exit velocity was 1.1, time 2 was 1.7, and time 3 was the fastest at 2.1. Facility design limited collecting exit velocities at the remaining collection times.

Genotype Effects

There was an effect of 1125 and 1204 genotypes on live weight (Table 3 and Table 5, respectively) that illustrates the steers that were homozygous for the major allele had greatest ($P < 0.05$) weights compared to heterozygotes or those with homozygous minor allele (1204 genotype) genotypes. Mean balking scores were also affected by 1204 genotype (Table 6). Animals that were homozygous for the major allele in SNP 1204 (TT) had the lowest ($P < 0.05$) mean balking scores at 1.2, which differed from the CC genotype at 1.8. Heterozygotes (CT) were intermediate. Mean hot carcass weight was also affected by genotype in SNPs 1125 and 1204 (Tables 3 and 5, respectively). Animals that were homozygous for the major allele (AA, 1125 genotype; or TT, 1204 genotype) had heavier ($P < 0.05$) hot carcass weights than the heterozygotes (AC for 1125 genotype, CT for 1204 genotype) or for the homozygous minor allele (CC) in the case of 1204 genotype, which was similar to CT.

Chute Scores

There were genotypes by time interactions on chute scores in both 1125 and 1204 genotypes (Figs 1 and 2). Angus animals ($n = 18$) comprised 86% of the homozygous steers and had higher ($P = 0.02$) mean chute scores than the AC genotype, of which 83% were Hereford-Angus crossbred animals ($n = 24$). Collection time three illustrated the peak chute score mean and differed ($P < .0001$) by genotype, with AA genotypes having a higher score than the AC genotypes, 2.3 vs. 1.8, respectively.

There was a collection time by genotype interaction on chute score for the 1204 genotype. Those that were homozygous for thymine (TT, $n = 18$) were all Angus breeding, while the majority of heterozygotes (CT, 72%, n = 16) and homozygotes for cystine (CC, 89%, n = 16) were Hereford-Angus crossbred steers. Collection time three illustrated the peak chute score mean and differed ($P < 0.05$) by genotype, with TT genotypes having a higher score (2.5) than the CT or CC genotypes, 1.8 and 1.6, respectively.

Discussion

University animals are subject to more handling events than typically in beef cattle production. These steers were handled 5 to 6 times prior to weaning, and 8 times from weaning until slaughter. This acclimation to frequent handling may have contributed to the low chute scores and slow exit velocity at weaning. Additionally, this herd has been selected for temperament to enable safe handling by numerous researchers. Cattle that are raised extensively, with infrequent exposure to handling experiences or humans will have more variable behavioral scores and exit velocities (cite source).

Numerically, balking scores rose steadily during collection times 1 to 3 and dropped at time 4, possibly reflecting adaptation to a new environment or to handling in various facilities with differing personnel. A noticeable increase in chute score was apparent in both breeds at collection time 3. Although balking means were similar in all collection times except time 4, balking behavior means were numerically highest for collection time 3. Collection time 3 was the first data collection period after relocation to Texas from Arkansas. This relocation was a strong environmental change in landscape, temperature, humidity, and diet. Previously, the steers had been in the Ozark Mountains of northwest Arkansas with hills, trees, and valleys. The plains of Texas offered no shade, windbreaks, or potential hiding from predators compared to their environment in Arkansas. Perhaps this new environment elicited a slightly higher mean balking score, more agitation in the chute, and the fastest EV at the first handling since relocation. Interestingly, behavior at the next collection date reflected the lowest mean balking score and chute score mean returned to previous levels. This may be due to an adaptation to the environment, acclimation to handling, and return to previous overall temperament.

While circulating levels of heat shock proteins were not measured in this study, the polymorphisms themselves may be an indicator of altered gene function. Consideration should be given to the possibility that these polymorphisms, seen especially in the Hereford-Angus crossbred steers, change HSP 70 gene function or production of stress proteins, and result in decreased reactivity to stressors such as handling. Our results indicate that animals with highest % of polymorphisms showed lower responses in reaction to restraint as measured by chute scores. While balking behavior has not been determined to be an indicator of overall temperament, animals with polymorphisms in the 1204 SNP genotype showed more balking at entrance to the scale. However, these polymorphisms were also associated with decreased HCW. No differences by genotype were shown in exit velocity.

Polymorphisms in the HSP 70 gene promoter region may provide insight to behavior and performance. A change of environment affected behavior. Animals adapted to that change, and returned to their previous behavior patterns. While we cannot attribute behavioral differences solely to genotype, it is a factor to be considered in combination with environment as related to phenotype. Our results do not agree with Tulloh (1961) in that Angus steers in this study did not refuse to move compared to HX steers. However, in agreement with Tulloh (1961), the HX steers that may be considered difficult to handle due to balking behavior did not necessarily have a bad temperament based on mean chute scores or mean exit velocities.

