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Bezerra, Victor Henrique Esterlino Ferreira Brusin

UNIVERSITY OF CALGARY

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by

Victor Henrique Esterlino Ferreira Brusin Bezerra

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ABSTRACT – EFFECTS OF THE APPLICATION ON NEONATAL BEEF CALVES OF TACTILE STIMULATION AND UMBILICAL ANTISEPTIC ON THEIR PRE-WEANED WELFARE

In the western Canadian beef cow-calf production systems, beef calves are generally kept with their dams in extensive outdoor grazing systems. Due to this, the contact between stockpeople and calves is infrequent. Often, when it does happen, the calf is subjected to potentially aversive experiences associate with capture and restraint, as well as painful procedures such as injections, castration, and branding. Young calves are also at risk of getting various diseases, including neonatal calf diarrhea (NCD), bovine respiratory disease (BRD), and umbilical infections, with negative consequent effects on their welfare. Practices like tactile stimulation and the application of umbilical antiseptic have the potential to improve human-animal relationship, calf health, and productivity, and these practices remains understudied in the context of beef calf processing in western Canada.

This thesis aims to evaluate the effects of the application of tactile stimulation and umbilical antiseptic on neonatal beef calves on their welfare level at the pre-weaned stage, using three different welfare indicators: behaviour, health, and productivity. Four experimental groups (TSUA, calves with tactile stimulation and application of umbilical antiseptic; TS, calves with tactile stimulation but no application of umbilical antiseptic; UA, calves without tactile stimulation but with application of umbilical antiseptic; and C, control calves without tactile stimulation or application of umbilical antiseptic) were evaluated to assess the association of these interventions with reactivity when interacting with humans in handling facilities, risk of disease treatment, mortality, and average daily gain. Of all the animal welfare indicators evaluated, only one behavioural indicator differed among treatments; flight speed was significantly lower for calves from TS group, indicating less reactivity, compared with other experimental groups. Other indicators were not significantly different among treatments. Overall, this study demonstrated limited evidence that tactile stimulation and umbilical disinfection, as applied in this study, impacted the welfare of pre-

weaned Angus beef calves.

PREFACE

Victor Brusin was involved in study design, data collection, statistical analysis, interpretation of results, and writing of the manuscripts under the guidance of his supervisor Dr. Maria Camila Ceballos, co-supervisor Dr. Claire Windeyer, and supervisory committee members Dr. Jennifer Pearson and Dr. Ed. Pajor.

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I am very grateful to animals, the source of my inspiration to do this project.

Thank you to Dr. Maria Ceballos. Some years ago, we had a conversation about you becoming my supervisor and I your student. It was just a dream. Today, we can say we dreamed together, and we got it. Thank you so much for the opportunity to become your student and to learn from you everyday. One day, I would like to be like you.

Thank you to the Margaret Gunn Endowment for Animal Research, the funder of this project, for financial support.

DEDICATION

To Rita Lee, one of the greatest singers in Brazil and an activist for animal rights, I bring here the prayer she created for the animals.

“My dear Francis of Assisi, protect the dogs and cats abandoned on the streets. Protect the sacred cows from barbecue and their raw meats. Protect the monkeys from yellow fever madmen. Protect horses and cattle from the slaughterhouse, saddle and spur. From butchers, protect pigs. From bullfighters, protect the bulls. Free the innocent animals condemned in your slaughterhouses. Protect exotic animals from smugglers. Protect rats, rabbits, hamsters and monkeys from cruel research. Protect the calves from the cowardly ropes of rodeos. Release the birds from their cruel captivity to the heavens. Protect the sweet beasts from the bestial safaris. Protect the furs of seals, foxes, and chinchillas from human vanities. Protect the chickens, ducks and turkeys in your inhumane aviaries. Protect the sweet sheep and goats from their bloodthirsty barbarians. Protect all pets from their greedy breeders. Protect lions, bears, elephants and tigers from merciless circuses. Protect porpoises, dolphins and whales from the swimming pools's prisons. Protect animals held in zoos. Protect the animals from the animals catcher. Protect animals and children from all inhumanity. Protect the pure in heart from all brutality. Amen."

Rita Lee, another autobiography.

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CHAPTER 1 – LITERATURE REVIEW

1. INTRODUTION

1.1. Animal welfare

The science of animal welfare emerged as a multidisciplinary research field in the 1970’s (Duncan, 1970; Dawkins, 1977; Wood-Gush et al., 1975). The World Organisation for Animal Health (WOAH) (OIE, 2021) defined animal welfare as “the physical and mental state of the animal in relation to the conditions in which it lives and dies”. An animal experiences good welfare if they are healthy, comfortable, well nourished, safe, not suffering from unpleasant states such as pain, fear, and distress, and are able to express behaviours that are important for their physical and mental state (OIE, 2021). Broom (1991) explained that animal welfare is a continuum varying from very good, where the individual is well adapted to its environment, to very poor, where the individual faces extreme, potentially even fatal, challenges. The Farm Animal Welfare Council (FAWC, 2009) proposed the "five freedoms of animal welfare", stating that animals will have good welfare if they are i) free from thirst, hunger, and malnutrition; ii) free from pain, injury, and disease; iii) free from discomfort; iv) free from fear and distress; and, v) free to express their natural behaviours. The five freedoms have been used internationally and became a conceptual reference in research, focusing on keeping animals free from harmful conditions. However, the term "freedom" anticipates that animals should not experience any negative state, such as hunger and thirst, but these physiological mechanisms are important for survival because they motivate animals to behave in specific ways to obtain life-sustaining resources, such as seeking and consuming food and water (Mellor, 2016). For this reason, it is recommended to use the concept of “needs” as a general reference for assessing animal welfare (Broom, 2011). A “need” is a requirement that is part of the basic biology of an animal to obtain a particular resource or respond to a particular environmental or bodily stimulus (Fraser and Broom, 1998). These needs are related to health, physiology, performance, resistance to diseases, and the possibility of expressing natural behaviours (Broom, 2021). Therefore, it is essential to provide animals with conditions that allow them to experience a high degree of welfare, where they have the resources that allow them to meet their needs (Broom and Molento, 2004). Accordingly, to evaluate animal welfare, it is necessary to consider not only the evaluation of negative conditions the animal may face but also the possibility of meeting their needs and experiencing positive emotions (Mellor and Beausoleil, 2015; Mendl et al., 2010). Mellor and Reid (1994) proposed the Five Domain model to evaluate animal welfare effectively and systematically. The most up to date Five Domain Model involves: 1) nutrition, 2) physical environment, 3) health, 4) behavioural interactions, and 5) mental state. The first four domains focus attention on the physical or functional states, while the fifth is related to the affective experience of the animal (Mellor et al., 2020). For a broad assessment of welfare conditions, indicators that assess both negative and positive experiences in each domain must be included (Mellor et al., 2020).

The importance of animal welfare is also reflected as a societal demand (Cornish and McGreevy, 2016) and directly impacts international trade, with various countries increasingly demanding minimum standards of animal welfare to export or import animal-origin products (Molento, 2005). The WOAH (OIE, 2019) recommended that the 178 member nations adopt 11 general principles for the welfare of animals in livestock production systems to guide the development of standards for various animal species. These are as follows: 1) Genetic selection should always take into account the health and welfare of animals; 2) Animals chosen for introduction into new environments should be suited to the local climate and able to adapt to local diseases, parasites, and nutrition; 3) The physical environment, including the flooring substrate should be suited to the species so as to minimise risk of injury and transmission of diseases or parasites to animals; 4) The physical environment should allow comfortable resting, safe and comfortable movement including normal postural changes, and the opportunity to perform types of natural behaviour that animals are motivated to perform; 5) Social grouping of animals should be managed to allow positive social behaviour and minimise injury, distress, and chronic fear; 6) For housed animals, air quality, temperature, and humidity should support good animal health and not be aversive. Where extreme conditions occur, animals should not be prevented from using their natural methods of thermo-regulation; 7) Animals should have access to sufficient feed and water, suited to the animals' age and needs, to maintain normal health and productivity and to prevent prolonged hunger, thirst, malnutrition, or dehydration; 8) Diseases and parasites should be prevented and controlled as much as possible through good management practices. Animals with serious health problems should be isolated and treated promptly or killed humanely if treatment is not feasible or recovery is unlikely; 9) Where painful procedures cannot be avoided, the resulting pain should be managed to the extent that available methods allow; 10) The handling of animals should foster a positive relationship between humans and animals and should not cause injury, panic, lasting fear, or avoidable stress; and, 11) Owners and handlers should have sufficient skill and knowledge to ensure that animals are treated in accordance with these principles. The latter 2 principles focus on human-animal relationship, which are important for animal welfare (OIE, 2019) and primarily related to fear (Rault et al., 2020).

1.2. Animal temperament: assessment and importance

Fear, aggression, and anxiety are animal temperament traits that are also impacted by human-animal relationship. Animal temperament is characterized by individual behavioural differences expressed early in life and relatively consistent over time (Bates, 1989, Réale et al., 2007). Although the term 'animal temperament' is widely used, there is a lack of consensus among researchers that leads to the use of the terms' 'personality' or 'personality trait' to also describe consistent individual differences in behaviour (MacKay and Haskell, 2015; Setser et al., 2023). Personality is defined as "an individual's behavioural responses to a complete range of possible stimuli and situations" (Setser et al., 2023). Personality trait refers to "a consistent behavioural response to a particular stimulus, with a range of possible responses across a population" (e.g., an individual can be more or less reactive) (Setser et al., 2023). Within the context of livestock production, the way an animal responds to handling (e.g., a beef calf restrained inside the squeeze chute) is commonly referred as animal reactivity, which can be measurable and used as the animal's behavioural response to a particular fearful situation and can be interpreted as an indicator of fearfulness (Reale et al., 2007; Stamps and Groothuis, 2010; MacKay and Haskell, 2015). These situations in which behavioural responses are measured to assess 'personality traits' or 'animal temperament' can be called 'personality tests' or 'temperament tests' (MacKay and Haskell, 2015; Setser et al., 2023).

Animal temperament differences can be identified through the exhibition of various animal responses, including exploration and sociability, aggressiveness, aversion to new situations, and reactivity, all of them within specific situations (Réale et al., 2000, 2007). The expression of cattle temperament is complex. Its evaluation usually uses indicators that assess one or more aspects at a time, with reactivity being the most used characteristic for evaluating beef cattle temperament (Benhajali et al., 2010; Boivin et al., 1992; Fordyce et al., 1988; Parham et al., 2019; Sebastian et al., 2011). Reactivity measures the animals' level of reaction to some type of handling, particularly stimuli caused by the presence of humans (Boivin et al., 1992). The decision of which trait to use depends on the objectives of the assessment, facilities, and personnel, as well as the type of cattle being evaluated (Kilgour et al., 2006). In animal production systems, it is possible to use a various temperament tests (Setser et al., 2023) that evaluates different traits, including open-field (Grandin and Deesing, 2022; Setser et al., 2023), novel environment (de Passillé et al., 1995; Forkman et al., 2007), novel object (Forkman et al., 2007), novel person and avoidance distance (Forkman et al., 2007; Waiblinger et al., 2006; Welfare Quality ®, 2009), startle (surprise) (Finkemeier et al., 2018), restraint (crush score) (Ceballos et al., 2016; Fordyce et al., 1982; Grandin, 1993), and flight speed (Burrow et al., 1988) tests. Temperament tests have the potential to be used by livestock producers to select cattle for temperament traits of their interest (Grandin and Deesing, 2022).

