

THESIS

TEMPERAMENT AND MATERNAL BEHAVIOR OF BEEF CATTLE

Submitted by

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In partial fulfilment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2023

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ABSTRACT

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This thesis contains both a literature review and an observational study about temperament and maternal behavior of beef cattle. The literature review covers maternal behavior, maternal protectiveness, temperament, and stress in beef cows. The aim of the experiment was to compare the distance maternal protective behaviors were performed against measurements of temperament. The results of the study showed very little correlation between how the cow defends her calf and her behavior in the chute. The overall aim of this thesis was to address the paucity of data regarding how maternal protectiveness and temperament are related and if there is an inadvertent increase in the potential for predation if breeding for a docile temperament.

ACKNOWLEDGEMENTS

I would like to say a big thank you to everyone who has helped me in my journey here at Colorado State. Dr. Grandin has truly changed my life with the opportunity to come and work under her. This was most definitely a once in a lifetime opportunity and I will forever be grateful to her for what she has done for me.

I would like to thank my committee members: Dr. Terry Engle, Dr. Shawn Archibeque, and DVM Franklin Garry. I have truly appreciated your support over the last two years.

I can never thank my family enough for supporting me and always encouraging me to dream big. I would not be who I am today without my family and their unending love.

Lastly, I would like to give a big shout out to my cohorts, who happen to be my best friends and closest advisors. I undoubtedly could not have done this without you all. Thank you, Melissa, Paxton, Lily, Lauren, and Claire.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iii
CHAPTER 1: REVIEW OF LITERATURE.....	1
Introduction.....	1
Maternal Behavior in Beef Cattle.....	2
Maternal Behavior.....	2
Importance of Maternal Behavior.....	5
Factors that Impact Maternal Behavior.....	6
Maternal Protectiveness in Beef Cattle.....	9
Measurements of Maternal Protectiveness.....	9
Maternal Protection and Humans.....	10
Human Injury.....	11
Predators.....	12
Temperament in Beef Cattle.....	14
Definition of Temperament.....	14
Importance of Temperament.....	14
Stress in Cattle.....	17
Conclusion.....	20
LITERATURE CITED.....	21
CHAPTER 2: INVESTIGATING THE RELATIONSHIP BETWEEN MATERNAL PROTECTIVENESS AND TEMPERAMENT IN BEEF CATTLE.....	33

Study Overview.....	33
Introduction.....	34
Materials and Methods.....	35
Maternal Data Collection.....	35
Temperament Data Collection.....	36
Statistical Analysis.....	37
Results.....	37
Discussion.....	40
Conclusions.....	43
LITERATURE CITED.....	45
Tables.....	48
Figures.....	55

CHAPTER 1: REVIEW OF LITERATURE

Introduction

Temperament and maternal protection are both important traits for beef cattle in terms of profit, welfare, and safety (Haskell et al., 2014; Turner et al., 2011). Throughout the domestication of cattle, temperament has been a critical factor for selection. As the early cattle were domesticated, it was the calmer, less temperamental animals that were kept in the herd (Adamczyk et al., 2013). Maternal behavior of cattle is made up of four components (Grandinson, 2005): the ability to create a bond between the mother and the offspring, nursing behavior, responsiveness/ attentiveness, and protectiveness. Maternal protection is the behavior of the dam when she defends her calf from perceived threats (Lent, 1974; Vicentini et al., 2022). If a dam is not protective of her calf, then the calf is at greater risk of injury or death (Barrier et al., 2012); this attribute cannot be overstressed in areas of high predation. For a ranch to be economically profitable, cows must adequately convert grass to muscle and deliver a living calf every year (Willham, 1972). However, in many beef operations there is little to no supervision over the herd or defenses against predators, thus it is up to the dam to protect her offspring. While it is valuable for cows to display protectiveness of their offspring in some environments, like in open grazing land, it can pose as a risk to human safety when ranchers need to process the calf or other handling procedures (Buddenberg et al., 1986; Hoppe et al., 2008).

Human contact can be perceived as a threat, which can cause stress in cattle with more an excitable temperament (Le Neindre et al., 1998, 2002). Stress can have many impacts on cattle behavior, efficiency, and immunity (Burdick et al., 2011). Stress is triggered through the hypothalamic-pituitary-adrenal axis and sympathomedullary system (Butcher and Lord, 2004). High lactic acid concentration in the blood may also be a sign of stress, because muscles are reliant

on increased anaerobic metabolism associated with sympathetic adrenal responses. The intent of this review is to examine maternal behavior, including maternal protectiveness and temperament, with a brief overview of how predators, human interactions, and stress in beef cattle play a role in their behavior.

Maternal Behavior in Beef Cattle

Maternal Behavior

Katja Grandinson (2005) describes four important components of maternal behavior: the ability to create a bond between the mother and the offspring, nursing behavior, responsiveness/attentiveness, and protectiveness. However, maternal behavior can be described as several other behaviors that the cow performs to or with her calf during late gestation, birth, lactation, and the weaning period (Dwyer, 2008). von Keyserling and Weary (2007) wrote a review of maternal behavior of beef cattle, which included the behaviors pre, during, and post calving, and the hormones circulating in each phase.

Pre-Calving Behavior

Maternal behavior begins before the calf is born; cattle isolate themselves from herd mates before calving (Lidfors et al., 1994a). Age, parity, and environment will impact the distance cows will travel from their herd mates to calve (Flörcke and Grandin, 2014). However, when cattle are housed indoors (Edwards, 1983) or in a greater stocking density (Owens and Edey, 1985), the observations of cows separating from the herd are less noted.

Other smaller pre-calving behaviors are key hints of the impending calving. The monitoring of increased movement of the tail and lying bouts can help predict impending parturition (Krieger et al., 2018). On the day of calving, cattle are more active and restless; they have more frequent lying

bouts, however they last shorter amounts of time (Jensen, 2012). During the last two hours before calving, it is typical to see more head turns toward the abdomen (Jensen, 2012). The frequency of rumination can also be an indicator of upcoming parturition. Cattle ruminate when resting and their increase in activity limits time for resting and therefore decreases rumination (Clark et al., 2015). While these actions can be observed by a handler or stock person, technology is developing to monitor those behaviors and send notifications to alert caretakers of a calf's upcoming arrival. (Crociani et al., 2022).

Licking

On average between one to seven minutes after birth, the cow will start to lick her calf (Ramli, 1987; Le Neindre, 1989; Houwing et al., 1990; Vandenheede et al. 2001). Licking the calf stimulates breathing, circulation, urination, and defecation (Metz and Metz, 1986). Edwards and Broom (1982) observed that during the post-partum period, first-time heifers were less intense in the licking of their calf, while multiparous cows were more intense but lick the calf for a shorter portion of time compared to heifers. Pre-calving docility, measured using Boivin et al., 1992 isolation test, and time spent licking the calf have different genetic heritability (Le Neindre et al., 2002). The title of the Le Neindre et al., paper claims 'Docile Limousine Cows Are Not Poor Mothers'(2002); which indicated that more excitable temperament was thought to be related to being a good mother before this paper.

Placentophagia

Placentophagia is the term used to describe the ingestion of birth membranes and fluids after parturition, as seen in many non-aquatic placental mammals (Kristal, 1991). Most cows will eat their placenta after calving (Selman et al., 1970; Illmann and Spinka, 1993). Other mammal species(i.e. dogs, rats, and mice) will consume donor placenta at any time, however, pregnant cows

will only show interest in amniotic fluid about 12 hours before calving, and placenta and amniotic fluid up to 24 hours post-partum (Kristal, 1991; Machado et al., 1997).

Cows may consume the placenta because it could attract predators to the newborn calf, so eating the placenta would eliminate the potential attractant (Kristal, 1980). Placentophagia can also offer opioid-mediated antinociception, which means the ingestion of the placenta could mediate the bodies reception to pain from calving (Corpening et al., 2000). However, it is important to note that the ingestion of placenta without ongoing analgesia will not produce analgesia (Kristal, 1991), thus if a cow is eating the placenta of another cow, it will not receive the pain relieving effects.