The question arises if these polymorphisms increase HSP70 protein production which may allow for easier adaptation, and therefore, less stress, to a new environment with decreased response to handling.

Conclusions

Polymorphisms in the HSP 70 gene promoter region may provide insight to behavior and performance in cattle. A change of environment affects behavior, even if animals are docile, but they adapt. In some SNPs in the HSP 70 gene that we analyzed, performance and behavior was affected, not only by breed type, but by molecular genotype. Perhaps this information will attribute to genetic knowledge of cattle breed-type differences.

References

Burrow, H.M., Seifert, G.W., Corbet, N.J., 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17, 154-157.

Burrow, H.M., 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abst., 65(7):477-495.

Devlin, T. M. (Ed.) 2011. Textbook of biochemistry: with clinical correlations. 7th ed. John Wiley and Sons, Inc., Hoboken, NJ.

Grandin, T. 1980. Livestock behaviors related to handling facilities design. Int. J. Stud. Anim. Prob. 1:33-52.

Grandin, T. 1993a. Behavioral principles of cattle handling under extensive conditions. In Livestock Handling and Transport (T. Grandin, ed.) pp 43-57. CAB International, Wallingford, UK.

Grandin, T. 1993b. Behavioral agitation during handling is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Hohenboken, W.D., 1987. Behavioral genetics. Vet. Clin. North Am.: Food Anim. Prac. 3:217-229.

Huang, S.Y., M.Y. Chen, E.C. Lin, H.L. Tsou, Y.H. Kuo, C.C. Ju, and W.C. Lee. 2002. Effects of single nucleotide polymorphisms in the 5'-flanking region of heat shock protein 70.2 gene on semen quality in boars. Anim. Repro. Sci. 70:99-109.

Klug, W.S., M.R. Cummings, C.A. Spencer, M.A. Palladino. 2013. Essentials of genetics, 8th ed. Pearson Education, Inc., Glenview, IL.

Lindquist, S., and E.A. Craig. 1988. The heat shock proteins. Annu. Rev. Genet. 22:631-677.

Murphey, R.M., F.A.M. Duarte, M.C.T. Penedo. 1980. Approachability of bovine cattle in pastures: Breed comparisons and a breedxtreatment analysis. Behav. Genet. 10:171-181.

Rosenkrans, C., Jr., A. Banks, S. Reiter, and M. Looper. 2012. Calving traits of crossbred Brahman cows are associated with Heat Shock Protein 70 genetic polymorphisms. Anim. Repro. Sci. 119:178-182.

Stricklin, W.R., C.E. Heisler, and L.L. Wilson. 1980. Heritability of temperament in beef cattle. J. Anim. Sci. 57:Suppl 1, 109.

Tulloh, N.M., 1961. Behavior of cattle in yards. II. A Study of temperament. Anim. Behav. 9:25-30.

Turner, C.M., A.H. Brown, Jr., M.A. Brown, C.D. Steelman, and C.F. Rosenkrans, Jr. 2013. Associations among heat shock protein 70 genotype, forage system, and horn-fly infestation of beef cattle. Prof. Anim. Sci. 29:237-241.

Voisinet, B.D., T. Grandin, J.D. Tatum, S.F. O'Conner, and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

Wu, C., 1984. Activating protein factor binds in vitro upstream control sequences in heat shock gene chromatin. Nature 311:81-84.

Table 1. Data collection conditions, time numeration, animal age, date, time of day, location, *n*, and behavior scores.

Conditions	Collection time	Age, mo	Date	Time	Location	*n*	Behavior Scores: Balk	Chute	Exit velocity
Weaning	1	7.3	May12	AM	AR	60	✓	✓	✓
Prior to shipping	2	9.4	July 12	AM	AR	60	✓	✓	✓
Native forages 42 d, sorghum-sudan 30d	3	12	Sept12	AM	TX range	60	✓	✓	✓
Entry to feedlot	4	13.2	Oct12	AM	TX range	60	✓	✓	
Feedlot	5	16.7	Feb13	AM	TX feedlot	30	✓	✓	
Plant	6			PM	TX plant	30	✓		
Feedlot	7	17.6	Mar 13	AM	TX feedlot	30	✓	✓	
Plant	8			PM	TX plant	30	✓		

Table 2. Distribution of SNP in a 539-bp amplicon of the bovine heat shock protein 70 promoter and coding regions.