The open-field test puts the animal in either a new (novel environment) or known (open-field) environment (Grandin and Deesing, 2022; Setser et al., 2023) and measures exploration, activity, boldness-shyness, and fearfulness traits (de Passillé et al., 1995; Forkman et al., 2007). The temperament traits measured in this test may vary based on whether the animal had a free choice to enter and expose itself willingly, measuring exploration or activity (Carter et al., 2013). If the entry is forced and the animal has no choice, the traits measured would be fearfulness and boldness (Carter et al., 2013; de Passillé et al., 1995). In the open-field test, the animal is placed alone in an arena, either willingly or forcibly, and various activities are measured (Grandin and Deesing, 2022), including the time to enter (if the choice is free), the sections of the arena explored, time spent exploring, vocalizations, escape attempts, and locomotion (de Passillé et al., 1995; Forkman et al., 2007). The open-field test has several advantages for testing large animals because it is easily applicable (e.g. it is possible to apply in the farms' pens), and allows the measurement of other behaviours (e.i. vocalizations, locomotion, and latency to enter in the pen) (Kilgour, 1975). This test should be carefully interpreted, because it was created to evaluate fear responses in rodents in open spaces, but livestock species, such as beef cattle, are usually in open spaces (Boissy and Bouissou, 1995; Kilgour, 1975). Because of that, it is suggested that the open field test might also measure fear of isolation, given that livestock species are herd animals (Forkman et al., 2007). Thus, it is recommended to use this test in conjunction with the novel-object test, for a better interpretation of the all personality traits measured in the open-field test (Canario et al., 2013; Forkman et al., 2007).

The novel object test puts the animal in contact with an unfamiliar object (Forkman et al., 2007; Grandin and Deesing, 2022; Setser et al., 2023) and measure traits like exploration, boldness-shyness, and fearfulness in the animal (Forkman et al., 2007). However, it is recommended to use this test in conjunction with other tests when the goal is to measure only "fearfulness", to being able to differ this trait from "exploration" (Forkman et al., 2007). The animal is led to a familiar or unfamiliar environment and placed with a new object, for example an umbrella (Forkman et al., 2007). Then, behavioural measurements such as latency to approach, time interacting, time attentive toward the object, and the distance from the object are collected

(Forkman et al., 2007; Setser et al., 2023). The novel object test has several advantages, standing out as a straightforward method that eliminates the need for external motivational rewards or punishments (Antunes and Biala, 2012). Its efficiency shines through in its quick completion time, ensuring animals undergo the test without experiencing undue stress (Antunes and Biala, 2012). A notable strength lies in its ability to assess recognition memory after just one trial, presenting a clear advantage over alternative methods (Antunes and Biala, 2012).

The avoidance distance (AD) and novel person (NP) tests puts an animal or group of animals in contact with an unfamiliar (for both) or familiar (for AD) person, causing a mix of responses related to fear, panic, and exploration. Both tests indicate the degree of fear in relation to humans (Breuer et al., 2000) and is important in livestock animals because of the necessity of handling them for management (Setser et al., 2023). The AD measures the maximum distance the animal allows the observer to approach before it moves away (Hemsworth et al., 2000; Waiblinger et al., 2006; Welfare Quality ®, 2009). When the animal enters the pen, the observer remains stationary on the opposite side of the pen entrance, waiting for the animal to stand still before starting the test (Waiblinger et al., 2006). Then, the person approaches the animal slowly (e.g., one step per s) with the hands down and arms held close to the body. When the animal expresses any withdrawal reaction (e.g., the animal moves away), the observer stops and measures the distance to the animal's nearest front hoof (Hemsworth et al., 2000; Waiblinger et al., 2006; Welfare Quality ®, 2009). To conduct the NP test, the observer stays still for a period of time, and several animal activities could be measured such as latency to approach the person, time interacting with the person, time spent being attentive toward the person, distance from the person, the type of interactions with the person, and vocalizations (Forkman et al., 2007). There are several handling involved in livestock species and the avoidance distance test is especially important when applicable to those animals because it can measure the response of the cattle to a human approach (Forkman et al., 2007). Then, using that is possible to standardize practices of handling based on how the animal reacts to the humans' presence (Forkman et al., 2007). Both tests involve human presence (Beausoleil et al., 2008; Boissy et al., 2005), however, usually, animals undergoing these tests also have visual access to conspecifics and the measurement of fear of humans could conflict when they have access to their peers (Beausoleil et al., 2008; Boissy et al., 2005).

The startle or surprise test involves intentionally startling the animal by performing a sudden action or playing an auditory stimulus (Finkemeier et al., 2018; Grandin and Deesing, 2022). This test measure traits like boldness-shyness and fearfulness (Finkemeier et al., 2018). This test is less frequently used than the novel object test; however, to measure the fearfulness traits by animals to a ‘threat’ (Yuen et al., 2017). The startle test might use the sudden opening of an umbrella, dropping a heavy chain, a person stamping their feet, a puff of air in the face of the animal, dropping a ball into an arena, or auditory cues mimicking the presence of or signalling predators (Favati et al., 2014; Lauber et al., 2006; Neave and Zobel, 2020; Vandenheede and Bouissou, 1993). Several animal activities can be measured such as latency to unfreeze, latency to approach item after startling, time spent frozen, or vigilance after startling (Finkemeier et al., 2018). The main advantage of the startle test is that measures “fearfulness” or “boldness” traits by individuals in a ‘threat’ situation (Yuen et al., 2017). However, one disadvantage regarding the use of the startle test is the exposition of the animal to a ‘threatening’ situation which can have negative impacts on them such as putting the animal under stress (Yuen et al., 2017).

The restraint test uses a person or equipment such as a squeeze chute to restrain the animal (Grandin and Deesing, 2022). This test measure traits like fearfulness and reactivity of the animal (Grandin and Deesing, 2022). The main advantage specifically for beef cattle inside the squeeze chute, the temperament trait measured is reactivity to restraint, reflecting a fearfulness trait of the animal in a common husbandry management (Forkman et al., 2007). Usually, visual scores are used to assign predefined scores to assess how cattle react in the restraining handling situations (Tulloh, 1961). Higher values indicate more reactive animals and lower values signify less reactive ones (Fordyce et al., 1982; Grandin, 1993). The reactivity score inside the squeeze chute is the most commonly used in research and consists of evaluating the degree of disturbance of the animal when it is restrained in the squeeze chute (Ceballos et al., 2016; Fordyce et al., 1985; Grandin, 1993). The scores are applied according to the intensity and frequency of movement, breathing, kicking, and the position of the animal in the squeeze chute, such as lying or standing (Ceballos et al., 2016; Fordyce et al., 1985; Grandin, 1993). Different scores assess different animal reactions inside the squeeze chute. Among them, the 'crush score' (Cafe et al., 2011; Ceballos et al., 2016; Sant'Anna et al., 2013), 'movement score' (Fordyce et al., 1988; Grandin, 1993), 'agitation score' (Paranhos da Costa, 2002), and the 'ease of neck restraint score' (Hall et al., 2011). For a congregated interpretation of these scores, it is possible to add them together and creates a composite score with the extreme value representing animals more reactive and less value animals less reactive (Highfill et al., 2010; Meagher, 2009). Several animal activities can also be measured such as latency to unfreeze, activity within a box or chute, steps, kicks, or tension (Forkman et al., 2007). A disadvantage of these tests is that humans scores the response of the animal, which could be subjective and introduce potential observer bias (Setser et al., 2023). This can be avoided by training and making sure observers have inter and intra observer reliability (Vogt et al., 2017).

The flight speed measures the speed at which the animal leaves the squeeze chute or a space in which the animal is contained towards an open space (Burrow et al., 1988; Grandin and Deesing, 2022). This test measures fearfulness after leaving a restraining environment (Grandin and Deesing, 2022). The flight speed is assessed by measuring the time each animal takes to exit the squeeze chute and cover a known distance. Once the animal passes this knowledge distance, the exit time is recorded in seconds and converted into speed in m/s. Faster animals are considered more reactive than slower animals (Burrow et al., 1988). While electronic measurement of flight speed incurs higher costs, a distinct advantage lies in the heightened objectivity of these measurements (Hoppe et al., 2010). However, the reaction of animals leaving a restraining environment such as the squeeze chute, if repeated several times, a learning process can happen (such as habituation) due to the repeated exposure (Gibbons et al., 2011). It is suggested to use the flight speed test together with other tests such as restraint, open-field or novel object tests (Gibbons et al., 2011).

These reported tests help understand cattle temperament, which is very important for livestock farming, as it is related to productive performance, work safety, and animal welfare (Réale et al., 2007; Stamps and Groothuis, 2010). For example, Bruno et al. (2016) measured two temperament traits, novel objective chute score and flight speed, and described their interaction with mixed-breed steers' growth performance. Steers with low flight speed had greater average daily gain (ADG) and dry matter intake (DMI; amount of feed consumed per day) compared with faster ones. Also, steers with higher novel objective chute scores had greater DMI compared with the lower ones. The authors conclude that calmer steers had better growth performance than excitable ones (Bruno et al., 2016).

Temperament studies provide a basis for how animals perceive management, and this can be used to increase management efficiency with management techniques that are based on animal welfare (Boissy and Bouissou, 1995). Ceballos et al. (2018) developed research with Nellore heifers to understand the relationship between human-animal interaction, reactivity (a temperament trait), stress response, and reproductive indicators. The reactivity was measured using flight speed and composite reactivity scores. The more excitable heifers had lower chances of getting pregnant compared with calmer ones. Also, the highest levels of cortisol were found in the most reactive heifers. The authors concluded that poor quality handling practices were associated with heifers being more reactive and could increase the risk of animal accidents (Ceballos et al., 2018). Using correct handling practices when interacting with animals over time has the potential to reduce animal's fear of humans and encourage approach and interaction (Hemsworth et al., 1993, Hemsworth, 2003; Hemsworth and Coleman, 2010; Rault et al., 2020)

1.3. Human-animal relationship

Human-animal relationship within livestock production systems are related to any contact between handlers and animals and may involve different aspects, such as tactile, visual, olfactory, and auditory perception (Hemsworth and Coleman, 2010). Various factors affect human-animal relationship such as the frequency, duration, and nature of the interactions between them (Hemsworth and Coleman, 1998). The nature of human-animal relationship can be positive, neutral, and negative, based on how the handler performs the tasks on the farm (Hemsworth and Coleman, 1998; Waiblinger et al., 2002).

Human-animal relationship is an important determinant of animal welfare (Boivin, 2018; Hemsworth and Coleman, 2010; Hemsworth et al., 2018; Pinillos et al., 2016). Negative human-animal relationship can negatively affect animal productivity, health, and behaviour, primarily through fear as the underlying mechanism (Hemsworth and Coleman, 1998; Hemsworth et al., 2018), which can hinder animal welfare. Fear is an aversive emotional state (Boissy, 1995). Fearfulness is considered a temperament trait defining the general susceptibility of an animal to react to a variety of potentially threatening situations (Boissy, 1995). Fear is triggered by environmental stimuli that are either novel or has specific fear-inducing attributes, such as by another animal, specific shapes, facial features, loud noises, or sudden movements, and stimuli that have been associated with previous aversive experiences (Boissy, 1995). Studies in experimental and commercial conditions with farm animals demonstrate that the quality of stockpeople’s interactions influences animals' perception of humans with direct impact in the fear the animals can feel from humans (Hemsworth and Coleman, 2011; Hemsworth, 2018; Rault et al., 2020).

Fear can be reduced through neutral or positive human-animal relationship (Hemsworth, 2018; Rault et al., 2020). A positive human-animal relationship is contingent upon the animal's positive perception of the human involved (Rault et al., 2020). Although evaluating this perception in practical terms is challenging, it is possible to assess based on behavioural changes such as the voluntarily approaching and seeking spatial proximity, signs of anticipation, pleasure, relaxation, prosocial or affiliative behaviours, and other indicators that reveal a rewarding experience stemming from the interaction with the human (Mellor, 2012; Rault et al., 2020).