Vocalization

Calves behave differently to other calves calls, but are partial to the call of their dam (Marchant-Forde et al., 2002). Calves are able to distinguish the sound of their mother's call when compared to a dam that is not their mother (Barfield et al. 1994). Seventy-eight percent of 341 dams in a study by Florcke et al. vocalized to their calf when approached by an unfamiliar vehicle, which the calf responded to by moving closer to the dam (2012). Vocalization can be used as a tool to assess welfare (Grandin, 1998). Vocalization can give clues to age, dominance status, sex, and reproductive status (Watts and Stookey, 2000). Watts and Stookey (2000) reviewed the literature of cattle vocalization which includes evidence for the information content of cattle vocalization and approaches to the study of vocal communication and described the need for future research to better understand cattle vocalization.

Weaning

Weaning is considered an integral part of raising livestock in order to give the mother time to recover from lactation and its effects on decreasing body condition score (BCS) to prepare for the next offspring (Newberry and Swanson, 2008). Weaning is a stressful time for calves and cows (Orihuela and Galina, 2019; Wilson et al., 2017; Enriquez et al., 2011; Lynch et al., 2010; Lefcourt

and Elsasser, 1995). Not only is there physical separation from the dam, but also a loss of a food source (i.e., dam's milk), and in most cases, calves are moved to a new environment and must create a new social order (Newberry and Swanson, 2008; Weary et al., 2008). Cows and calves display behavior that can be indicators of stress such as pacing, vocalizing, and less time eating and drinking (Veissier and Le Neindre, 1989). In one study, multiparous cows showed increased pacing and less rumination after weaning compared to primiparous cows (Ungerfeld et al., 2011). It was concluded that the more extreme behavior of the multiparous cows was due to the more intense mother-offspring bond (Ungerfeld et al., 2011).

There is a growing interest in finding a better weaning system that improves welfare for both cows and their calves (Price et al., 2003, Enríquez et al., 2011, Ungerfeld 2015). One such technique to decrease stress is two-stage weaning, which allows the cow and calf to remain together, but does not allow the calf to suckle from the dam because of the plastic nose flap. With this technique calves showed fewer behavioral signs of stress compared to traditionally weaned cattle (Haley et al., 2005), and a later study found the two-stage process to have a positive effect on the wellbeing of the dams (Ungerfeld et al. 2015). Fenceline weaning, where the calves and the dams are separated through a fence, but can still see, hear, and have limited physical contact, has also shown to reduce stress in calves (Price et al., 2003). While weaning is considered a major source of stress for calves, it is still considered a necessary practice to ensure reproductive efficiency (Enríquez et al., 2011).

Importance of Maternal Behavior

One of the best ways to ensure profitability for a beef production system is to ensure cows are adequately converting grass to muscle and are delivering a living calf every year (Willham, 1972). The survival of the calf hinges on the maternal behaviors of the mother (Dwyer, 2008).

The efficiency of the cow herd, also known as maternal productivity, has long been considered the most correlated with total system efficiency (Archer et al., 1999). The profitability and efficiency of an operation is directly tied to the number of calves born and raised in a breeding cycle and the ability of the dam to reproduce annually (Grossi et al., 2008). Both reproductive and maternal behaviors are linked with productivity and profitability (Chenoweth et al., 2014). Cow-calf producers are trying to rise to the challenge of meeting the consumers demands, while also being sustainable to the environment and the herd as a whole (Tess, 2002).

Factors That Impact Maternal Behavior

Age/Parity Impact on Maternal Behavior

First parity cattle, commonly called heifers, are known to have greater levels of dystocia when compared to second parity cattle (Meijering, 1986). Dystocia can be described as prolonged and difficult parturition, which typically needs human intervention to keep both mother and offspring alive (Lombard et al., 2007; Zaborski et al., 2009). Meijering (1986) classified dystocia into two main classifications of causes: indirect and direct. Indirect would include causes such as phenotypic effects dependent on the cow and calf, non-genetic effects, and genetic effects (Meijering, 1986). Direct causes would include malpresentation and uterine torsion (Meijering, 1986). Dystocia can negatively impact calf survival rates with dystocia scores of 2 (mild dystocia) and 3 (severe dystocia) resulting in 2.3 and 15.4 times (respectively) more likely to be stillborn (Lombard, 2007). Calves born from severe dystocia have also proven to be slower to stand up and attempt suckling (Adams et al., 1995; Odde, 1988; Hickson et al., 2008). Cow deaths increase by 4% with cows who had extreme dystocia (Dematawena and Berger, 1997). Dystocia in heifers is largely attributed to feto-pelvic incompatibility (FPI) (Tierarzt et al., 1967). FPI is the combination

of a large calf and a small pelvic area (Meijering, 1984). Calving at 2 years old, compared to 3 years old, increases the efficiency of the beef herd; however a younger cow will have a smaller pelvic area, as they are not fully grown, which could lead to more FPI (McMillan and McCall, 1991). Odde (1988) described how cows with more severe dystocia had reduced mothering scores, which means they acted hesitant, or physically abused the calf.

Age is also directly related to parity, which means that the primiparous cattle are younger and less experienced compared to the multiparous cattle. Age also impacts the distance in which a cow will separate from the herd before calving (Florcke and Grandin, 2014). While this separation from the herd could allow for better maternal bonding without interference from conspecific, it also could leave the pair vulnerable to predation (Florcke and Grandin, 2014).

Breeds Impact on Maternal Behavior

Six breed groups (Angus, Charolais, Hereford, Heritage Angus, Polled Hereford, and Red Polled) had postpartum maternal response measured for twenty-five years (Sandelin et al., 2005). The breed, year, age of dam, and calf BCS has a statistically significant difference on the maternal behavior (Sandelin et al., 2005). In this study Red Poll cows had greatest (least favorable) numerical score (2.47 ± 0.11), while Angus and Heritage Angus had the least (most favorable) numerical score (2.10 ± 0.05 and 2.09 ± 0.10 , respectively; Sandelin et al., 2005). Hoppe et al., (2008) saw significant temperament differences between the German Angus (2.78, S.E. 0.05) and Simmental (2.29, S.E. 0.05) using a score of 1 to 5 to categorize the behavior of the dam during the tagging process: 1, the cows stands very quietly or rather shows indifference; 2, The cow stands quietly and observes, slightly excited; 3, the cow is excited and occasionally paws the ground; 4, the cow is nervous and tries to interfere with handling procedure; and 5, the cow is dangerous and tries to push the handler away. Hoppe et al. (2008) also saw differences in heritability of German

Angus (0.14, S.E 0.08) and Simmental (0.42, S.E. 0.05) cows for maternal protective behavior. This could be explained due to breeding and production changes, dairy cattle have been selected for less intense maternal behavior than beef cattle, because dairy calves are cared for by farm employees so the dams can produce milk for human consumption (Edwards and Broom, 1982). Therefore, any cross of a dual purpose or milk breed, such as Simmental, could impact the maternal behavior, or lack thereof.

Exposure to Predators

The presence of wolves increases cattle excitability and fear- related physiological stress response that have been exposed to cattle, but not present in cattle unfamiliar with this predator (Cooke et al., 2013). Cooke et al. (2017) reported that cattle that had experience with wolves had greater expressions of *c-Fos proto-oncogene* in the hippocampus and amygdala when compared to cattle that had no previous experience with wolves when exposed to wolf urine and recorded wolf howls. The expression of *c-Fos proto-oncogene* is a key biological marker which is downregulated during stress-related psychological disorders elicited by fear, such as in post-traumatic stress disorder (PTSD; Cooke et al., 2017). Cooke et al. also described how the previous experience with predators, or lack thereof, will change the behavior of cattle (2013) because of fear- related psychological disorders that could impact welfare and productivity (2017). It is well studied that cattle undergoing stress, whether physical or mental, causes loss of productivity (Grandin et al., 1998). Thus, cattle, even when not actively being preyed upon, could still be experiencing stress which could result in a loss of productivity (Cooke et al., 2017)

In wild ungulates such as elk (*Cervus elaphus*) the vigilant behavior is impacted by both predation risk and conspecific risk (Lung and Childress, 2006), and can be seen in cattle depending upon conspecifics and the environment (Kluever et al., 2008). Cows with calves showed vigilant

behavior more than twice as often when compared to cows with no calves (Kluever et al., 2008). Cows also showed different levels of vigilance depending on the size of the group they are in and the level of visibility in their surrounding environment (Kluever et al., 2008). Dams that lost calves to predation had a decrease in foraging behavior and increase in vigilance for about 10 days before returning to base line (Kluever et al., 2008). Cattle can also become laxer in antipredator behavior around heterospecific that are also preyed upon (Kluever et al., 2009).