			Genotype distribution[3] by genotype and breed						
			Homo		Hetero		Homo		
			Breed		Breed		Breed		
Region	Polymorphism[1]	Frequency[2]	Angus	HX[4]	Angus	HX	Angus	HX	MAF[5]
Promoter	C895D[6]	58	26	17	0	11	0	0	10
Promoter	A1125C	69	18	3	5	24	0	0	29
Promoter	G1128T	35	25	12	1	19	0	0	18
Promoter	T1204C	69	18	0	6	16	2	16	50
Coding	G1851A	5	24	30	0	3	0	0	3
Coding	G2033C	5	26	29	0	3	0	0	3

[1]Single nucleotide polymorphism occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele.
[2]Percentage of steers with that SNP in our population of 60 steers.
[3]Number of steers that were homozygous for the primary allele (Homo), heterozygous (hetero), and homozygous for the minor allele (homo).
[4]Hereford-Angus crossbred.
[5]Minor allele frequency expressed as a percent.
[6]D represents deletion of cytosine.

Table 3. Effect of collection time on live weight, and 1125 genotype on live weight and hot carcass weight.

Collection time	Weight, kg	SE
1	224^{e}	5.8
2	274^{d}	5.8
3	357^{c}	5.8
4	373^{b}	5.8
5	623^{a}	6.2
7	627^{a}	6.5
P <.0001		

^{ab}Within a column in a category, means without a common superscript differ ($P < 0.05$).

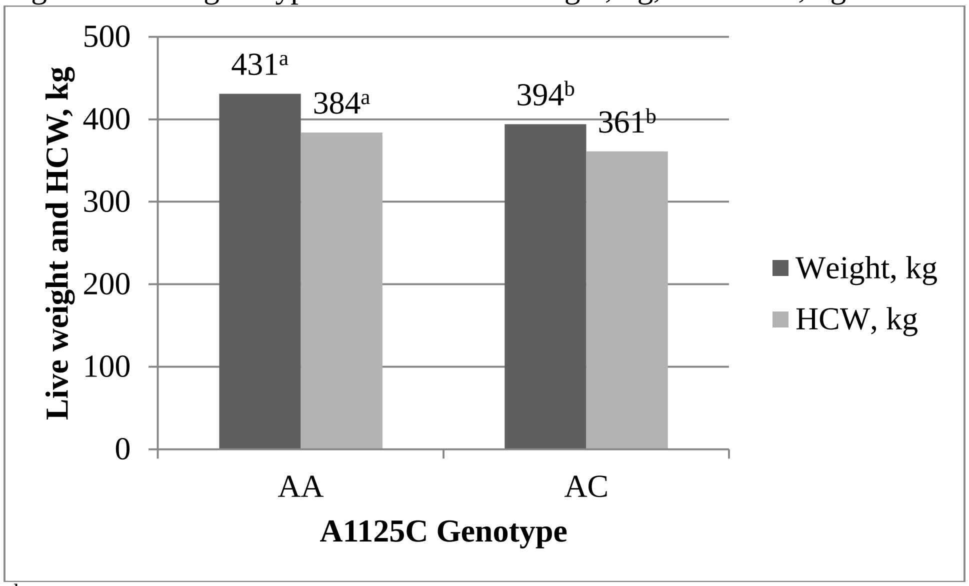
Table 4. Effect of collection time on mean balking score and exit velocity by 1125 genotype.

Collection time	Balking Score	SE
1	1.5[b]	0.13
2	1.6[ab]	0.13
3	1.8[a]	0.13
4	1.1[c]	0.13
5	1.4[bc]	0.17
7	1.4[bc]	0.20
$P = 0.0026$		
	Exit Velocity	
1	1.1[c]	0.11
2	1.7[b]	0.11
3	2.1[a]	0.11