Establishing a relationship is a progressive process, strengthened through repeated interactions over time (Rault et al., 2020). Associative learning can expedite the development of a positive human-animal relationship, as the animal links humans to positive elements, whether through classical conditioning (i.e., the presence of humans or their simultaneous association with a positive event) or operant conditioning (i.e., engaging with humans results in positive consequences) (Brajon et al., 2015; Gácsi et al., 2001; Lensink and Boivin, 2000; Rault et al., 2019, 2020; Schmied et al., 2008; Tallet et al., 2005). Handling strategies that reinforce positive human-animal relationship, such as gentle handling, food reward, and brushing has been studied in production systems (Bertenshaw et al., 2008; Heird et al., 1986; Tallet et al., 2018). Rault et al. (2019) investigated the possible changes in brain activity in pigs undergoing different interactions with a human (i.e., stroking the pig's abdomen by a human, human presence, isolating the pig individually, and being in the home pen as a baseline) (Rault et al., 2019). The brain activity was lower when stroking the pig's abdomen than other treatments and elicited a distinct behavioural response suggesting the animal was calm, stable, and at rest (Rault et al., 2019). The authors suggested that the lower brain activity linked with those behavioural changes indicates positive welfare state (Rault et al., 2019). Schmied et al. (2008) developed a study stroking different body regions of dairy cows to investigate its effects on avoidance and approaching behaviour. Results demonstrated that stroking, particularly the neck, reduced avoidance and increased approach reactions to humans (Schmied et al., 2008).

It may be easier to develop a positive human-animal relationship with young animals, because they typically have had fewer negative experiences with humans, are more curious, explore more, and may have a greater learning ability than adult animals (Foster et al., 2012; Hemsworth and Barnett, 1992; Nowak and Boivin, 2015; Scott, 1963, 1992). Researchers who applied tactile stimulation to young animals report positive effects on the development, behaviour, and health of dairy calves (Silva-Antunes and Paranhos da Costa, 2021), piglets (de Oliveira et al., 2015), foals (Schmidek et al., 2018), and mice (Costa et al., 2020).

1.4. Tactile Stimulation

Tactile stimulation is "the mechanical contact between two or more individuals of the same or different species that are perceived as either positive or negative" (Bolognesi and Gonçalves, 2019). In rats, tactile stimulation was characterized as a method of sensory stimulation to the skin that mimics maternal behaviour, licking, and grooming (Mychasiuk et al., 2013). Those maternal behaviours are common among mammals, including cows who perform mechanical and systematic contact by licking their calves (Schmied et al., 2005; Schmied et al., 2008). These behaviours contribute to establishing the bond between dam and calf (Paranhos da Costa and Cromberg, 1998). In addition, they stimulate mechanoreceptors within somatic structures, for example, the skin (BVSC, 2022; Somjen, 2013). There are two main types of mechanoreceptors in the skin: the Meissner corpuscle, which is sensitive to light touches, and the Pacinian corpuscle, which is the touch pressure receptor. These two mechanoreceptors have non-neural specialized cells, called C fibres, associated to the nerve fibres' ends (BVSC, 2022; Somjen, 2013). Pacinian corpuscle has a nerve ending surrounded by layers of cells and extracellular fluid (BVSC, 2022; Somjen, 2013). When the skin receives a mechanical pressure, for example tactile stimulation, the Pacinian corpuscle generates an electrical stimulus and transmits it to the brain (BVSC, 2022; Somjen, 2013). This electrical stimulus goes through somatic afferent pathways to the central nervous system (Somjen, 2013). These nerve impulses favour the development of neurons and the formation of synapses, resulting in the ability to drastically reorganize the neuronal morphology and synaptic connectivity of the individual’s developing brain (Mychasiuk et al., 2013; Somjen, 2013).

Newborn farm animals become conscious either at birth or shortly thereafter (Mellor and Diesch, 2006). Early in their life, animals acquire the capacity to interpret their experiences as harmful and aversive or as positive and rewarding depending on the intensity, duration, and frequency of the external stimuli (Chen and Sato, 2017; Mellor and Diesch, 2006; Westerath et al., 2014). They can perceive tactile stimulation as a process similar to licking from their mothers, characterized as allogrooming or social grooming. These behaviours have the potential to increase basal oxytocin concentrations, resulting in a positive experience for the animal (Chen and Sato, 2017; Westerath et al., 2014). Therefore, tactile stimulation can be used as associative learning to develop a positive human-animal relationship (Chen and Sato, 2017; Mellor and Diesch, 2006; Westerath et al., 2014).

Tactile stimulation has the potential to promote good welfare (Costa et al., 2020; de Oliveira et al., 2015; Kowalik et al., 2017; McBride et al., 2004; Silva-Antunes and Costa, 2021) particularly during the first phase of animals' lives, when this handling strategy may have positive effects on the relationship between humans and animals, reducing fear and stress, and facilitating future handling (Becker and Lobato, 1997; Boivin et al., 2003; Waiblinger et al., 2006). Oliveira et al. (2015) examined whether early human handling using tactile stimulation would improve piglets' reactions in a standardized fear test. In litters where all or half of the piglets received tactile stimulation, piglets allowed more physical contact with a familiar or an unfamiliar person compared with litters that no piglets received tactile stimulation. Furthermore, piglets in litters where they all received tactile stimulation vocalized less than piglets in litters when none received tactile stimulation. Thus, providing early human interaction may diminish fear of humans in piglets, leading to their increased activity and reduced vocalization in a new environment (de Oliveira et al., 2015). Lensink et al. (2000) examined the effects of positive human contact on veal calves on their reactivity. Providing veal calves regular additional contact by a stockperson stroking and letting calves suck their fingers were highly effective in reducing calves' fear of humans, leading to less avoidance and more human-animal interactions (Lensink et al., 2000).

Although tactile stimulation has the potential to promote positive human-animal interaction, implementing this practice within the beef production system could be challenging.

Human contact with calves is minimal, as calves remain with their mothers from birth until weaning, typically in extensive outdoor grazing systems (Fraser, 2005). However, there is an opportunity for interaction between the stockpeople and newborn calves when calves are handled within the first week of life for identification and vaccination (Orey, 2008). These management practices can potentially result in a negative human-animal relationhip (Adcock and Tucker, 2018). Nonetheless, at this same moment, there is an opportunity to improve interactions with calves by applying positive tactile stimulation and testing the performance of potential preventive health management practices, such as the application of umbilical antiseptic.

1.4.1. Effects of tactile stimulation on reactivity

Jago et al. (1999) compared dairy calves that were hand-fed, handled involving stroking, or both. Calves fed without human presence readily approached and engaged with unfamiliar individuals, spending a significant amount of time in their vicinity during arena tests compared with the others groups, suggesting that handling with stroking could play a crucial role in shaping subsequent interactions between humans and calves. Interaction between humans and animals could be potentially modified more effectively when associated with food (Jago et al., 1999). Vicentini et al. (2023) investigated the effects of gentle tactile stimulation prior to calving on dairy cows' maternal care during post-calving period and maternal protective behaviour towards handlers during the first handling of calves. Results suggested that pre-calving training protocol involving tactile stimulation for the first milking was associated with reduced movement and lower maternal defence, which could have a potential benefit for the handler's safety during routine processing of calves (Vicentini et al., 2023).

Jago et al. (1999) compared the effects of different types of contact involving brushing, approaching, or any contact between stockpeople and calves. Their results suggested that brushing and stockpeople approaching improved the human-animal relationship with calves, whereby calves approached humans more. In an unpublished study (Cerezo, 2023), the short-and long-term effects of tactile stimulation on the welfare of newborn Nellore calves were evaluated. The findings indicated that calves receiving tactile stimulation for 1 minute exhibited a more positive emotional state, compared to those that did not. This implies that incorporating this practice, even for just one minute during initial handling procedures, holds promise for enhancing calves' responsiveness to stockpeople (Cerezo, 2023).

1.4.2. Effects of tactile stimulation on health

Silva et al. (2017) applied a package of management practices in dairy calves, consisting of the application of umbilical antiseptic, colostrum feeding, and brushing for 5 minutes, with no effects on the odds of calves being treated for pneumonia and bovine anaplasmosis (Silva et al., 2017). Also, an investigation of the same package of management practices reported that calves who received the handling practices had a decrease frequency of antibiotic treatments and number of calf deaths (Silva-Antunes and Costa, 2021). The authors suggested that the act of brushing improved the interaction between handlers and calves, which made it possible for the handlers to identify the clinical signs of diseases earlier, allowing for early veterinary intervention, when necessary (Silva-Antunes and Costa, 2021). These studies demonstrate that tactile stimulation, often combined with other management practices, may have an effect on health parameters. Therefore, more studies are needed to confirm its influence on this parameter.

A study focusing on physiological parameters reported that calves that experienced tactile stimulation, performed by two handlers who brushed them once a day, had lower serum lactate concentrations after brushing when environmental enrichment was present compared when the environmental enrichment was not present (Miranda et al., 2023). This suggests that tactile stimulation was important for reducing lactate levels, which can be produced immediately after a sudden fright or tension (Sandhu et al., 2000; Stockham and Scott, 2013), positively influencing the welfare of calves in an enriched environment (Miranda et al., 2023). Waiblinger et al. (2004) reported similar results when investigating the effects of previous positive handling and of gentle interactions during rectal palpation with sham insemination in dairy cows. Previously handled animals had lower heart rates, kicked less when alone, and tended to display less restless behaviour, which may mean that positive interactions can be used to reduce the stress reactions of cows during the rectal palpation (Waiblinger et al., 2004). It is well established that stress is associated with health (Broom et al., 1993; Moberg, 1985).

1.4.3. Effects of tactile stimulation on productivity

Weight gain in rats can be influenced by the physical effects of massage-like stroking, potentially through vagal activation of the gut's endocrine system, which could optimize the digestive process, enhance nutrient assimilation, and support anabolic metabolism and growth, (Holst et al., 2005). Lürzel et al. (2015) applied 3 min of gentle interactions (i.e., stroking and gentle talking) per day during the first 14 days of dairy calves' life, reporting a significant effect on the ADG, which was higher for stroked calves compared with control calves. The authors hypothesized that one potential mechanism that could result in a greater weight gain in stroked calves is that they may allocate more energy towards growth rather than fear responses towards humans, which ultimately results in lower energetic costs (Carter, 1998; Ferguson et al., 2001; Field, 2001; Turner et al., 2011). Conversely, Lensink et al. (2000) evaluated gentle contacts with veal calves, consisting of the stockperson stroking and talking to calves and letting them suck their fingers directly after milk feeding and reported no influence on ADG or cold carcass weight (Lensink et al., 2000). The authors supposed that the short time they applied the contact may not have been enough if the animals were also exposed to a several practices that could be classified as negative contacts, such as slapping (Lensink et al., 2000). Beef calves undergo diverse management practices, particularly in the initial days of life during which calves are often processed on a beef cow-calf operation (Chamorro et al., 2016; González et al., 2010). This crucial time, often involving handlings practices like vaccination, castration, and branding, exposes the calves to different types of contact with the stockpeople (González et al., 2010) and with different effects on their welfare (González et al., 2010; Kleinhenz et al., 2021; Meléndez et al., 2018; Mellor and Stafford, 2004).

1.5. Welfare challenges in beef cow-calf operations

In western Canada cow-calf production system, beef calves are generally kept with their dams in extensive outdoor grazing systems (Endres and Schwartzkopf-Genswein, 2018; Jenkins and Ferrell, 2002). This means that human-animal interactions are infrequent and often isolated to a few, potentially aversive events. Within this aspect of the system, various welfare and health issues may occur, including difficulty experienced during calving process, exposure to extreme weather conditions, predation, and disease (Endres and Schwartzkopf-Genswein, 2018; Murray et al., 2016).

Pre-weaning calf morbidity and mortality in cow-calf operations are important concerns for producers (Murray et al. 2016), which can contribute negatively to calf welfare. Diseases such as neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) affect between 2.4% to 36% and 3% to 11% of beef calves, respectively (Murray et al., 2016; Waldner, 2001; Waldner et al., 2013, Pearson et al., 2019). Waldner et al. (2013) also reported that before weaning, 26% of all herds treated more than 5% of calves for NCD, 28% treated more than 5% of calves BRD, and 9% treated more than 5% of calves for umbilical infections. These studies demonstrate that beef calves are subject to being affected by diseases resulting in compromised health in the pre-weaning period.