Maternal Protectiveness in Beef Cattle

As mentioned previously, protection of the offspring is listed as one of the four components of maternal behavior (Grandinson, 2005). An important component of maternal style is defensiveness because the calf is under the dam's constant supervision to provide protection from predators or threatening conspecifics (Lent, 1974; Vicentini et al., 2022). Depending on how the cow perceives the human (predator, source of food, or social partner) post-calving cows can have a range of reactions (Le Neindre et al., 1998). While a protective dam is critical in an extensive grazing systems, it can be dangerous to stockman trying to handle the calf. In the European Union ranchers must ear tag calves within 20 days of birth in order to comply with the law (European Council Regulation No 820/97), thus waiting for the mother to become less protective (Veissier et al., 1990) is not an option.

Measurements of Maternal Protectiveness

Maternal protectiveness in cattle has been measured in many ways such as Pérez-Torres et al. (2014) held calves on one side of the fence and *Bos Indicus* dams on the other side. The calves were walked individually in front of the dam, selected randomly, and then the response of the mother was scored on a scale of 1-5, which ranged from 1- standing quietly, to 5- the cow is very

aggressive and tries to get through the fence. In this experiment the scores were then reduced into smaller categories of 1- does not react and 2-5- does react (Pérez-Torres et al., 2014). For the first two collections at 30 and 60 days postpartum only 10% of the cattle had no reaction, while the other 90% did react to either an alien calf or any calf presented (Pérez-Torres et al., 2014). Morris et al. (1994) evaluated 5,973 *Bos taurus* dams within 24 hours of partition and rated the protective behavior of the mother on a scale of 0-5. A score of 0 indicated the dam stood quietly and may lick the calf, 1 was generally quiet, offers token resistance, 2 slightly excited and occasionally pawing the ground, 3 excited vigorous tail swishing, bellows loudly, 4 very disturbed and tries to interfere with tagging process and handler only feels safe when cow is always watched, and 5 the cow is dangerous and unmanageable (Morris et al., 1994). The results showed 23% scored a 1 or 2, 42% scored a 3, 24% scored a 4, and 11% scored 5 (Morris et al., 1994). Hoppe et al. (2008) used a slightly modified scale of Morris et al., (1994) by changing score 1 to nonresponding. In this study 4% of the German Angus cattle and 15% Simmental scored a 1, and 16% of German Angus and only 7% Simmental scored either a 4 or 5; the Simmental were less reactive/protective than the German Angus dams. As previously discussed, this is likely due to the dual-purpose nature of the Simmental breed.

Florcke et al. (2014) used a circling approach, similar to a predator approaching, in an unfamiliar vehicle to the cows and measured individual defense patterns of the mothers to the newborn calves. She collected data on the distance in which the dam alerted to the vehicle, got between the vehicle and the calf, moved away from the vehicle, vocalization, and any signs of aggression (Florcke et al., 2014).

Maternal Protection and Humans

Livestock can remember painful procedures; it is critical to minimize painful procedures the first time an animal is exposed to a new person or piece of equipment (Grandin, 2021). However, on many ranches, the first encounter a calf has with a human can involve a painful procedure such as ear tagging, vaccination, castration. Yet, unlike mature cattle, it is commonly believed that very young infant animals do not remember the experience and will not make an association with the person performing the procedure (Grandin, 2021).

Multiparous cows tend to be more aggressive in the protection of their calves from humans (Vicentini et al., 2022). Nevertheless, cows that are difficult to handle in their first parity are likely to remain that way in the future (Turner et al., 2013). Humans can be perceived to the dam as a threat to the calf, and this perception can put people in danger (Le Neindre et al., 1998, 2002). While it is imperative for a dam to protect her calf, specifically when the calf is very young and has no defenses, it can pose a great risk if the dam feels the need to be aggressive toward humans (Buddenberg et al., 1986; Hoppe et al., 2008).

Human Injury

In 2020, 368 farmers and farm workers died because of a work related injury, which resulted in a rate of 18 deaths per 100,000 workers (CDC The National Institute for Occupational Safety and Health). A review by McCurdy and Carroll in 2000, found that up to one-third of agriculture-related injuries are due to livestock (McCurdy and Carroll, 2000). In 2017 the average age of a U.S. farm producer was 57.5 years (2017 Census of Agriculture Farm Producers).

It was hypothesized as a person aged, they would experience a greater rate of injury from working with livestock (Gelberg et al., 1999). However, this study from Kentucky and New York found no differences for injuries related to animals for young (18-59) or old (≥ 60) farmers which was 12.8% and 10.8% in New York and 19.8% and 18.5% in Kentucky, respectively (Gelberg et

al., 1999). Cattle that show unpredictable behavior or are aggressive should be culled from the herd (Watts et al., 2014).

Predators

Conflict between people and wolves over domesticated animals likely occurred around the same time ungulates were domesticated (Mech, 1995). Predatory species and humans compete against each other for biological resources such as wild game, fish, or livestock (Schwerdtner and Gruber, 2007). Wolves were viewed as evil and vile animals and have historically been hunted as threats (Young and Goldman, 1944). Wolves became protected in the Continental United States by the Endangered Species Act of 1973 (Mech, 1995). At that time, any remaining wolves survived in the most remote and well-preserved wilderness, which led researchers and lay people alike to believe wolves could not live outside this range (Mech, 1995). In hindsight however, it is more obvious that wolves can live in many environments, but at the time they had been exterminated from anywhere else (Mech, 1995). Since that time, the question has shifted from how to save the species to how is the best way to manage the species (Mech, 1995).

The price for predation typically falls on the unfortunate landowners whose property overlaps with a predator's territory (Fourli, 1999). Because of this, many people in those situations have a more negative attitude toward predators than the general public (Ericsson and Heberlein, 2003). In the livestock industry, it is imperative to understand the price that predators can have on livestock producers (Naughton-Treves et al. 2003; Schwerdtner and Gruber, 2007). Predators can create stress and cause significant damage, which can lead to human-wildlife conflicts (Yom-Tov et al., 1994; Cozza et al., 1996; Woodroffe et al., 2005). The federal government has assistance programs to compensate ranchers who lose livestock to confirmed wolf and other endangered predators (FSA LIP, Farm Bill 2018). While compensation programs can be useful to recoup the

monetary cost, it does not change or improve the attitudes of livestock producers towards wolves (Naughton-Treves et al., 2003). This compensation also typically covers the direct effect of livestock killed and does not include the profit lost from indirect effects such as reduced reproductive rates, or reduced average daily gain (Steele et al., 2013).

One of the most contentious and controversial issues in the Western United States is the reintroduction and management of predators (Bruskotter, 2013; Wilson, 1997). Since 1973 wolves had been protected under the Endangered Species Act until 2020 when they were considered recovered and taken off the list, and management was given to the states and tribes in which the wolves were located. Colorado State Statute 33-2-105.8, which passed on November 3, 2020, directed Colorado Parks and Wildlife to develop a plan to reintroduce wolves into the state, west of the Continental Divide. However, on February 10, 2022, the U.S. Fish and Wildlife Service vacated the 2020 final delisting rule, stating all gray wolves are now back under federal protection of the Endangered Species Act; except for those already in the Northern Rocky Mountain population. As Colorado fell outside of the Northern Rocky Mountain population range, the jurisdiction of the wolves has returned to the federal government. In order for the state statute to be put in place, the Colorado Parks and Wildlife will need to file an Environmental Impact Statement as part of the National Environmental Policy Act. This process is known to take a long time and could potentially set the project back by years. For ranchers this delay in the project is an optimal time to prepare themselves and their herds for the upcoming reintroduction. This time also gives ranchers another opportunity to talk to their legislators about policies surrounding the wolves.

Throughout domestication and selective breeding, domesticated animals appear to have lost some anti-predator behaviors (Price, 1984; Diamond, 2002). Domesticated animals have

smaller brains and less acute sensory organs compared to their non-domesticated relatives (Diamond, 1996; Clutton-Brock, 1981). This is likely due to selective breeding for other traits such as feed efficiency, growth, and other more economically important traits. (Diamond, 1996, 2002; Clutton-Brock, 1981). Compared to wild ungulates, like bison which spend 9.6-18.9% of their peak grazing time being vigilant of their surroundings (Laundré et al., 2001), cattle only spend around 3% of peak grazing time being vigilant (Kluever et al., 2008). All of these changes could lead to an increase in predation rates on domesticated cattle.