$P < .0001$

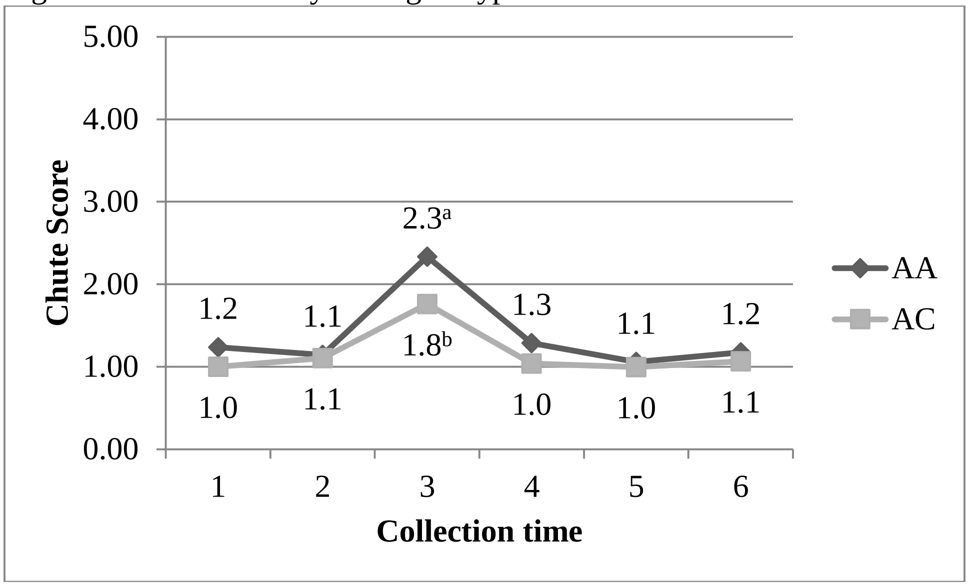
[abc]Within a column in a category, means without a common superscript differ ($P < 0.05$).

Fig 1. A1125C genotype effects on live weight, kg, and HCW, kg.



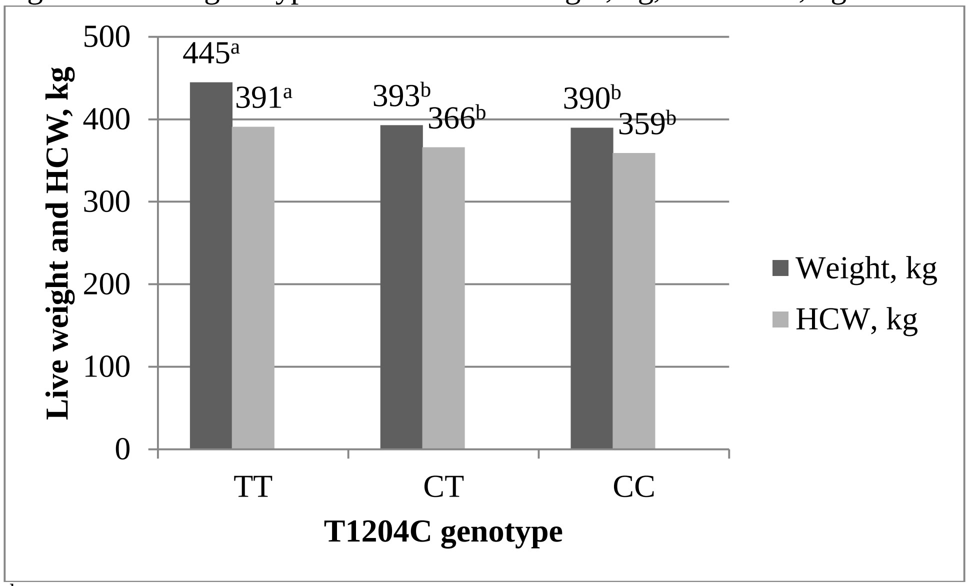
[ab]Within a column in a category, means without a common superscript differ ($P < 0.05$).

Fig 2. Collection time by 1125 genotype interaction on chute score.



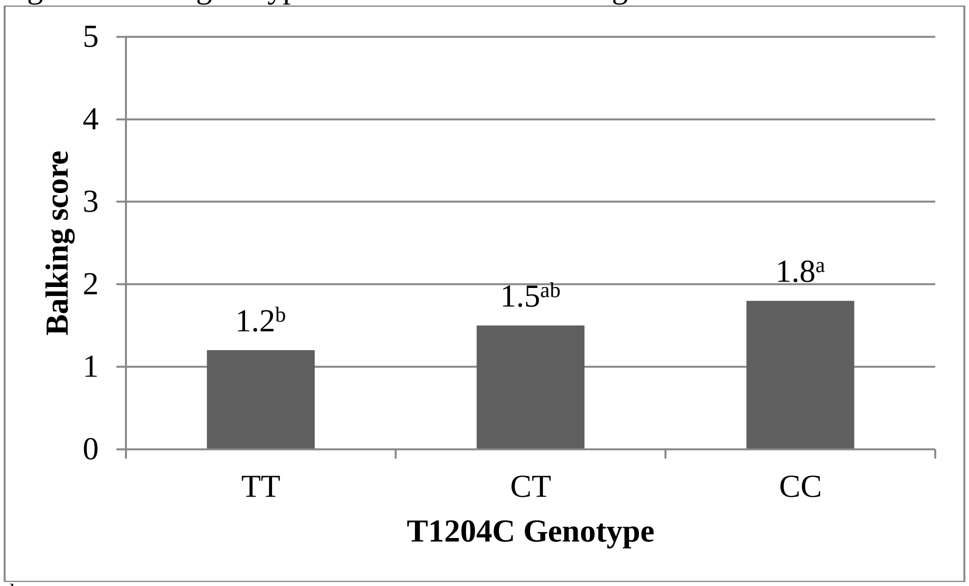
P = 0.02

Fig. 3. T1204C genotype effects on live weight, kg, and HCW, kg.