1.6. Beef Calves Umbilical Structures

The umbilical cord is an important connection between the placenta and the fetus during gestation to transfer nutrients, oxygen, water, and waste (Rings and Anderson, 2009). The umbilical cord structure contains the umbilical arteries, umbilical veins, and urachus. These structures rupture and contract during the birthing process, exposing them to the environment

(Rings and Anderson, 2009). Once umbilical structures are exposed to the environment, they become a potential entrance for pathogens that are in the environment, increasing the risk of infections (Lorenz et al., 2011; Mee, 2008; Rings and Anderson, 2009; Steerforth and van Winden, 2018).

The infection of umbilical structures is known as omphalitis (Madigan and House, 1996). When a calf has omphalitis, the umbilical arteries, veins, and the urachus are affected (Trent and Smith, 1984). The infection of any of these structures may manifest with overt signs of inflammation, heat, swelling, purulent discharge, and pain and can significantly contribute to neonatal morbidity and mortality (Miessa et al., 2003; Virtala et al., 1996). Umbilical infection can also contribute to depression of the calf's immune system, favouring major pre-weaning diseases, such as NCD and BRD (Pardon and Deprez, 2018).

There are negatives consequences resulting from poor umbilical health, such as the risk of mortality (Duffield et al., 2018; Retskii et al., 2007) and reduced ADG (Johnson et al., 2018; Virtala et al., 1996). Consequences of umbilical problems can be present even at slaughter, as demonstrated by Thomas and Jordaan (2013) with veal calves. They studied veal mortality and post-slaughter wastage, and omphalitis was related to 54% of post-slaughter wastage and 23% of pre-slaughter calf mortality.

There are various management practices to prevent omphalitis, such as having a clean calving pen (Mee, 2008) and using an umbilical antiseptic (Grover and Godden, 2011; Robinson et al., 2015; Wieland et al., 2017). The practices can have a positive impact on calves' health, reducing the risk of animals becoming contaminated by pathogens and contracting diseases (Grover and Godden, 2011; Mee, 2008; Robinson et al., 2015; Wieland et al., 2017). Umbilical antiseptic (i.e., navel dip) is used by 40% of dairy producers (Renaud et al., 2017). In contrast to the dairy industry, only 9.1% of western Canadian beef producers use this management on their farms (Waldner et al., 2013), and there is a lack of scientific information related to this practice in beef calves.

1.6.1 Effect of the use of umbilical antiseptic on reactivity

The correct handling of newborn calves during the newborn calf processing is essential to ensure their health (Murray et al., 2016). Beef calf management typically involves briefly separating calves from their mothers for identification, vaccinations, or some medical treatment (Chamorro et al., 2016; González et al., 2010). Silva-Antunes and Costa (2021) applied a package of management practices in dairy calves that included applying umbilical antiseptic, colostrum feeding, and brushing for 5 five minutes, and demonstrated that calves who received this package had a decrease in the frequency of antibiotic treatments. It is important to highlight that in that study the application of umbilical antiseptic was applied together with other practices that could had contributed to the results.

Practices involving injections for health treatment may be perceived by calves as a negative interaction with humans. Calves' previous experiences with stockpeople not only influence their human-animal relationship perception, but the management context also has an influence (Brajon et al., 2015; Fureix et al., 2009; Hemsworth et al., 1996; Lensink et al., 2000). A study carried out with pigs, applying an injection procedure over a 3-week period, demonstrated that this procedure was aversive to pigs and has a negative impact on their welfare (Hemsworth et al., 1996). Based on this, if the need to treat calves can be reduced, calf management involving negative interaction (e.g. restraining and injection) can become less frequent, then human-animal interaction quality could be improved.

1.6.2. Effect of the use of umbilical antiseptic on health and productivity

Typically, antiseptic compounds are used to clean, sanitize, and improve the rate of umbilical stump healing while reducing the risk of infection for the animal (Mee, 2008). There are discrepancies in the literature regarding the optimcal procedures to improve umbilical health (Renaud et al., 2018; Scott et al., 2019), and there are conflicting results regarding its effectiveness (Donovan et al., 1998; Place et al., 1998; Windeyer et al., 2014).

The most commonly recommended product for umbilical cord disinfection after birth is 7% iodine (Robinson et al., 2015), because it is bactericidal, sporicidal, cysticidal, and virucidal (OIE, 2001). In addition, iodine at a concentration of 7% is strong enough to kill most pathogens within a short time of contact with the umbilical stump (Imdad et al., 2013). A commercial product called Navel Guard (Sirius Chemical Group, McDonough, GA) was compared to 7% iodine solution, low iodine concentration (0.5 to 2%) solution, or not dipping the umbilicus at birth to prevent umbilical infections (Grover and Godden, 2011). The results demonstrated that 10.3% of calves in Navel Guard group experienced navel infections, whereas this proportion was 28.3% higher in calves that did not receive navel dipping, meaning the odds of developing an umbilical infection were approximately 3.5 times higher in calves that did not receive umbilical dip compared to calves that were dipped with Navel Guard. Furthermore, there was no statistically difference among calves that received any of the navel antiseptics on the risk of the calves be treated for umbilical infection (Grover and Godden, 2011). Wieland et al. (2017) investigated the effect of 3 navel dips including Navel Guard, 7% iodine, and 2% chlorhexidine gluconate on umbilical infection, health events, and ADG in neonatal dairy calves. There were no significant statistical variations observed among the three navel dipping groups in terms of preventing umbilical infections or on the incidence of BRD, NCD, or arthritis (Wieland et al., 2017). However, the ADG differed among groups, with highest ADG among calves receiving 7% iodine compared to Navel Guard or chlorexidine (Wieland et al., 2017). Fordyce et al. (2018) compared the effect of 7% iodine with a dry dip formulated using an antibacterial peptide nisin mixed with talc (3.105 g of nisin per 100 g of talcum powder on a weight per weight basis), liquid nisin (64 µg/mL), and 4% chlorhexidine mixed with alcohol in a 50:50 solution on the umbilical cord healing rate, incidence of infection, and age at umbilical cord detachment using newborn Holstein heifer calves. There were no statistical differences between the four experimental groups on the umbilical cord drying rate or days for the umbilical cord detachment (Fordyce et al., 2018). Calves receiving iodine and liquid nisin had a higher incidence of umbilical infection compared with calves receiving nisin and 4% chlorhexidine mixed with alcohol in a 50:50 (Fordyce et al., 2018). In contrast, Van Camp et al. (2022) evaluated one dose of 7% iodine and detected no effects in reducing external umbilical infections within the initial 30 days of their calves, compared to a lack of treatment, suggesting that administering a single application may not be effective for preventing external umbilical infections (Van Camp et al., 2022). There is a lack of studies evaluating the effect of the use of umbilical antiseptics on beef calves in the literature.

It is possible to find discrepancies about the effectiveness of navel dipping application and its impacts on productivity indicators. Windeyer et al. (2014) developed an observational study to investigate factors associated with the risks of morbidity and mortality, and growth, in commercial dairy heifers' calves. This study demonstrated that mortality risk was increased in calves treated for BRD, and navel dipping is included as one of the factors associated with an increased risk of BRD, with calves who did not receive navel dipping at birth having a 17% risk for BRD while calves that received it had a 31% risk (Windeyer et al., 2014). They concluded that animals that received navel dipping at birth are more likely to be at risk for BRD, and calves treated for BRD were at greater risk for mortality (Windeyer et al., 2014). However, Perez et al. (1990) developed an observational epidemiological study on 63 commercial Dutch dairy farms and reported that dairy calves not receiving umbilical cord care at birth were at greater risk of BRD. Although navel disinfection is generally recommended, its effects still need to be better studied, mainly in beef cow-calf operations.

Examining the management practices of beef calves in western Canada, we have delved into the utilization of umbilical antiseptic and of tactile stimulation. However, despite our exploration, a comprehensive understanding of the potential synergies or conflicts arising from the simultaneous implementation of both practices remains elusive. The gaps in existing knowledge become evident when considering the multifaceted aspects of animal welfare, specifically focusing on behavioural responses, health outcomes, and overall productivity.

1.7. Overall aim of the thesis

To address the knowledge gaps outlined above, this thesis provides an evaluation of the effects of the application on neonatal beef calves of tactile stimulation and umbilical antiseptic on pre-weaned beef calves' reactivity when interacting with humans in handling facilities, pre-weaning risk of disease treatment and mortality, and average daily gain from birth to weaning in a western beef cow-calf operation.

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CHAPTER 2 – EFFECTS OF THE APPLICATION ON NEONATAL BEEF CALVES OF TACTILE STIMULATION AND UMBILICAL ANTISEPTIC ON THEIR PRE-WEANED WELFARE

Abstract: Beef calves may be exposed to various challenges during their life, such as some routine farm handling that has the potential to negatively affect human-animal relationship, and health problems such as neonatal calf diarrhea (NCD), bovine respiratory disease (BRD), and umbilical infections, which can result in reduced growth and increased risk of mortality. Tactile stimulation and the application of umbilical antiseptic could be practices used to promote positive human-animal relationship and reduce health problems. The objectives of this thesis are to investigate the impacts of the newborn calf tactile stimulation and the application of umbilical antiseptic on 1) calves` reactivity when interacting with humans in handling facilities, and 2) calves' risk of disease treatment and mortality, and average daily gain (ADG). Data collection was carried out at W.A. Ranches at the University of Calgary, located in Alberta, Canada. A total of 120 calves (65 males and 55 females), randomly divided into a 2x2 factorial design, were enrolled into one of four experimental groups: TSUA) calves with tactile stimulation and application of umbilical antiseptic; TS) calves with tactile stimulation but no application of umbilical antiseptic; UA) calves without tactile stimulation but with application of umbilical antiseptic, and C) control calves without tactile stimulation or application of umbilical antiseptic. Data collection occurred during three handling events: event 1 (during calving season, May 2022; E1), event 2 (near spring processing, June 2022; E2), and event 3 (near weaning, at two different days in October 2022; E3). The welfare of calves was assessed using three indicators: behaviour, health, and productivity. Behaviour was assessed with four tests: newborn calf reactivity during weighing inside the cage (RDW), reactivity of the calves inside the squeeze chute (RSC), flight speed (FS), and avoidance distance (AD). Occurrence of treatment for disease and deaths from birth to pre-weaning were recorded as health indicators. Lastly, calves' individual ADG in two periods (ADG1 and ADG2) were used as a productivity indicator. A principal component analyses was performed to analyse the behaviours composing RDW, generating 4 principal components PC1 (pressing the head against the cage, leg movement, and tail flicking), PC2 (head movement, ear movement, and standing), PC3 (high pitch vocalization), and PC4 (low-pitch vocalization) with eigenvalues exceeding 0.95 and together explained a total of 68.5% of the total variance. To evaluate the differences in calves' PC's scores, RSC, FS, AD, and productivity indicators (ADG1 and ADG2) among experimental groups, generalized linear mixed models were performed. To analyse health indicators, Fisher's tests were performed to investigate the association between the experimental groups (TSUA, TS, ND, C) and the occurrence of NCD, BRD, or both (DBRD). From all behavioural indicators, only FS had a significant association with the experimental groups ($P<0.001$), with the lowest mean ± SE for TS (1.10 ± 0.03) compared with TSUA (1.23 ± 0.03, $P = 0.01$), UA (1.22 ± 0.04, $P = 0.02$), and C (1.22 ± 0.03, $P = 0.02$), which did not differ from each other ($P>0.05$). Regarding health and productivity indicators, there was no significant association between experimental groups (TSUA, TS, UA, C) and calves treated for NCD, BRD and DBRD ($P > 0.05$) or ADG1 and ADG2 ($P > 0.05$). There were only two deaths, and these were due to unrelated diseases and therefore not analyzed. In conclusion, there is limited evidence that tactile stimulation and umbilical disinfection, as applied in this study, impacted the welfare of pre-weaned Angus beef calves.