Temperament in Beef Cattle

Definition of Temperament

Temperament is typically described as an individual trait influencing an animal's behavioral response to human handling (Tulloh, 1961; Burrow, 1997). In as early as 1980 Elder et al. stated that temperament was an important quality when selecting cattle for purchase. In 1982, Fordyce et al. began to measure the temperament of cattle starting with four different methods. Two of these methods chute score test and exit speed, will be discussed later; breathing score, and bellowing kicking and kneeling score were also used at that time, but are not as common now (Fordyce et al., 1982). Later that decade in 1988, Burrow et al., declared the flight speed test to be an accurate and easily implementable test to measure temperament. More recently, temperament of cattle as an expected progeny difference (EPD) has been developed and researched as an important trait for over 25 years (Hyde, 2004; Le Neindre et al., 1995).

Importance of Temperament

Temperament can affect virtually all aspects of cattle production, including growth, reproduction, and immunity (Burdick et al., 2011). Cattle with excitable temperament are harder

to work with and pose safety hazards to both handlers and themselves (Grandin, 1993). Voisinet et al. (1997a) found temperament to be a critical factor in average daily gain (ADG), meaning cattle with a calmer temperament had improved ADG and cattle with more excitable temperament had a reduced ADG in a feedlot setting. Voisinet et al. also found feedlot cattle with more excitable temperament to have a greater incidence of borderline dark cutting, a meat quality defect as a result of chronic stress (1997b).

Heifers with excitable temperament tend to gain less weight and experienced delayed puberty compared to heifers with adequate temperament (Cooke et al., 2019). In adult cows, the measurements and physiological response of temperament influenced the probability of cows becoming pregnant during breeding season (Cooke et al., 2009).

Cattle with calmer temperaments are less susceptible to disease, and had a better immune response (Oliphint, 2006). In a feedlot setting, Fell et al. (1999) found calves with a more excitable temperament to have greater morbidity and reduced ADG. The study suggested that looking at the relationship between behavioral traits and immunological measurements may be useful in predicting suitability of individuals for the feedlot environment (Fell et al., 1999).

Measurements of Temperament

Hair Whorl

A hair whorl occurs when the backwards slope of the hair is reversed (Craft, 1934). Craft (1932) wrote a paper on the types of hair whorls found on mammals and the inheritance of hair whorls in swine. Since the late 1980s horse trainers have noted a correlation to type of hair whorls and temperament (Tellington-Jones and Bruns, 1985; Baker, 1990; Friedly, 1990). In 1995, Grandin et al. (1995) successfully correlated the height of the hair whorl on the face of the cattle

to agitated behavior during restraint. The center of the spiral was the reference point (Grandin et al., 1995). The height of the hair whorl was marked ‘high’ if the center was above the top of the eye, ‘middle’ if the located between the top and bottom of the eye, and ‘low’ if it fell below the bottom of the eye (Grandin et al., 1995). Cattle with none or multiple whorls was also recorded (Grandin et al., 1995). Florcke et al., added abnormal hair whorl (without a clear center) to improve the scale in 2012.

Chute Score

A chute score is used to describe the movement of cattle when restrained in a head gate (Tulloh, 1961), which is an indicator of temperament (Burrow and Corbet, 2000). Originally chute score was a seven-point scale used to describe and rank the behavior of animals in the chute (Fordyce et al., 1988). Grandin (1993) condensed the score to a 5-point scale, which would redistribute the 6 and 7 to 4 and 5. Then in 1995, the scale was once again condensed to better express the smaller availability of movement shown in a hydraulic squeeze chute on a scale of 1-4 (Grandin et al., 1995).

Exit Score

Exit score, or exit speed in some literature (Lanier and Grandin, 2002), is a score that describes the gait of an animal when coming out of a restrain for a set distance. When chute score and exit score were first being implemented, they were thought to measure the same thing, however now chute score is a measurement of fear and exit score is a measurement of anger (Grandin and Deesing, 2022). The original scale for exit score was 1=walk, 2=trot, 3=run, and 4=jump (Lanier and Grandin, 2002), however, in 2013 Vettters et al., proved that jump was not a continuous score and was removed from the scale. Then the scale was expanded to avoid middle group bias to 2=trot and 3=canter and 4=run (Vettters et al., 2013). The Beef Improvement Federation (BIF) uses

another way to measure exit score; instead of using the gate of the animal, BIF use more general terms on a scale of 1-6, with 1= slow and 6=very fast (BIF, 2018).

Exit Velocity

Exit score and Exit Velocity are so highly correlated that they measure the same behavior ((Lanier and Grandin, 2002; Vettters et al., 2013; Parham et al., 2019). Exit velocity, or flight speed, is measured by using infra-red laser to measure how much time an animal takes to cover a set distance and then velocity is calculated from those measurements (Burrow et al., 1988). This test is one of only a few tests for temperament that is objective (Burrow and Corbet, 2000). The distance in which the lasers are set apart has varied in different experiments; from 1.7 meters (Burrow et al., 1988) to 2.9 meters (Sebastain et al., 2011). Exit velocity is highly correlated to temperament as calmer animals exit the chute at a slower speed and more agitated animals leave at a faster speed (Sebastain et al., 2011).

Isolation Test

Le Neindre et al. (1995) first tested 10- to 11-month-olds calves to see if the handler could maneuver the heifer in a corner away from herd mates. Then, hold her there for 30 consecutive seconds, and then stroke the heifer, all in under two minutes. This experiment has become the foundation for most current isolation test. However, this test could put the handler at a greater risk of injury (Kilgour et al., 2006).

Stress in Cattle

Hypothalamic-Pituitary-Adrenal Axis

For the present study, the definition of stress will be “a state in which homeostasis is threatened or perceived to be so; homeostasis is attempted to be re-established by a complex repertoire of behavioral and physiological adaptive responses of the organism” (Chrousos and

Kino, 2005). Stress response begins when a stressor triggers the body through the hypothalamic-pituitary-adrenal axis (HPA axis) system and sympathomedullary system (Butcher and Lord, 2004). Stressors can be either internal or external, and disrupts homeostasis (Aguilera, 1998; Black, 2002). When the stress response is triggered, the higher brain centers stimulate neurons in the paraventricular nucleus of the hypothalamus, which causes a synthesis and secretion of corticotrophin-releasing hormone and vasopressin (Carrasco and Van De Kar, 2003; Plotsky, 1991; Gibbs and Vale, 1982). When greater concentrations of corticotrophin-releasing hormone and/or vasopressin gather in the hypothalamic-hypophyseal portal, blood exposes the anterior pituitary glands corticotrophs to neurohormones that activate the adrenal axis (Burdick et al., 2011). Once stimulated by corticotrophin-releasing hormone, corticotrophs within the anterior pituitary synthesize and secrete adrenocorticotrophic hormone into blood circulation (Carrasco and Van De Kar, 2003; Webster Marketon and Glasre, 2008). Through the endocrine mechanisms, adrenocorticotrophic hormone triggers the production of glucocorticoids by the adrenal cortex (Carrasco and Van De Kar, 2003; Makara et al., 1981). Once adrenocortical stimulation by the adrenocorticotrophic hormone glucocorticoids are released and distributed through the circulatory system and act systematically to produce a variety of effects depending on a number of factors (Burdick et al., 2011). Those factors include the amount of hormone secreted, the duration of secretion, peripheral blood concentration of cortisol binding globulins, the relative abundance of glucocorticoid receptors in the target tissue, specific tissue in which they exert their effect, and the extent of excretion of glucocorticoid metabolites (Burdick et al., 2011).

Glucocorticoids supply negative feedback to both the hypothalamus and the pituitary by stopping synthesis and/or secretion of corticotrophin-releasing hormone, vasopressin, and adrenocorticotrophic hormone (Aguilera, 1998). However, glucocorticoids can also cause an

increase of expression in vasopressin V1b and V1a receptors (Watters et al., 1996; Scott and Dinan, 1998). This suggests that corticotrophin-releasing hormone and vasopressin are regulated in different ways (Burdick et al., 2011).

Glucocorticoids can bind to both the mineralocorticoid receptor and the glucocorticoid receptor (Burdick et al., 2011). When the mineralocorticoid receptor becomes fully saturated then glucocorticoids will bind to the glucocorticoid receptor which initiates many of the effects on the hypothalamic-pituitary-adrenal axis and other body systems (Carrasco and Van De Kar, 2003). The mineralocorticoid can be found in the limbic brain areas as well as heart, kidney, and colon (Burdick et al., 2011). The attachment of the glucocorticoids to the glucocorticoid receptor makes modifications to gene expression to several genes, each being tissue specific (Burdick et al., 2011).