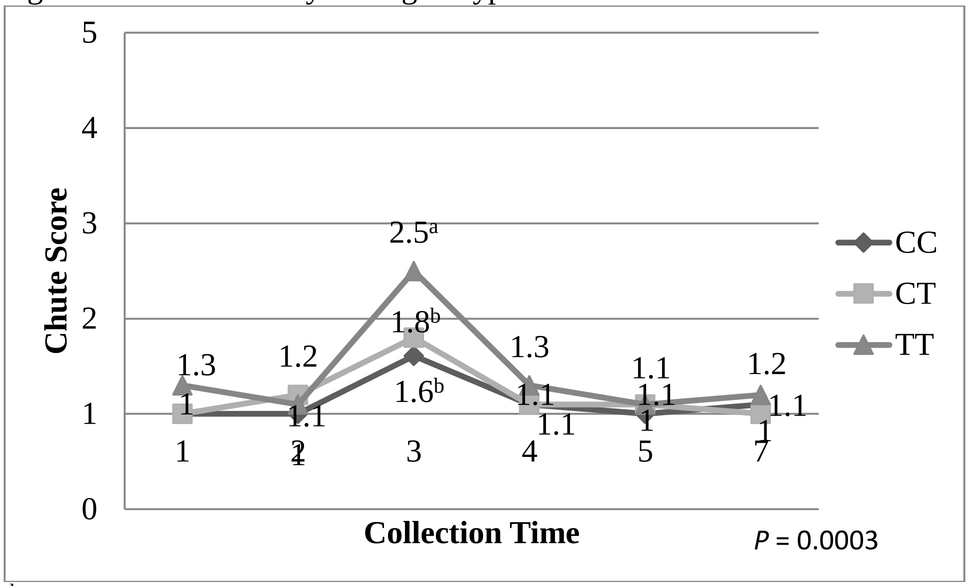
[abc]Within a column in a category, means without a common superscript differ ($P < 0.05$).

Fig 4. T1204C genotype effects on mean balking scores.



[abc]Within a column in a category, means without a common superscript differ ($P < 0.05$).

Fig 5. Collection time by 1204 genotype interaction on chute score.



[ab]Within a category, means without a common superscript differ ($P < 0.05$).

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MEMORANDUM

TO: Hayden Brown

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 9, 2009

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : November 8, 2012

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#10013- "GENETIC CONSIDERATIONS FOR BEEF CATTLE PRODUCTION IN CHALLENGING ENVIRONMENTS** ". You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **11-08-2012,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

Center for Food Animal Wellbeing

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE OF AGRICULTURAL, FOOD & LIFE SCIENCES

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April 22, 2014

To Whom It May Concern:

I am writing this letter to certify that Michelle Lynn Thomas conducted the work and wrote in excess of 51% of chapter 5 of her dissertation entitled “Balking Incidence and Behavioral Responses during Handling in Fed Cattle with Genetic and Carcass Implications”.

Please contact me if you have further questions or concerns.

Sincerely,

Yvonne Vizzier Thaxton, Ph.D.
Professor and Director, Center for Food Animal Wellbeing

Chapter 6: Conclusion

This study illustrates the application of the scientific method to validate general assumptions. People who interact with cattle in various environments often form opinions, based on experiences, about behavior. Very little, if anything, is known about the genetic background of the animals upon arrival at the processing plant. Coat color and physical characteristics can only offer a suggestion of breeding. The study began with evaluating behavior at a particular point in the processing facility. After gaining initial information, the study progressed to evaluating behavioral differences in cattle with known breeding in several handling facilities and environments throughout the lifetime of university steers. Behavioral differences, based on breed-type, were discovered. Molecular investigation of those same steers led to discovery of behavioral differences based on genetic components. This study began with assessing behavioral responses to handling from simple coat color and characteristic markings to the molecular genetics level, for a complete investigation of a relevant topic. This information adds to the scientific body of work dedicated to bovine behavior.