Keywords: bovine, animal welfare, behaviour, health, performance, human-animal relationship

2.1. INTRODUCTION

In beef cow-calf production systems in western Canada, beef calves are typically maintained alongside their dams in expansive outdoor grazing environments (Endres and Schwartzkopf-Genswein, 2018; Jenkins and Ferrell, 2002). Due to this, the interaction between stockpeople and calves is infrequent, generally occurring when calves are submitted to necessary routine handling procedures such as ear tagging, castration, vaccinations, and disease treatments (Boissy et al., 2005; Raussi, 2003). Studies in experimental and commercial conditions with farm animals demonstrate that the quality of interactions with stockpeople influences animals' perception of humans (Hemsworth, 2018; Hemsworth and Coleman, 2011; Rault et al., 2020), and these interactions with humans may potentially be perceived by calves as aversive (Hemsworth et al., 2018).

Human-animal relationship is an important factor influencing animal welfare (Boivin, 2018; Hemsworth et al., 2018; Hemsworth and Coleman, 2010; Pinillos et al., 2016). Animal welfare is defined as the physical and mental state of an animal in relation to the conditions it lives and dies (Broom, 1986). A calf experiences good welfare if it is healthy, well-nourished, safe, not suffering from unpleasant states such as fear and distress, and able to express behaviours that are important for its physical and mental state (OIE, 2023). Negative human-animal relationship affects animal behaviour, health, and productivity, primarily through fear as an underlying mechanism directly related to stress (Hemsworth, 2018; Hemsworth and Coleman, 2011; Rault et al., 2020).

Stress generated by aversive handlings practices affect animals' productivity (Hemsworth et al., 2018; Hemsworth and Coleman, 1998) and the immune system, resulting in higher susceptibility to disease (Blecha, 1988; Hemsworthlt et al., 1993; Siegel and Gross, 2000). Fear of humans can be reduced through neutral or positive human-animal interactions (Hemsworth, 2018; Rault et al., 2020). The application of tactile stimulation has positive effects on the development, behaviour, and health of dairy calves (Silva-Antunes and Costa, 2021), piglets (de

Oliveira et al., 2015), foals (Schmidek et al., 2020), and mice (Costa et al., 2020), but little is known about its effect on beef calves.

The most common pre-weaning diseases of beef calves are NCD, BRD, and umbilical infections (Murray et al., 2016; Waldner, 2001; Waldner et al., 2013, Pearson et al., 2019). Newborn calves are exposed to infections through the umbilical cord from environmental pathogens (Grover and Godden, 2011; Mee, 2008; Robinson et al., 2015; Wieland et al., 2017). Umbilical infection can contribute to calves' immune system depression, potentially increasing the risk of NCD and BRD (Pardon and Deprez, 2018). The use of umbilical antiseptic has been reported as a management strategy to prevent umbilical infections (Grover and Godden, 2011; Robinson et al., 2015; Wieland et al., 2017).

The effect of a package of management practices, consisting of the application of umbilical antiseptic, colostrum feeding, and brushing for 5 minutes, on dairy calf health was previously evaluated (Silva-Antunes and Costa, 2021). Calves that received the package had a decreased frequency of antibiotic treatments and fewer calves died compared to calves that did not receive it (Silva-Antunes and Costa, 2021). The act of brushing may have improved the interaction between handlers and calves, making it easier for handlers to identify clinical disease signs, allowing early veterinary intervention when needed (Silva-Antunes and Costa, 2021). The short- and long-term effects of the application of one minute of tactile stimulation during the first handling of Nellore calves on their welfare has also been assessed (Cerezo, 2023). Calves receiving tactile stimulation exhibited a more positive emotional state when receiving the stimulation, and had higher ADG compared with calves that did not receive the intervention. The results of these studies suggest that incorporating these practices may have a positive effect on different welfare indicators such as behaviour, health, and productivity. However, there is a lack of studies examining the impacts of tactile stimulation and the use of umbilical antiseptics on beef calf welfare in the western Canadian context.

2.2. OBJECTIVES

The overall aim of this study was to evaluate the effects of the application on neonatal beef calves of tactile stimulation and umbilical antiseptic on their pre-weaned welfare. The specific objectives were to: 1) investigate the impacts of the newborn calf tactile stimulation and application of umbilical antiseptic on the reactivity of pre-weaned beef calves when interacting with humans in handling facilities, and 2) investigate the impacts of the newborn calf tactile stimulation and application of umbilical antiseptic on the risk of disease treatment, mortality, and average daily gain in pre-weaned beef calves.

2.3. HYPOTHESES:

Calves receiving tactile stimulation and application of umbilical antiseptic are expected to demonstrate less fearful reactions when interacting with humans in handling facilities and have lower risk of being treated for disease, lower mortality, and higher ADG compared to the other experimental groups.

2.4. MATERIALS AND METHODS

2.4.1. Location and Animals

The study was approved by the University of Calgary Veterinary Sciences Animal Care Committee (AC22-0029) and was conducted in accordance with guidelines established by the Canadian Council on Animal Care.

The research was conducted at the W.A. Ranches at the University of Calgary, located in Alberta, Canada, between May and October 2022. Mature cows (i.e., multiparous dams of parity 2 to 13) were monitored daily in two maternity pastures during the third trimester of pregnancy, with 88 and 131 cows in each pasture. Only calves without obvious signs of disease, born from eutocic calvings, and consuming colostrum on their own within the first 24 hours after birth were enrolled in the study. Within 8 - 24 hours after calving, each calf was manually captured by two stockpeople using a utility terrain vehicle (UTV) equipped with a calf cage (0.9 m wide x 1.2 m long, and 0.9 m from the floor to the top of the cart). A total of 120 calves (65 males and 55 females) were randomly divided using a 2x2 factorial study design. They were enrolled into one of four experimental groups: TSUA - calves with both tactile stimulation and application of umbilical antiseptic (bulls = 16, heifers = 14); TS - calves with tactile stimulation but no application of umbilical antiseptic (bulls = 16, heifers = 14); UA - calves without tactile stimulation but with the application of umbilical antiseptic (bulls = 16, heifers = 14), and C – control calves without tactile stimulation or application of umbilical antiseptic (bulls = 17, heifers = 13).

Data collection occurred during three handling events: event 1 (during calving season, May 2022; E1), event 2 (near spring processing, June 2022; E2), and event 3 (near weaning, at two different days in October 2022; E3). At E1, the primary handler approached the cow-calf pair and observed physical signs such as responsiveness and abdominal fill of the calf, and udder fill of the cow (i.e., if the teats were small and thin) to ensure the calf had consumed colostrum prior to enrollment (based in the ranch's protocol). After ensuring the calf met the inclusion criteria, the same handler captured the calf using a calf hook and moved it into the cage, while the secondary handler provided occasional support by opening the cage and, if needed, protecting the primary handler from the dam. Then, the calf received the interventions according to its assigned experimental group (TSUA, TS, UA, or C). Each calf was identified with an ear tag with a visual identification number (ID) and a radio-frequency identification (RFID) tag and received 2ml of an intranasal multivalent modified live viral vaccine against bovine herpesvirus type 1, bovine respiratory syncytial virus, and parainfluenza virus type 3 (InForce™ 3, Zoetis, NJ, USA), according to the on-farm protocol of the ranch. Four different handler pairs participated in animal enrollment at E1: Pair 1 – handler 1 and 2; Pair 2 – handler 1 and 3; Pair 3 – handler 3 and 2; and Pair 4 – handler 3 and 4.

Cows and calves were moved to the processing facilities at E2 when the calves were an average 40 ± 3.4 (mean ± SE) days of age and E3 when the calves were 158 ± 3.6 (mean ± SE) days of age. At each event, calves were separated from their dams, the dams were placed in a pasture distant from the processing facility without physical and visual contact with the calves, and the calves were placed in a pen next to the processing facility. They were then individually moved into a hydraulic squeeze chute inside the processing facility for weighing and behavioural tests, as described below. After the behavioural tests at both events, calves were reunited with their dams and returned to pasture.

2.4.2. Experimental group description

Randomization was blocked by the four experimental groups (TSUA, TS, UA, and C) so during sequential enrollment over time, the distribution of birth dates would be balanced across experimental groups. Calves were stratified by sex, and random allocation to treatment group was performed within each stratum. The same trained researcher applied all experimental interventions. Calves received the following interventions according to the assigned experimental group:

Application of Umbilical Antiseptic and Tactile Stimulation (TSUA)

The researcher entered the cage and applied tactile stimulation for two minutes by gently but firmly touching the calf’s body using movements from the neck to the hind end of the calf, including the front and hind legs, repeating this movement for the entire tactile stimulation period. After the researcher left the cage, each calf was weighed using an electronic scale attached to the cage, and its behaviour was recorded for one minute using a video camera (GoPro HERO 10, GoPro Inc., CA, USA) attached to the cage. After the one-minute period, the researcher entered the cage again and applied the umbilical antiseptic using a teat dip cup (Blisstime, model BF-102, ASIN B00YE2CCH4, USA) filled with a 7% iodine solution (Iodine Tincture Stronger, Dominion Veterinary Laboratories Ltd, MB, Canada). The navel structures were immersed and soaked in the solution, dipping in and out of the teat cup for 15 seconds (Grover and Godden, 2011).

Tactile stimulation and no application of umbilical antiseptic (TS)

The researcher entered the cage and applied tactile stimulation to the calf as described for TSUA. After the researcher left the cage, each calf was weighed as described for TSUA. After the one-minute period, calves belonging to this group stayed in the cage for an additional 15 seconds without the researcher's presence inside the cage to match the time frame the calves spent within the cage among experimental groups.

No tactile stimulation and application of umbilical antiseptic (UA)

The researcher entered the cage and stayed there for two minutes without interacting with the calf. After the researcher left the cage, each calf was weighed as described for TSUA. Then, calves belonging to this group received umbilical disinfection as described for TSUA.

No tactile stimulation and no application of umbilical antiseptic (C)

The researcher entered the cage and stayed there for two minutes without interacting with the calf. After the researcher left the cage, each calf was weighed as described for TSUA. Then, calves belonging to this group stayed in the cage for an additional 15 seconds without the researcher's presence inside the cage to match the time frame the calves spent within the cage among experimental groups.

2.4.3. Animal Welfare Indicators

2.4.3.1. Behaviour indicator

At E1, the reactivity of the newborn calf during weighing (RDW) inside the cage was recorded. At E2 and E3, within the ranches' handling facilities, the following reactivity tests were applied: the reactivity of the calves inside the squeeze chute (RSC), flight speed (FS), and avoidance distance (AD). Behavioural indicators were measured as follows:

2.4.3.1.1. *Reactivity Assessment During Weighing (RDW)*

Videos of 1 min duration were assessed by one trained observer using the ethogram described in Table 1. For TSUA and TS, calf behaviour was recorded after the tactile stimulation application, when the researcher left the cage, and for UA and C, it was recorded after the researcher left the cage, after staying in the cage for two minutes without interacting with the calf.

2.4.3.1.2. *Reactivity Inside the Squeeze Chute (RSC)*

Calves' reactivity was recorded immediately upon entrance into the squeeze chute without using any additional restraining devices (i.e., head gate or squeezing the sides) (Ceballos et al., 2016; Grandin, 1993) using a high-definition video camera (FDR-AX53, SONY, SONY of Canada LTD., ON, Canada). The RSC measures the degree of movement, tension, body posture, breathing, kicking, and vocalizations, as described in Table 2. It is stipulated that 4s of videos should be used to assess the reactivity score for adult cattle (Grandin, 1993; Hoppe et al., 2010; Tulloh, 1961). Twenty-five videos (i.e., calves) were assessed for 4 sec at E2. However, due to the lack of behaviour variability inside the chute during this time, these videos were also evaluated for 30 seconds (Hearnshaw and Morris, 1984). After comparing the videos of the same calves for these two periods of time, more than 50% of the videos had greater behaviour variability for RSC (Table 2) in 30 seconds video compared to 4 seconds. Thus, for E2, videos were analyzed for 30 seconds. In E3, the same comparison was made using 25 videos. However, at this time, more than 98% of the 4-second videos had the same variability for each score as the 30-second videos. Thus, for E3, the videos were analyzed for 4 seconds. The RSC was assessed by two trained observers blinded to experimental groups. The inter-observer and intra-observer reliability was calculated using Kappa (k) coefficient (Kraemer, 1980) of the RSC measures presented in Table 2. After a training session with 45 videos, 45 different videos were used to evaluate inter-observer and intra-observer reliability.