Sympathomedullary System

The sympathetic nervous system is triggered by stressors and often appears parallel, or even before, the stimulation to the hypothalamic-pituitary-adrenal axis (Burdick et al., 2011). Once stimulated, noradrenergic neurons in the brain and postganglionic sympathetic neurons innervating peripheral organs secrete norepinephrine into circulation (Burdick et al., 2011). That includes organs such as the heart, kidneys, vasculature, gut, and adipose tissue (Burdick et al., 2011). The secretion of norepinephrine and other catecholamine increase blood pressure, heart rate, and respiration rates in all species (Burdick et al., 2011). Nerve impulse in higher cortical centers located within the brain relay messages through the limbic system resulting in the secretion of norepinephrine, serotonin, and acetylcholine, which activates the paraventricular nucleus (Burdick et al., 2011; Black, 2002). Preganglionic sympathetic fibers innervating the adrenal medulla stimulate the production and secretion of epinephrine and norepinephrine via acetylcholine (Butcher and Lord, 2004; Burdick et al., 2011). Increasing concentrations of epinephrine in the

brain will set off alarms and limit neurovegetative activities as well as signal the hypothalamic-pituitary-adrenal axis (Tsigos and Chrousos, 2002).

Interactions between the HPA Axis and the SMS

The responses of the hypothalamic-pituitary-adrenal axis and the sympathomedullary system are extremely concordant, and have the ability to affect each other (Burdick et al., 2011). Connections between norepinephrine and corticotrophin-releasing hormone in the brain are reciprocal, which allow each hormone to activate the other (Tsigos and Chrousos, 2002). Catecholamines and glucocorticoids work together to raise cardiovascular output and catabolic effects, however they can also inhibit other systems like reproduction and immune systems (Butcher and lord, 2004; Chrousos and Gold, 1992).

Blood Lactate

Increased stress hormones such as epinephrine and cortisol will increase gluconeogenesis, proteolysis, and increased sensitivity of lipids to lipolytic hormones (Harvey et al., 1984, Boles et al., 2012). The increase in these systems leads to increases anaerobic metabolic processes in the muscle, and that results in the high concentrations of lactic acid in the blood (Boles et al., 2012). In one study average concentration of blood lactate in cattle is between 2.5 mmol/L and 3.0 mmol/L; the range of the samples spanned from 0.3mmol/L-25.0mmol/L (below detectable limits) and 11.3mmol/L (Boles et al., 2015). In Burfeind and Heuwieser (2012) their average blood lactate concentration was 1.83mmol/L. Variation in temperament as well as exercise can also impact lactic acid (Holmes et al., 1972). Blood lactate in combination with rectal temperature are strong candidates to predict exit velocity in steers, which as described above is an indicator of temperament (Williams et al., 2019). Blood lactate measurements have historically been run in a lab, however advancements in technology has developed hand held lactate readers which can be

run anywhere and only require a single drop of whole blood with up to $r= 0.9736$ correlation to lab run analysis (95% confidence interval: 0.9562–0.9841, Buczinski et al., 2014).

Conclusion

Maternal behaviors play a critical role in profitability and viability of calves. Maternal protection is a crucial component of maternal behavior (Grandinson 2005), however it can be dangerous if the dam interprets the rancher as a threat to her calf (Buddenberg et al., 1986; Hoppe et al., 2008). Temperament has been, and will continue, to be a deciding factor for ranchers to use as they continue to strive for better genetics. As the world begins to care more about food animal welfare (Fernandes et al., 2019) reducing stress for the animals will be a top priority (Etim et al., 2014). Stress can have many negative effects on cattle production such as behavior, efficiency, and immunity of cattle (Burdick et al., 2011). Understanding stress in the brain involves many systems like the hypothalamic-pituitary-adrenal axis and the sympathomedullary system. Blood lactate can be a blood biomarker for stress as blood lactate builds up in the blood when the body is working in an anerobic state under stress. The intent of this literature review was to examine maternal behavior, including maternal protectiveness, temperament, and a brief overview of predators and stress in beef cattle on subsequent behavioral outcomes.

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CHAPTER 2: INVESTIGATING THE RELATIONSHIP BETWEEN MATERNAL PROTECTIVENESS AND TEMPERAMENT IN BEEF CATTLE

Study Overview

Observations were made on 73 dams on two ranches in Colorado and one in Wyoming during the spring 2022 calving season to investigate the relationship between temperament and calf protection. The objective was to compare temperament scores to the distances of protective behavior performed by the dam as the observer moved closer to the pair. The protective actions of the cow were: Approach (alerted by raising her head to observer's presence), Protective (moved between observer and calf), Vocalization (called to calf), and Closest (closest distance the observer reached before the pair moved away). Temperament scores included chute score, exit score, exit velocity, and blood lactate. One hundred percent of the dams alerted to the observer's presence, twenty-five percent moved between the observer and the calf, and sixty-three percent vocalized to their calf. Thirty-five (47.9%) dams allowed the observer to touch the calf, categorized as Touch, while the other thirty-eight (52.1%) walked away from the observer before contact with the calf, categorized as No Touch. In a linear model using Touch/No Touch as the predictor and the continuous variables of Approach, Protect, Vocalize, Closest, exit velocity, and blood lactate; only closest and exit velocity had significant differences ($P < 0.05$) between the means. Dams that allowed the observer to touch the calf, on average, exited the chute with lesser velocity compared to those that did not allow observer to touch calf. Blood lactate and exit velocity were predictors in a mixed model with Ranch as a random factor with Touch/No Touch as the outcome; no significant difference was found. The Kruskal-Wallis test was performed individually on Approach, Protection, Vocalization, and Closest with predictor values of Ranch, date of birth

(DOB), and hair whorl. A significant difference appeared between the medians of Closest when grouped by hair whorl; dams with abnormal hair whorls moved away from the observer at a greater distance. When divided by Ranch, there were differences in the average chute score.

Keywords: Temperament, Hair Whorl, Maternal Protection

Introduction

Temperament, the way cattle react to human handling (Tulloh, 1961; Burrow, 1997) has been and is still used as a critical selection tool for breeding cattle (Elder et al., 1980; Parham et al., 2021). Temperament can impact virtually all aspects of cattle production, including growth (Voisinet et al., 1997), reproduction, and immunity (Burdick et al., 2011), which carry economic implications. Cattle with excitable temperaments are also harder to handle and pose safety hazards to both handlers and themselves (Grandin, 1993). Thus, the cattle industry has been breeding for more docile and, therefore, profitable animals (Hyde, 2004; Le Neindre et al., 1995).

The profitability of a ranch depends largely upon the ability of each dam to produce a calf every year (Willham, 1972); however, for this to happen, each dam needs to have strong maternal behavior to increase the calf's chance of survival. Maternal protection is considered one of the four fundamental components of maternal behavior (Grandinson, 2005); the other three components include creating a bond between the mother and the offspring, nursing behavior, and responsiveness/attentiveness (Grandinson, 2005). The calf is under the dam's constant supervision to provide protection from predators or threatening conspecifics (Lent, 1974; Vicentini et al., 2022). Cattle, like most mammals, react to pain, stress, or threats in three distinct ways: flight, fight, or freeze (Anderson and Muir, 2005). There are individual differences in how a cow protects her calf (Florcke et al., 2012); many (99.1% in one study) dams will face the perceived threat, then

move between the threat and the calf (Florck et al., 2012). The dam's vocalization was another common (78%) protective behavior because the vocalization signals the calf to come closer (Florcke et al., 2013). This study hypothesizes that cattle with a more docile temperament will have less drive for maternal protectiveness against humans.

Materials and Methods

This study was granted exemption by Colorado State University Institutional Animal Use and Care Committee. IACUC protocol #3057.