2.4.3.1.3. Flight Speed (FS)

Flight Speed (FS) was measured at E2 and E3. This behavioural test was performed as the animals exited the squeeze chute (Burrow et al., 1988). The measurement was obtained using two pairs of photoelectric cells, 2 meters apart. This equipment was installed in the corridor at the exit of the squeeze chute to record the time each animal took to travel the 2-meter distance. The flight speed (m/s) was calculated dividing the distance traveled in meters by the time it took to travel in seconds. Faster animals were considered more reactive (Burrow et al., 1988).

2.4.3.1.4. Avoidance Distance (AD)

Avoidance distance is the maximum distance the animal allows the observer to approach before it moves away. The same trained researcher measured the avoidance distance at E2 and E3 in a holding pen adjacent to the processing area. At E2, the holding pen measurements were 15.3 m x 15.5 m x 15.3 m x 12.5 m, and at E3, they were 13.9 m x 14.5 m x 14.8 m x 14.8 m. The observer was positioned approximately in the center of the holding pen. When calves exited the squeeze chute after the flight speed test evaluation, they entered the holding pen, and two minutes of familiarization time inside the pen were given. After two minutes, the observer waited for the calf to be still and facing him, and then walked slowly towards the calf. The observer approached at a speed of approximately one step per second, with the arm extended at an angle of approximately 45° to the body. The approach continued toward the calf until signs of withdrawal occurred or until the observer touched the calf's body. If the animal did not retreat, the avoidance distance was recorded as 0 m. The withdrawal movement was defined as the animal recoiling or turning away (Hemsworth et al., 2000; Welfare Quality ®, 2009).

2.4.3.2. Health Indicators

Health evaluations and treatments were performed by ranch staff following the ranch's health protocol as defined by their veterinarians. Ranch staff visited the pastures daily to monitor animal health, identified calves with symptoms of disease, and administered treatments. They recorded the ID, sex, tentative diagnosis, treatment date, medication used, route of administration (i.e., subcutaneous, subcutaneous by the base of the ear, oral, intramuscular, or dart), and number of treatments administered. On ranches in western Canada, the risk of calves being treated for NCD occurs primarily in the first 30 days of life and for BRD in the first 60 days of life (Waldner et al., 2022; Woolums et al., 2013). So, only treatments that occurred for NCD before 30 days of life and BRD before 60 days of life, or for both BRD and NCD together (DBRD) were included in the statistical analyses. If any calf died, the animal ID, sex, tentative diagnosis, and date of death were recorded.

2.4.3.3. Productivity Indicators

Calves were weighed at each event. Calves' body weight at E1, E2, and E3 was used to calculate calf average daily gain between E1 and E2 (ADG1) and between E1 and E3 (ADG2), as follows: the total weight gain divided by the number of days between the two events (E2 Weight – E1 Weight)/number of days between E1 and E2, or E3 Weight – E1 Weight)/number of days between E1 and E3).

2.4.4. Statistical Analysis

The statistical analyses for health, productivity, and RDW test were performed in R software with RStudio integrated development environment (version 4.2.3; Rstudio, Inc., Boston, MA, USA). The functions and packages used are presented in the format (package::function) corresponding to the computer programming language in R. The statistical analyses for AD, FS, and RSC were performed in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Alpha of 5% was considered the threshold for statistical significance.

2.4.4.1. Description of Study Sample

A statistical analysis was conducted to identify potential confounding variables that could affect the relationship between independent and dependent variables. Possible confounding factors included sex (male, female), pen (1, 2), stockperson (1 – 4), cow parity (2 – 13), and week of enrollment (1, 2). Chi-square test was used to assess the association of the categorical variables sex, pen, stockperson, cow parity, and animal enrollment with the experimental groups. This analysis helped ensure that the effects observed in the experimental groups are not due to any confounding factor but rather the result of the specific interventions being studied.

2.4.4.2. Behavioural Indicators

Intra-observer reliability - Reactivity Assessment During Weighing (RDW)

Intraclass correlation coefficient (ICC) was performed to evaluate the intra-observer reliability of RDW behaviours at E1. The ICC was calculated using the observation of 45 randomized videos repeated twice, with an interval of 15 days between observations. The classification proposed by Altman (1990) was used to interpret the reliability of each RDW behaviour as follows: poor (0 to 0.20), reasonable (0.21 to 0.40), moderate (0.41 to 0.60), good (0.61 to 0.80), and very good (0.81 to 1.0).

Reactivity Assessment During Weighing (RDW)

The RDW test was assessed using videos collected during E1, when calves had not received the umbilical antiseptic application. Therefore, TSUA and TS groups were categorized as With Tactile Stimulation (WTS), and UA and C groups categorized as No Tactile Stimulation (NTS).

The RDW behaviours were analyzed using a principal component analysis (PCA) with a correlation matrix and without rotation (Jeffers, 1978). The PCA reduces the number of variables by examining the matrix of correlation coefficients between all measurements and infers principal components (PC), which may help classify the data (Beale et al., 1967; Ringnér, 2008). Four principal components (PC) with eigenvalues >0.95 were identified. Terms with loading values >0.6 were considered major contributors to each PC. Factor loadings for each animal from each PC were extracted, and the adherence to a normal distribution for each PC was assessed using Shapiro-Wilk tests (stats::shapiro.test). The PCs had normal distribution after square root transformation. Raw and standardized residuals were plotted. The PCs had normal link function. The association of birth weight, sex, pen, enrollment week, and stockpeople pair with factor loadings for each animal from each PC were tested individually using ANOVA (aov::stats), and only these with significant association were included in the final model. To analyze the variation of factor loadings for each animal from each PC between the experimental groups, a general linear mixed model (GLMM) was fitted (stats::glm), including the experimental group (TSUA, TS, UA, C) as the variable of interest. For each PC, means were compared using *post hoc* Bonferroni correction (emmeans::lsmeans).

Intra and Inter-observer reliability for Reactivity Inside the Squeeze Chute (RSC)

Kappa analyses was performed to evaluate inter and intra-observer reliability of RSC behaviours at E2 and E3. Kappa (k) coefficient (Kraemer, 1980) was calculated using the observation of 45 randomized videos repeated twice, with an interval of 15 days between observations. The classification proposed by (Landis & Koch, 1977) was used to interpret k as follows: poor (0 to 0.19), slight (0.21 to 0.39), moderate (0.40 to 0.59), substantial (0.60 to 0.79) and almost perfect (0.80–1.00) agreement.

Avoidance Distance (AD), Flight Speed (FS), and Reactivity Inside the Squeeze Chute (RSC)

Linear mixed models for repeated measures were fitted using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Adherence to a normal distribution for RSC, FS, and AD was assessed using Shapiro-Wilk tests. The AD and FS had normal distribution after square root transformation, but RSC did not. Raw and standardized residuals were plotted. The AD and FS had normal link function and RSC lognormal link function. Before identifying the final models, the association between each dependent variables (i.e., AD, FS, and RSC) and birth weight, sex (male and female), calves treated for IBK, NCD, BRD, medical interventions inside the squeeze chute (only for AD and FS), and day of data collection during E3 were individually tested. All final models included experimental groups (TSUA, TS, UA, C), event (1 and 2), and the interaction between experimental groups and event as variables of interest. The random effect of calf was included to account for repeated measures. Means were compared using a *post hoc* Tukey test.

2.4.4.3. Health

A dataset consisting of information on whether or not the calves were treated (Yes = Treated, No = Not Treated) was created for three dependent variables: NCD, BRD, and DBRD. A contingency table was created (base::table) to examine the number of calves treated for NCD, BRD, and DBRD in each experimental group. Fisher's exact tests (stats::fisher.test) were performed to investigate the association between the experimental groups (TSUA, TS, UA, C) and the occurrence of NCD, BRD, or DBRD.

2.4.4.4. Productivity

Adherence to a normal distribution for ADG1 and ADG2 was assessed using Shapiro-Wilk tests (stats::shapiro.test). The covariates of sex, pen, birth weight, NCD, BRD, DBRD, and treatment for IBK were tested individually using ANOVA (aov::stats) to understand their association with ADG1 and ADG2. The following covariates were treated as categorical: NCD (treated, not treated), BRD (treated, not treated), DBRD (treated, not treated), IBK (treated, not treated), pen (1, 2), and sex (male and female). Birth weight (BW) was treated as continuous variable. Only those covariates with a significant association with ADG1 and ADG2 were included in the final models. To compare the outcomes ADG1 and ADG2 among experimental groups, general linear mixed model (GLMM) were fitted (stats::glm) individually, including the experimental groups (TSUA, TS, UA, C) as variable of interest. Finally, post hoc tests for multiple comparisons were conducted using the Bonferroni correction (lsmeans::lsmeans and multcomp::cld).

2.5. RESULTS

2.5.1. Description of Study Sample

There was no significant association (P > 0.05) of experimental group with any evaluated potential confounding factor (Table 3).

2.5.2. Reactivity Indicators

2.5.2.1 Reactivity Assessment During Weighing (RDW)

Based on ICC coefficients, intra-observer reliability for RDW behaviours were very good (leg movement (0.92), head movement (0.89), ear movement (0.97), presses head against the cage (0.92), tail flicking (0.97), jumping (1.00), low pitch vocalization (0.87), high pitch vocalization (0.88), kneeling (1.00), lying (1.00), standing (1.00), and stomping (1.00)).

The four PCs generated, with eigenvalues exceeding 0.95, explained a total of 68.5% of the total variance (Table 4). The PC1 explained 27.6% of the variance and included the behaviours pressing the head against the cage, leg movement, and tail flicking with positive loadings above 0.6. The PC2 explained 17.0% of the variance and included the behaviours head movement, ear movement, and standing with positive loadings above 0.6. The PC3 explained 13.1% of the variance and included high pitch vocalization. The PC4 explained 10.8% of the variance and included low-pitch vocalization. The PCA is depicted in Figure 1.

The PC1 did not differ among experimental groups (mean ± SE: WTS - 1.10 ± 0.09 and

NTS - 1.33 ± 0.11; P > 0.05). However, there was a significant association between PC1 and birth weight (P = 0.005). For each unit of birth weight (kg) increase, PC1 decreased by 0.04 units. For PC2, there was no significant difference between experimental groups (P > 0.05) WTS (mean ± SE: 0.75 ± 0.07) and NTS (mean ± SE: 0.67 ± 0.07). There was a significant association between stockpeople pair and PC2 (P = 0.02), with the highest mean ± SE for Pair 1 (mean ± SE: 0.88 ± 0.06) compared with Pair 3 (mean ± SE: 0.50 ± 0.13, P = 0.04) but did not differ (P > 0.05) with other pairs (Pair 2 - mean ± SE: 0.83 ± 0.09; Pair 4 - mean ± SE: 0.64 ± 0.12), nor did they differ between them. Regarding PC3, it did not differ between the experimental groups (P > 0.05) WTS (mean ± SE: 0.89 ± 0.07) and NTS (mean ± SE: 1.08 ± 0.09). Finally, PC4 did not differ between the experimental groups (P > 0.05) WTS (mean ± SE: 0.84 ± 0.07), and NTS (mean ± SE: 0.67 ± 0.06).

Reactivity Inside the Squeeze Chute (RSC)

Based on Kappa coefficients, intra-observer reliability for observers 1 and 2 was almost perfect (Movement (0.93 and 0.87), Tension (0.84 and 0.93), Body Posture (1 and 1), Kicking (1 and 1) and Balking (1 and 1), respectively). Inter-observer reliability between observers 1 and 2 was almost perfect (Movement (0.88), Tension (0.84), Body Posture (1), Kicking (1) and Balking (1)).

Distributions of RSC are presented in Appendix A. The RSC did not differ between experimental groups (P > 0.05) mean crude values ± SE: TSUA (4.71 ± 0.09), TS (4.56 ± 0.07), UA (4.45 ± 0.09), and C (4.68 ± 0.08) (Fig. 2). There was a significant association between event and RSC (P = 0.002) with lower mean crude values ± SE for E3 (4.52 ± 0.01) compared with E2 (4.67 ± 0.01). There was no significant effect of the event-by-group interaction on RSC (P>0.05).

Flight Speed

The FS distribution is presented in Appendix A. The FS (m/s) differed between experimental groups (P = 0.03) with lower mean crude values ± SE for TS (1.35 ± 0.05) compared with TSUA (1.59 ± 0.05, P = 0.01), UA (1.63 ± 0.05, P = 0.02), and C (1.59 ± 0.05, P = 0.02), which did not differ among them (P > 0.05) (Fig. 2). There was a significant association between event and FS (P<0.001) with a lower FS mean ± SE for E2 (1.02 ± 0.005) compared with E3 (2.06 ± 0.006). There was no significant effect of event-by-group interaction on FS (P>0.05).

Avoidance Distance

The AD distribution is presented in Appendix A. There was no significant association of treatment, event, or their interaction with AD (P > 0.05). The AD (m) did not differ between experimental groups (P > 0.05) mean crude values ± SE: TSUA (4.58 ± 0.08), TS (4.60 ± 0.01), UA (4.55 ± 0.09), and C (4.74 ± 0.10) (Fig 2).

2.5.3. Health Indicator

The diseases that calves were treated for were BRD, bloat, coccidiosis, depression, footroot, NCD, and infectious bovine keratoconjunctivitis (IBK). There were no treatments for umbilical infections recorded. The distribution of the number of calves treated and for which disease they received treatment, as well as the number of dead calves and reason are described in Table 5. Only calves treated for NCD in the first 30 days of life (n = 3) and calves treated for BRD in the first 60 days of life (n = 4) were included in the health analyses. There was no significant association between experimental groups (TSUA, TS, UA, C) and calves treated for NCD, BRD, and DBRD (P > 0.05) (Table 6). Two calves from the study died, but the causes of death were not related to the study's research question. Because of this, no further analysis was conducted for mortality.

2.5.4. Productivity Indicator

The distribution of ADG1 and ADG2 are presented in Appendix B. There was no significant association between the experimental group (TSUA, TS, UA, C) and ADG1 ($P > 0.05$) with mean ± SE (kg/day) for TSUA (1.31 ± 0.13), TS (1.28 ± 0.13), UA (1.29 ± 0.13), and C (1.28 ± 0.12). There was a significant association between ADG1 and BRD treatment ($P < 0.02$) and sex ($P < 0.007$). Calves not treated for BRD had lower ADG1 (1.04 ± 0.02) compared with treated calves (1.54 ± 0.24). Female calves had lower ADG1 (1.23 ± 0.12) compared with males (1.34 ± 0.12).

There was no significant association of the experimental groups (TSUA, TS, UA, C) with ADG2 ($P > 0.05$) with mean ± SE for TSUA (1.24 ± 0.02), TS (1.23 ± 0.02), UA (1.28 ± 0.02), and C (1.28 ± 0.02). There was a significant association between ADG2 and sex ($P<0.003$). Female calves had lower ADG2 (1.22 ± 0.02) compared with males (1.29 ± 0.02).

2.6. DISCUSSION

To our knowledge, this is the first time employing different types of welfare indicators to assess the effects of tactile stimulation and the application of umbilical antiseptic early in life on pre-weaned beef calf welfare in western Canada. One of the several behavioural indicators evaluated (i.e., flight speed) suggested that tactile stimulation can potentially reduce calves' reactivity. However, there was no effect of tactile stimulation or umbilical disinfection on other behavioural indicators nor on health and productivity outcomes.

There was no association of tactile stimulation and the application of umbilical antiseptic with any of the PCs generated from RDW. The PCs included the behaviours pressing the head against the cage, leg movement, tail flicking (PC1), head movement, ear movement, standing (PC2), and high and low-pitch vocalizations (PC3 and PC4, respectively). Head and ear movements are generally characterized as a seeking expression in herd animals on pastures (Grandin and Deesing, 2022). Tail flicking is related to flight response and can communicate a sign of danger, such as a possible predator, to conspecifics (Kiley-Worthington, 1976).

Conversely, this behaviour is also present when calves are playing and nursing, which are activities generally associated to positive emotions (Jensen et al., 1998; Jensen and Kyhn, 2000). Pressing the head against the cage could be interpreted as calves' attempting to escape the cage (Grandin, 2008; König von Borstel et al., 2018). Vocalization between cows and calves is one of the initial evolutionary mechanisms that stimulate vocal development of calves (Brudzynski, 2014). These vocalizations help cows' early recognition of calves by calling them actively through vocal calls and receiving calves’ vocalization as answers (Kiley, 1972). In contexts with stressful stimuli such as the calf being separated from its dam, calves' locomotor activity tends to increase as signs of distress and anxiety (Flower and Weary, 2001; Hudson and Mullord, 1977; Veissier et al., 1989; Weary and Chua, 2000). Social isolation could induce severe psychological stress in various livestock species such as cattle, sheep, goats, and horses, expressed through behavioural changes such as increase in vocalization, locomotion, attempting to escape, and tail movements (Boissy and Le Neindre, 1997; Cockram et al., 1994, Lyons et al., 1993; Lyons et al., 1993; Wolff et al., 1997). Studies indicate that situations involving separation can induce more frequent tail movements in calves (Lee et al., 2009; Schwartzkopf-Genswein et al., 1997; Turner et al., 2020). Also, the frequency of head movements tends to rise in cattle undergoing periods of isolation and stressful handling (Turner et al., 2020). Studies have demonstrated that the urgency of identification between cow-calf after separation causes an increase in vocalizations (Kiley, 1972; Manteuffel et al., 2004; Mendl et al., 2010) related to negative states, such as anguish, fear, and frustration (Daros et al., 2014; Hopster and Blokhuis, 1994). A possible explanation for calves' behaviours not differing among experimental groups is that the separation from their dams may have resulted in separation distress, which overwhelmed any positive experience that might have been generated from tactile stimulation. However, it is important to note that some of the exhibited behaviours may also be associated with play, exploration, or pain response (Gleerup et al., 2015; Jensen et al., 1998; Jensen and Kyhn, 2000; Müller et al., 2019), so further research should explore more deeply the calves’ experience throughout the intervention. Within the context of this study and considered together, these findings may provide evidence that the cow-calf separation at this early stage of life, even for a few minutes, could result in calves expressing behavioural changes linked with distress of this situation.

There was an association between the stockpeople pair and the expression of PC2, where calves handled by Pair 1 presented higher values for PC2, consisting of head movement, ear movement, and standing, compared to calves handled by Pair 3. Schmidek et al. (2020) demonstrated that after experiencing positive handlings, almost 60% of foals accepted human approach and presented seeking behaviours that involved ear and head movements. De Passillé et al. (1996) reported that dairy calves' behaviour changes were influenced by the consistency of new experiences with positive stimuli. The nature of interactions between farm animals and stockpeople affects animals' perception of humans (Hemsworth et al., 2018; Hemsworth and Coleman, 2011; Rault et al., 2020). Positive and rewarding experiences derived from their interaction with humans directly influence behaviours like voluntary approach, seeking, pleasure, relaxation, prosocial, or affiliative (Mellor, 2012; Rault et al., 2020). The quality of the handling practices used when capturing the calf was not evaluated in this study. However, based on the researcher's subjective experience during data collection, it is possible that Pair 1 performed more careful handling when capturing and putting the calves inside the cage, compared with Pair 3, which performed more aversive techniques, such as grabbing the calf by the leg. Pair 2 and 4 was composed of at least one of the stockpeople who formed Pair 1 and Pair 3, which may explain the lack of difference among these pairs. Human-animal relationship are classified as positive, neutral, or negative, and the way the handler performs any interaction with the animal influence its quality (Hemsworth and Coleman, 1998; Waiblinger et al., 2002). The impact of these interactions extends to various indicators, including animal behaviour (Hemsworth et al., 2018; Hemsworth and Coleman, 1998). Therefore, it may be that the way Pair 1 performed their handling practices had an impact on the relationship between them and calves, resulting in calves showing more the behaviours contained in PC2.

Lighter calves had higher PC1 values, consisting mainly of pressing the head against the cage, leg movement, and tail flicking, than heavier calves. Few studies in the literature have explored the differences of calves' behaviours associated with their birth weight. However, birth weight is one of the main factors affecting calf survival and performance from birth to weaning (Comerford et al., 1987; Murray and Leslie, 2013). Birth weight has been related to calves' vigour, ability to stand, and colostrum ingestion within the first hours of life (Comerford et al., 1987; Murray and Leslie, 2013). Although no calves from dystocic births were enrolled in this study, it is possible that heavier calves may have experienced some difficulty at birth, and this may have had an impact on their behaviour, leading them to exhibit less of the behaviours of PC1. Another explanation could be that the size of the cage could have influenced the behaviours that calves were able to perform. Although the relative size of smaller versus larger calves was unlikely to have restricted their ability to demonstrate many of the behaviours assessed, being confined within a small area may have had different impacts on calves of varying birth weights. It is known that the environment size where the animals are inserted, for example a pen, can influence how animals express their behaviours (Whalin et al., 2021). This finding suggests that more studies should be conducted to better understand the relationship between calves' birth weight and reactivity.

There were no differences in calves' reactivity inside the squeeze chute among the experimental groups. The RSC is a test that involves restraining the animal inside the chute and is commonly used in beef cattle, because this is a handling procedure that frequently occurs on farms for management practices such as vaccination (Setser et al., 2023). The RSC is used as a measurement of fearfulness and reactivity under these conditions (Setser et al., 2023). Nonetheless, while adult animals typically undergo handling procedures within a squeeze chute, young calves, such as those involved in the current study, typically receive vaccinations or medical treatments directly in the pasture, where they are manually restrained by stockpeople. This test involves the interaction between humans and calves, so animals' previous experiences with humans play an important role in its outcome (Boissy and Bouissou, 1995; Hemsworth, 1991). Studies have demonstrated that cattle receiving negative interactions during handling are more reactive compared with cattle that did not receive negative interaction (Ceballos et al., 2018; Macedo et al., 2011). In the present study, the results suggested that the young calves' reactions may not have been influenced by what they experienced earlier. Another explanation related to this result could be to the fact that adult animals typically have established personalities (Cabrera et al., 2021). The temperament of dairy heifers appeared to become established only after 6 months of age, although some individuals were affected by the change from one environment to another (Dennison, 1985). It is known that some factors affect the animals' behavioural response for example, breed and age (Grandin and Deesing, 2022). The RSC test, as evidenced by various published studies, effectively identifies individual variations in behavioural responses in adult beef cattle (Ceballos et al., 2018; Macedo et al., 2011; Sant'Anna et al., 2013). It is plausible that young calves, not having fully developed personalities, or not having many experiences inside the chute, might have not exhibit detectable variations in behaviour, particularly in a restrained environment. It is important to highlight that assessing personality within single life stages only provides a snapshot of an individual's behavioural repertoire (Cabrera et al., 2021). Therefore, personality assessment using the RSC test may not be able to identify behavioural variation in young beef Angus calves and perhaps it is a test that should only be used in more mature animals. Another viable interpretation for the observed results is the occurrence of an infectious bovine keratoconjunctivitis outbreak in the pasture where calves were kept until weaning. This condition required daily monitoring of the animals by the stockpeople on the pasture to identifying and treating affected calves. The frequent human presence in the pasture, with neutral interaction, may have led the calves to a habituation process to human presence (Aubé et al., 2023; Sharma and Phillips, 2019; Ujita et al., 2021), potentially influencing their behavioural responses, which might have contributed to negating the expected effects of the applied interventions.