Maternal Data Collection

Maternal protection observations were collected during the spring 2022 calving season on three commercial ranches in northern Colorado and southern Wyoming. The study ranches manage 450, 280, and 160 cattle, respectively, of Angus and Angus crossed (30% Hereford- 70% Angus) multiparous cattle. Primiparous cows were excluded from the study because of the change in management required; heifers are more prone to dystocia and mismothering (Meijering, 1986) and are under closer surveillance. Inclusion criteria included: the calf had to stand and walk, be within 24 hours after parturition, and before the calf had been processed (tagged, vaccinated, castrated) by ranch employees. Maternal protective behavior was assessed by taking four distance measurements with definitions found in Table 1 Approach, Protection, Vocalization, and Closest using a laser range finder (Impact 1000 Laser Rangefinder, Vortex Optics, Barneveld, WI, USA). Points of distances of data collection are modified for an on-foot approach from Florcke and others (2012). Behavioral signs of aggression (pawing, charging, head shaking) were also noted. The cow-calf pair was approached in a spiral pattern on foot to more closely replicate the approach of a predator (MacNulty et al., 2007), as shown in Figure 1. The observer wore a neon yellow

reflective safety vest to add novelty and distinguish themselves from normal handlers. The Closest category was broken down into two subgroups: Touched, indicating the observer touched the calf, or No Touch, in which the observer did not touch the calf. The dam's date of birth (DOB) was obtained through ear tag and ranch records.

Temperament Data Collection

Temperament data was collected on 7/27/22, 11/3/22, and 3/16/23. Ranch 1 was collected during a 30-day pregnancy check using a hydraulic chute (Cumming and Son, Ultraline, Garden City, Kansas). Ranch 2 was collected at a 60-day pregnancy check in a breeding box. Ranch 3 was collected during a research project that required the herd to go through a hydraulic chute (Daniels Manufacturing Co., Ultimate AH, Ainsworth, NE). Only Ranch 1 still had calves at side while temperament measurements were conducted. Temperament attributes were evaluated by measuring exit velocity, hair whorl, blood lactate, chute score, and exit score. Exit velocity was measured by placing electronic laser timers (Farm Tek Inc., North Wylie, TX, USA) 1.83 meters apart and then recording the time in which it took the cow to cross the laser barriers (Curley et al., 2006). Hair whorl was evaluated using the Grandin et al., 1995 system that was modified by Florcke et al. (2012), which includes six mutually exclusive categories: high (above the top of the eye), middle (between the top and bottom of the eye), low (below the bottom of the eye), multiple (more than one), abnormal (unclear center spiral), or none. Blood lactate was measured by collecting blood from the ear, tail, or jugular vein and testing it with a handheld Lactate Plus meter in real-time (Lactate Plus, Nova Biomedical, Billerica, MA, USA). Chute score was evaluated by one person using the 4-point scale developed by Grandin et al., 1995, which ranges from 1 (calm, no movement), 2 (restless, shifting weight), 3 (head throwing, squirming and occasionally shaking the squeeze chute), 4 (violently and continually shaking the squeeze chute) (Grandin et al., 1995).

Exit score was also evaluated by a single observer using a 4-point scale developed by Lanier and Grandin (2002) and updated by Veters et al. (2013), which included: 1 (walk), 2 (trot), 3 (canter), or 4 (run).

Statistical Analysis

Statistical analysis was performed using R 4.2.2 (R Core Team 2021). Descriptive statistics were performed by finding the minimum, mean, median, maximum, and standard deviation (SD) of continuous variables and the frequencies of the categorical variables. A correlation coefficient heat map was created for the continuous variables (e.g., Approach, Protection, Vocalization, Closest, exit velocity, and blood lactate).

Separate linear regression models were fit for maternal distances (Approach, Protective, Vocalize, and Closest) and temperament (exit velocity and blood lactate) with treatment (i.e., Touch/No Touch) as a fixed effect. The means and standard deviation were calculated for Touch and No Touch treatments and the P-value. Kruskal Wallis tests were performed to analyze non-parametric variables, including Approach, Protective, Vocalization, and Closest; DOB, Ranch, and hair whorl were used as predictors. Due to the nature of the Kruskal Wallis test, which can only test the effect of one predictor at a time, Ranch was included as a fixed effect for these tests. Additionally, linear mixed-effect models were fit for exit velocity and blood lactate using Ranch as a random effect and DOB and hair whorl as fixed effects. A model was created using blood lactate as the outcome and location as the predictor values. Model fit and ANOVA assumptions were checked using diagnostic plots, and ANOVA analysis was subsequently performed on the linear mixed models. The significance level was set at $P \leq 0.05$.

Results

Maternal Protection Descriptive Statistics

Seventy-three cow-calf pairs met the selection criteria and were approached by the observer. Table 3 summarizes the minimum, mean, median, and max distances in which the dams performed actions specified in Table 1. All dams alerted to the observer's presence and either walked away or allowed the observer to touch the calf. Table 2 shows the distribution of date of birth (DOB) and cows per farm evaluated. Table 5 shows distribution of chute score, exit score, and hair whorl.

Chute Score

Approximately 91.2% of the dams evaluated were scored as either a 1 or 2, meaning all dams had little to no movement while standing in the chute. However, there was a significant difference in the mean chute score by Ranch (P-value <0.005). Approximately 20% of dams on Ranch 2 had a chute score of 3, whereas Ranch 1 and 3 had no dams score above 2. No dams scored above a three on any of the ranches in this study. There was no significant difference for DOB and hair whorl for the chute score (P-value=0.063 and P-value=0.070, respectively).

Exit Score

All dams evaluated fell into the first two categories for exit score, indicating cows walked or trotted out of the chute. There was a tendency (P-value = 0.073) for the difference in the means by Ranch. All dams on Ranch 1 exited the chute with a score of 1; Both Ranches 2 and 3 had dams that had an Exit Score of two (n=7, n=2 respectively). DOB and hair whorl did not affect the exit score (P-value>0.05).

Hair Whorl

The hair whorl of the dams was evaluated using the methods described in Section #. The distribution of the hair whorl pattern was: 48.5% low, 22% middle, 16.2% none, 10.3% abnormal,

and 2.9% multiple. There were no high hair whorls observed. There was a significant difference ($P=0.042$) in the Closest distance associated with hair whorls. These means are listed in Table 6. Abnormal hair whorls were significantly higher than the other hair whorl categories for closest ($P\text{-value}>0.005$), meaning dams with abnormal hair whorls walked away at a farther distance from the observer.

Aggression

Only 9 of the 73 (12.3%) dams evaluated showed signs of aggression (previously listed in the Materials and Methods section) toward the observer. Seven of the nine cows that showed aggression allowed the observer to touch their calf.

Of all cows evaluated in this study, just under half allowed the observer to touch their calf ($n=35$, 47.9%). The remaining cows and calves retreated from the observer before reaching the calf ($n=38$, 52.1%). This is shown in Table 8.

Statistical Models

The results of linear models of continuous outcomes (Approach, Protective, Vocalize, Closest, exit velocity, and blood lactate) with binary Touch/ No Touch as the predictor outcome proved to have two outcomes with significant differences between the means. Since Touch was determined with zero meters between the calf and observer and No Touch was any distance greater than zero, a significant difference between Touch/ No Touch to closest is expected. Regarding exit velocity, the cows that allowed the observer to touch their calf had a higher exit velocity, meaning they exited the chute faster than the cows that did not allow the observer to touch the calf.

The residuals of the continuous values for temperament (exit velocity, blood lactate) were normally distributed; a mixed model was created using Ranch as a random factor that evaluated

the impact of DOB and hair whorl. Neither DOB nor hair whorl showed a significant difference between the means of exit velocity or blood lactate when Ranch was held as a random factor.

Blood for the lactate measurement was collected from the jugular vein, tail head, and vein on the top of the ear. Ranch 1 only used blood from the ear (n=14), and Ranch 3 only used blood from the tail (n=24), while Ranch 2 used blood from both the jugular (n=6) and ear (n=24). Comparisons of the predicted mean blood lactate showed significant variation by location. Blood from the ear had a mean of 2.13 ± 0.249 mmol/L (predicted mean \pm SE), jugular 4.28 ± 0.627 mmol/L, and tail 1.27 ± 0.313 mmol/L. The contrast of ear and jugular, as well as jugular and tail, were significantly different. No significant difference was found between the ear and tail.

Discussion

In Florke et al. (2012) 99.1% of the 341 Red Angus dams evaluated moved between the calf and the vehicle the observer was driving. In the current study, only 18 dams (24.66%) moved between the observer and the calf. The author speculates that the observer on foot was not as threatening to the cows as the unfamiliar vehicle. This is further supported by the average distance of Approach for all hair whorls on Red Angus in Florke et al. (2012) being 90.25m, while in this study, the average Approach is 65.51m in Angus. However, breed differences have been observed in the past and could explain some variations (Sandelin et al., 2005; Hoppe et al., 2008).

The percentage of aggressive cows was similar between Florke et al. (2012) and this study, 13.2% and 12.3%, respectively. Sandelin et al. (2005) found cattle dams that showed at least some aggression at the time of first processing/tagging had greater calf survivability scores than the other dams that showed no signs of aggression. However, a review by McCurdy and Carroll (2000) found up to one-third of agricultural-related injuries are due to livestock. Ranchers in an open field

have little protection against a dam trying to protect her calf from what she perceives as a threat. Aggressive dams should be among the first considered for culling (Watts et al., 2014).

In previous literature, multiparous cows tend to be more aggressive in protecting their calf from humans (Vicentini et al., 2022). Parity number, specifically cows with reduced parity, is a better predictor of maternal defense (i.e., cows with reduced parity have decreased maternal defensiveness) compared to pre-calving temperament (Turner and Lawrence, 2013). However, in this study, no significant difference in protection was found due to age.

One of the first papers exploring temperament and maternal behavior, Le Neindre et al. (2002) reported a weak correlation between temperament and maternal behavior in Limousin cows when looking at an isolation test for docility and licking time as a maternal behavior indicator. Turner and Lawrence (2007) reviewed the literature published at the time, which collected evidence that flightiness and strong maternal care were associated with heightened maternal defense in several species. Turner and Lawrence (2013) stated pre-calving temperament and maternal defensiveness are independent traits and are unrelated to the maternal behavior shown to the calf by the dam. However, pre-calving fear could impact calf growth (Turner and Lawrence, 2013).

Even though nearly 50% of the dams allowed the observer to touch their calf, it does not necessarily mean they would allow a predator to approach their calf. The dam's reaction to a person's approach on foot is not comparable to the reaction of the same dam to a predator. In a study in 2004, Welp et al. found that dairy cattle had increased time of vigilance when exposed to a dog compared to an unfamiliar human. The author speculates that the dams that allowed the observer to touch their calf were more intrigued by the novelty of the observer, and this curiosity outweighed their fear. The observer walked at a slow, consistent pace and was not intentionally

threatening. Cattle with more flighty temperament have faster exit velocity, but cattle that are flightier are also the first ones to approach novelty when the novelty was non-threatening.

Hirata and Arimoto (2018) performed the first novel object test on cattle in a pasture as a group. They found that highly exploratory or bold cattle consistently approached novel objects the first time they were presented (Hirata and Arimoto, 2018). *Bos indicus* cattle that differ in flight speed (exit velocity) also differ in personality traits (Müller and von Keyserlingk, 2006).

The variation in Chute Score could be due to the style of chute used at each Ranch. Ranch 1 and 2 used hydraulic chutes, while Ranch 3 chute score was evaluated in a breeding box. Breeding boxes do not have squeezing sides, thus giving the dam more room for movement. However, there was no significant difference in the mean chute score for Ranch 1 and Ranch 3, but there was a difference for Ranch 2. Ranch 2 had a hydraulic squeeze chute that had a neck extender attachment. This attachment is intended to hold the cow's head in a stable position away from its body so researchers or caretakers can easily access the neck, which is the only Beef Quality Assurance approved site for injections (NCBA, 2019). While the neck extender was not used during the chute scoring, many of the dams on Ranch 2 would fall to their knees on their front legs directly after getting caught by the head gate. The authors speculate that the dams were trying to avoid the neck extender by getting below the reach of the neck extender bars. Blood collection was performed after chute score was measured so location and pain of blood draw should not impact chute score.

The significant difference between locations where the blood was drawn on blood lactate could possibly be explained by the differences between arterial and venous blood. Blood collected from the tail is often a mix of arterial and venous blood because the coccygeal arteries and veins are adjacent. It is easy to puncture several coccygeal vessels, leading to mixing the blood (Tvedten

et al., 2000). Samples from the jugular are more consistent for collecting exclusively venous blood. The arterial blood in the tail sampled could have diluted the amount of lactate present in the sample, so in comparison, the jugular looks much greater. Another factor to consider in this analysis is the variation of n per sampling site. Out of the six jugular bleeds, one lactate mean was 7.3 and the other 9.8, two of the highest seen in the whole study. The individual animal variation could have also affected the difference of means for Blood Lactate by location.

By definition, temperament is cattle's behavior when handled by humans. To collect the maternal protectiveness data, an observer (human) was used to incite a reaction of the cows. Thus, this measurement of maternal protectiveness is, by definition, a measurement of temperament. While in the previous literature, these two ideas have been considered independently, in the future, it would be beneficial to develop a measurement of maternal defense that does not rely on humans. As predator reintroduction continues across the United States, more naïve herds will be exposed to predators, and selection for maternal protection from predators will be critical. Cooke et al., found cattle that had experienced predation had greater cortisol when exposed to wolf howling recordings and wolf urine (2017). This could have economic impacts that go beyond predation loss because stress inhibits many production factors (Cooke et al., 2017).

In mice, mothers that showed the fewest signs of fear of a novel object test also showed the most aggression toward a potentially threatening male intruder (Maestriperi and D'Amato, 1991). Genetic lines of mice showing low levels of anxiety also showed a greater aggressive response toward a male intruder during lactation (Parmigiani et al., 1999).

Conclusions

The aim of this study was to further investigate the relationship between temperament and calf protection of beef cattle. The objective was to compare temperament scores to the distances of protective behavior performed by the dam as the observer moved closer to the pair. The dams evaluated in this study that allowed the observer to touch the calf had a lower mean exit velocity compared to the dams that did not allow the observer to touch the calf. The number of dams that allowed the observer to touch their calf without retreating could be more about the exploration of a novelty than factors of temperament or quality of maternal protection. Cattle with abnormal hair whorls walked away from the observer at a greater distance. From this study, we can conclude that temperament and calf protection have little to no relationship. In the future, a better way to test maternal defense from predators needs to be explored. This study provides more information for ranchers to make decisions about herd selection.

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TABLES

Table 1. Maternal Protective Behaviors of Note

Specific protective behaviors that directed the observer on when to take distance measurements. These distances were set by Florcke et al., (2012).

Protective Behavior	Actions performed by dam as observer moves closer
Approach distance	when the cow first perceived the observer's presence and stopped performing previous behaviors. Followed by raising her head and following the movement of the observer. (Florcke et al., 2012)
Protective distance	when the cow walked between the calf and the observer. (Florcke et al., 2012)
Vocalization distance	when the cow vocalized toward her calf. (Florcke et al., 2012)
Closest distance	the closest distance between the observer and cow-calf pair before pair moved away or the observer touched the calf. (Florcke et al., 2012)
Touch	Observer touched the calf (Closest distance was zero)
No Touch	Observer did not touch the calf (Closest distance was not zero)

Table 2. Summary of Sampled Population Demographics

Description of distribution of ages and number of dams evaluated per Ranch.

DOB	n, %
2012	7, 10.3
2013	5, 7.4
2014	3, 4.4
2015	8, 11.8
2016	10, 14.7
2017	11, 16.2
2018	20, 29.4
2019	4, 5.9
Farm	n, %
1	14, 19.2
2	31, 42.5
3	28, 38.4

Table 3. Descriptive Statistics for Protective Behavior

Descriptive statistics for maternal protective behaviors of cows (n=73) in this study. The variation in the (n) is due to the dam's participation or lack thereof in Protection and Vocalization.

Maternal Protective Action Distance	Min, m	Mean, m	Median, m	Max, m	Standard Deviation	n, %
Approach ^a	6	65.507	50	545	70.658	73, 100
Protective ^b	5	15.167	8.5	92	20.475	18, 24.66
Vocalization ^c	2	15.717	9	94	18.543	46, 63.01
Closest ^d	0	10.822	3	67	18.357	73, 100

^aApproach distance: when the cow first alerted to observers presence and stopped previously performed action

^bProtective distance: when the cow moved between the observer and the calf

^cVocalization distance: when the cow vocalized to their calf

^dClosest distance: when the cow-calf pair started to retreat from the observer, or the observer touched the calf

Table 4. Descriptive Statistics of Blood Lactate and Exit Velocity

Description of the average Blood Lactate and Exit Velocity of all cattle observed. Exit Velocity has one missed value due to electronic timer error.

	(n)	Min	Mean	Median	Max	Standard Deviation
Exit Velocity ^a	67	0.316 m/s	1.074 m/s	0.9 m/s	3.091 m/s	0.564
Blood Lactate ^b	68	0.4 mmol/L	2.016 mmol/L	1.5 mmol/L	9.8 mmol/L	1.718

^aExit Velocity was measured using electronic laser timers(Farm Tek Inc., North Wylie, TX) across 1.83m

^bBlood Lactate was measured using hand held lactate meter (Lactate Plus, Nova Biomedical, Billerica, MA, USA) collected from either ear, tail, or jugular vein

Table 5. Distribution of Chute Score, Exit Score, and Hair Whorl (n=68).

Chute Score	
Score	n, %
1	27, 39.7
2	35, 51.5
3	6, 8.8
4	0, 0
Exit Score	
Score	n, %
1	59, 86.8
2	9, 13.2
3	0, 0
4	0, 0
Hair Whorl	
Category	n, %
Low	33, 48.5
Middle	15, 21.1
High	0, 0
Abnormal	7, 10.3
Multiple	2, 2.9
None	11, 16.2

Table 6. Closest Distance Average by Hair Whorl

The mean for the closest distance the observer could reach to the cow-calf pair in meters by hair whorl.

Hair Whorl	Mean for Closest	SE
Abnormal ^a	19.43	6.34
Low ^b	7.36	2.92
Middle ^c	16.53	4.33
Multiple ^d	0.0	11.85
None ^e	4.36	4.36

^aHair whorl does spiral does not have a clear center

^bHair whorl spiral is below the eye

^c Hair whorl spiral is between the top of the eye and bottom of the eye

^dMore than one hair whorl

^eNo hair whorl present on face

Table 7. Sample Size and Percent of Herd in Touch and No Touch Categories

Sample size and percent of observed in the Touch and No Touch categories.

“Treatment”	n, %
Touch	35, 47.9
Not Touched	38, 52.1

Table 8. ANOVA of Continuous Variables

Results of individual ANOVA models with treatment means, standard error (SE), and P-value for the continuous variables of Approach, Protective, Vocalization, Closest, Blood Lactate, and Exit Velocity.

Item	Treatment Means		SE	P-value
	No Touch	Touch		
Approach ^a , m	71.5	59.0	12.0	0.4560
Protective ^b , m	21.8	8.56	6.64	0.1780
Vocalization ^c , m	19.5	12.6	4.02	0.2113
Closest ^d , m	20.8	0	2.57	<0.0001*
Blood Lactate ^e , mm	2.03	2.00	0.301	0.9517
Exit Velocity ^f , s	0.882	1.285	0.093	0.0027*

^aApproach distance: when the cow first alerted to observer's presence and stopped previously performed action

^bProtective distance: when the cow moved between the observer and the calf

^cVocalization distance: when the cow vocalized to their calf

^dClosest distance: when the cow-calf pair started to retreat from the observer, or the observer touched the calf

^eExit Velocity was measured using electronic laser timers (Farm Tek Inc., North Wylie, TX) across 1.83m

^fBlood Lactate was measured using handheld lactate meter (Lactate Plus, Nova Biomedical, Billerica, MA, USA) collected from either ear, tail, or jugular vein

Table 9. Kruskal Wallis Test Results

Individual Kruskal Wallis test with approach, protective, Vocalization, and closest with date of birth (DOB), Ranch, and hair whorl

	Approach		Protective		Vocalization		Closest	
	KW Chi²	P-value	KW Chi²	P-value	KW Chi²	P-value	KW Chi²	P-value
DOB	12.749	0.078**	5.7442	0.452	8.441	0.295	7.5177	0.337
Ranch	0.746	0.689	4.4653	0.107	0.56323	0.755	0.056	0.972
Hair Whorl	2.0143	0.7331	1.5971	0.8093	2.6204	0.6232	9.9084	0.042*

Table 10. Table 10. Mixed Linear Model Test Results

Results of Statistical model with Exit Velocity and Blood Lactate as observed and date of birth (DOB) and hair whorl as predictors with Ranch as a random factor

	Exit Velocity		Blood Lactate	
	F value	P-value	F value	P-value
DOB	0.518	0.817	0.7211	0.6545
Hair Whorl	0.638	0.637	0.5945	0.6679

Table 11. Frequency of Chute and Exit Score per Ranch

Describes the frequency of chute and exit score for each of the three Ranches. Chute and Exit Scores are ordinal values that range from 1-4.

Farm	Chute Score^a	n, % per Ranch
1	1	10, 71.4%
1	2	4, 28.6%
1	3	0, 0%
2	1	3, 1%
2	2	21, 70%
2	3	6, 20%
3	1	14, 58%
3	2	10, 41.7%
3	3	0, 0%
Farm	Exit Score^b	n, % per Ranch
1	1	14, 100%
1	2	0, 0%
2	1	23, 76.7%
2	2	7, 23.3%
3	1	22, 91.7%
3	2	2, 8.3%

^aChute Score: 1 (calm, no movement), 2 (restless, shifting weight), 3 (head throwing, squirming and occasionally shaking the squeeze chute), 4 (violently and continually shaking the squeeze chute)

^bExit Score: 1 (walk), 2 (trot), 3 (canter), 4 (run)

Table 12. Distribution of Chute Score and Date of Birth by Hair Whorl

Distributions of chute score and date of birth (DOB) over hair whorl.

Chute Score	Abnormal^a	Low^b	Middle^c	Multiple^d	None^e
1	1	11	6	1	8
2	4	20	7	1	3
3	2	2	2	0	0
DOB	Abnormal	Low	Middle	Multiple	None
2012	0	3	2	0	0
2012	0	2	1	0	1
2014	0	2	1	0	0
2015	1	5	1	0	1
2016	0	4	2	1	2
2017	2	5	3	0	1
2018	4	9	3	1	3
2019	0	1	0	0	2

^aHair whorl does spiral does not have a clear center

^bHair whorl spiral is inferior to the eye

^c Hair whorl spiral is between the top of the eye and bottom of the eye

^dMore than one hair whorl

^eNo hair whorl present on face

FIGURES

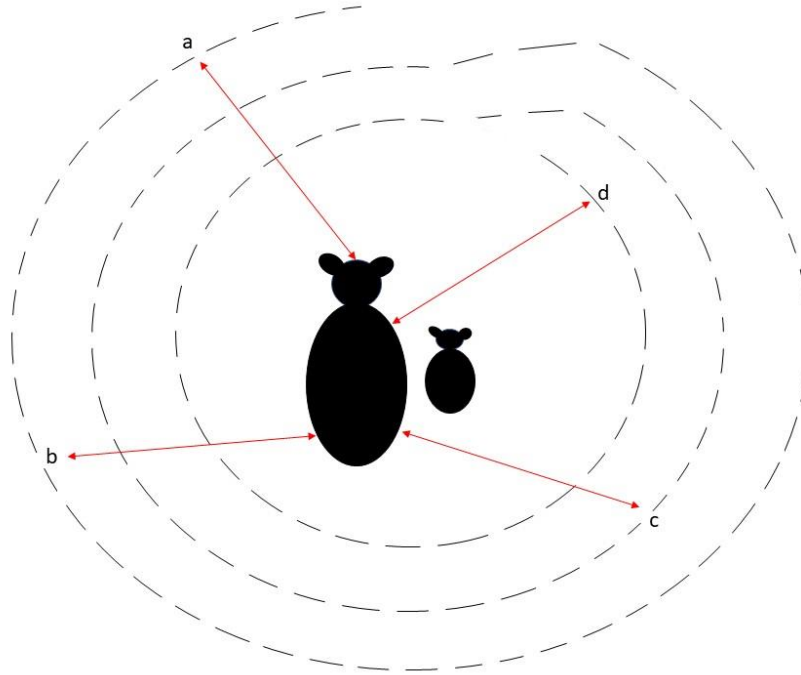


Figure 1. Illustration of the path the observer walked when collecting data on maternal protection. The observer wore a neon yellow reflective safety vest to add novelty and used a laser range finder (Impact 1000 Laser Rangefinder, Vortex Optics, Barneveld, WI, USA) to determine distance.

^aApproach distance: The distance where the cow first alerted to observers' presence and stopped previously performed actions.

^bProtective distance: The distance where the cow moved between the observer and the calf.

^cVocalization distance: The distance where the cow vocalized to their calf.

^dClosest distance: The distance where the cow-calf pair started to retreat from the observer, or the observer touched the calf.