Calves from TS group left the squeeze chute more slowly, indicating less reactivity compared with other experimental groups. According to these findings, calves that underwent just tactile stimulation were less reactive upon exiting a restrained environment. The literature contains limited studies regarding the direct impact of this practice on the flight speed test. A study conducted by Silva et al. (2017) contradicts our results, as it failed to observe differences in flight speed between a group of dairy heifers that received or did not received brushing, along with other management practices such as colostrum provision and umbilical antiseptic application. Early in animals' life, they have the capacity to interpret their experiences as aversive or positive, depending on the intensity, duration, and frequency of the external stimuli (Chen and Sato, 2017; Mellor and Diesch, 2006; Wit esterath et al., 2014). A plausible explanation for this result could be related to a positive memory that TS calves may have created, perceiving tactile stimulation as a positive stimulus early in their lives and influencing their behaviour later.

It was expected that the group subjected to both tactile stimulation and umbilical antiseptic application would exhibit similar albeit slightly less positive behaviours than the tactile stimulation only group. However, that was not the case in this study. Based on the researcher's personal experience, the application of iodine has the potential to create a stinging sensation in the treated region. It is known that if an animal has a negative experience with a management procedure, it is likely to perceive it adversely (Ceballos et al., 2018; Hemsworth and Coleman, 2011; Waiblinger et al., 2006). Therefore, maybe the umbilical antiseptic may have resulted in a negative experience for them, eliminating any possible positive effects of tactile stimulation for TSUA group.

Calves' RSC appeared to decrease from E2 to E3. Usually, cattle reactivity decreases from one event to another (Curley Jr et al., 2006; Hall et al., 2011, Sant'Anna et al., 2019).

Petherick et al. (2009) reported that repeated visits to the corral, even marked by mildly adverse handling, diminished behavioural reactivity, likely due to the increased predictability of the handling procedures over time. Ceballos et al. (2018) observed a reduction of Nellore heifers' reactivity from the initial to the second evaluation using flight speed and composite reactivity score. Sant'Anna et al. (2019) also reported a significant decrease in FS among Nellore cattle by the end of the confinement period. However, it should be noted that these studies evaluated reactivity more than twice. When calves were submitted to RSC test at E2, they entered the squeeze chute and no additional aversive interactions occurred, so perhaps at E3, based on calves' last experience, they predicted nothing aversive would happen and had lower reactivity inside the squeeze chute. Conversely, calves' FS increased from E2 to E3. Calves were approximately 40 days of age at E2 and about 158 days old at E3, with a clear difference in weight and size. As such, the FS at E3 might have been elevated simply due to the calf's larger size and inherent capability to move faster. Based on this, it is possible to hypothesize that perhaps the increase in FS was due to the calves' change in size and capacity to move faster, and RSC decreased because calves predicted that nothing aversive would happen inside the squeeze chute.

There were no differences in calves' avoidance distance among experimental groups and between events. This contradicts a previous study by Probst et al. (2012), who demonstrated that gentle touching of Limousin-crossbred beef calves reduced avoidance distance, resulting in calves with less fear reactions towards humans. Another study with lactating dairy cows suggests that periods of stroking during milking can positively influence their response to humans, reducing avoidance distances observed in the feeding place, barn, and milking parlor (Windschnurer et al., 2009). However, the animals in these two studies experienced tactile stimulation for longer periods of time over multiple days, which differs greatly from the protocol of the present study. It may be that only 2 minutes early in life, followed by possible aversive procedures such as vaccination and tagging, was not enough to influence how calves react toward humans later in their life. Furthermore, the relationship of avoidance distance with practices such as tactile stimulation varies according to the breed examined (Boivin, et al., 2008; Probst et al., 2013; Ujita et al., 2021). It is plausible that Angus calves do not perceive tactile stimulation the same as other breeds.

As mentioned before for RSC, calves and cows were frequently visited by the stockperson to follow the progression of the infectious bovine keratoconjunctivitis outbreak in the group. Visits were daily and involved walking among cattle in order to visualize each animal's face. It is known that avoidance distance is dependent on several factors such as the animals' breed, the situation the test was made, and previous interactions between animals and humans (Grandin, 1987; Grandin and Deesing, 2022; Purcell et al., 1988). Previous interactions with cows that were in frequent contact with handlers during feeding, watering, and cleaning had a considerable impact on cows' avoidance, resulting in more than half of the cows with zero avoidance distance (Sharma and Phillips, 2019). This frequent interaction may have influenced calves' behaviour. They may have habituated to the humans' presence. Habituation is "a non-associative learning process that results in decreased responsiveness to repeated stimuli" (Grissom and Bhatnagar, 2009). Numerous published studies have substantiated that animals become habituated with human presence, leading to a notable decrease in avoidance distance (Aubé et al., 2023; Sharma and Phillips, 2019; Ujita et al., 2021). The extent to which the calves habituated following the protocol remains uncertain. Nevertheless, since all calves in the study had increased contact with the stockpeople, this interaction might have played a role in neutralizing any possible impact of the interventions applied at E1, resulting in no discernible difference in avoidance distance.

Tactile stimulation and the application of umbilical antiseptic did not influence the risk of animals being treated for NCD or BRD. Two calves died, but the causes were unrelated to the experimental question, so no further analysis was conducted. There are very few studies aiming to understand the effects of these two practices in calves' health. A study that evaluated the effects of a package of management practices involving the application of umbilical antiseptic, colostrum feeding, and brushing dairy calves reported a decrease in the frequency of antibiotic treatments and the number of calf deaths (Silva-Antunes and Costa 2021). However, that project was conducted on a dairy operation, which poses several differences from the present study, which was conducted on a beef cow-calf operation where few treatments for BRD and NCD were required, and no umbilical infections were observed. Numerous studies have revealed a higher prevalence of pre-weaning diseases in dairy operations, likely associated with the system being highly intensive (Godden, 2008; Renaud et al., 2018; Uetake, 2013). Calves raised in an intensive system may experience more disease challenges associated with the quality of the cleaning routine of feed equipment and indoor housing, which can have negative effects on their health (Godden, 2008; Ring et al., 2018; Uetake, 2013). Hygiene of the environment, calves feeding routine, and diseases identification and treatment protocol can be considered some of the keys to control of pre-weaning diseases (Blanchard, 2012; Smith, 2012). In the present study, the calves were kept in large pastures with their dams, in a less intensive system, compared with dairy production systems, which likely explains why there were few calves treated for BRD, NCD, and umbilical infection. Also, it has been demonstrated that morbidity and mortality may be underestimated in beef cow-calf operations, because the maintenance of calf health records by cow-calf producers is not consistently done (Pearson et al., 2019). Furthermore, it was anticipated that clinical or subclinical umbilical infections may predispose calves to subsequent diseases (Pardon and Deprez, 2018) and that the use of umbilical antiseptic would reduce the occurrences of such infections. However, in this study, no calves underwent treatment for umbilical infections, which may explain why the experimental interventions showed no influence on the treatment of other diseases such as BRD and NCD.

These findings may also be explained by the sample size used in the study. Pearson et al. (2019) and Waldner et al. (2022) benchmarked 97 and 89 cow-calf operations in western

Canada, respectively, and identified that around 3% of calves were treated for NCD, 4-5% for BRD, and 2% for umbilical infection. In the present study, the study sample size was designed with a greater focus on behavioural indicators and on the logistics available (i.e., number of animals that could be held in the same pasture). Following a post-hoc size calculation, considering the results for the health indicator in the present study, it was determined that approximately 600 calves would be required for sufficient power. Each experimental groups each had 30 calves, which was probably was not enough to have the power to detect the effects of tactile stimulation and application of umbilical antiseptic on calf health in this study.

Overall, there are differences in the potential effects on the health of calves from the use of umbilical antiseptic (Fordyce et al., 2018; Grover and Godden, 2011; Wieland et al., 2017), tactile stimulation (Miranda et al., 2023; Waiblinger et al., 2004), and the both practices together (Silva et al., 2017; Silva-Antunes and Costa, 2021). It may be that tactile stimulation and application of umbilical disinfection may have different possible effects on the health of animals depending on the context in which they are being inserted.

There was no significant association of any treatment on ADG, as indicated by both ADG1 and ADG2. The influence of tactile stimulation and application of umbilical antiseptic on calves' performance, particularly beef calves, remains a relatively understudied area. Cerezo (2023) revealed a positive effect of one-minute tactile stimulation during processing of 3 days old beef Nelore calves' ADG. Additionally, Lürzel et al. (2016) observed a 6.6% increase in ADG of dairy calves following "gentle handling" practices, including brushing and gentle communication (i.e., low pitch verbal cues). It should be emphasized that the positive effects observed in Cerezo (2023) study may be linked to the producer's incorporation of tactile stimulation into their routines within a larger package of various animal welfare-related practices. These packages encompassed training about concepts of how to handle animals in the corral while respecting calves' behaviour and implementing management practices that could generate positive and neutral relationship between livestock farmers and animals, with a purposeful focused on low-stress handling practices (Ceballos et al., 2018). As such, it may be that tactile stimulation and umbilical disinfection alone, outside of a broader scope of change, may not be enough to affect ADG.

The sex of the calf had a significant influence on calves' ADG, with males having higher averages compared to females for both ADG1 and ADG2. This is anticipated, primarily because males undergo the synthesis of steroid hormones, leading to a more significant increase in muscle tissue (Marcondes et al., 2008; Perotto et al., 2001) and more food consumption, resulting in a more conversion of nutrients into muscle tissue (Nkrumah et al., 2004; Souza et al., 2020).

There was an influence of calves being treated for BRD on ADG1, with calves who were not treated for BRD having lower ADG than calves that were treated. Usually, BRD treatment is associated with lower ADG (Teixeira et al., 2013; Virtala et al., 1996; Wieland et al., 2017). Previous studies had a much larger number of animals treated for BRD and often had more than one farm included, which may have made it possible to find these associations. The present results are likely explained by the fact that only 4 calves were treated for BRD, whose averages were compared with 116 untreated calves, so this is likely a spurious finding related to low incidence of disease and small sample size.

It is important to emphasize that both tactile stimulation and the application of umbilical antiseptic were administered within the first 24 hours of the calves' lives. This timing was selected because it aligns with the farm stockpeople's routine when they head to the field to perform essential calf-processing tasks, including vaccination and calf identification. The first 24 hours are paramount for cow-calf bonding process and should ideally remain uninterrupted (da Costa and Cromberg, 1998; Mellor and Gregory, 2003; Simões and Stilwell, 2021). The cow engages in behaviours fostering the establishment of that bond (Mellor and Gregory, 2003; Simões and Stilwell, 2021). Studies demonstrating effects of tactile stimulation and application of umbilical antiseptic typically implemented these practices at least three days after birth or in older animals (Cerezo, 2013; Silva et al., 2017; Silva-Antunes and Costa, 2021). A plausible explanation for the lack of influence of the practices applied on the animal welfare indicators in this study could be because the experimental protocols were applied in the sensitivity moment between the cow and calf, and also together with other practices, that could have been perceived as aversive for the calves, such as vaccination, application of umbilical antiseptic, and tagging the calves' ear with their ID. Consequently, any potential effects from the treatment may have been negated, contributing to the observed outcomes.

2.7. CONCLUSION

Tactile stimulation was associated with the potential to decrease only one of the four reactivity tests evaluated, namely flight speed, demonstrating inconclusive effects of this practice on calves’ reactivity in handling facilities. The proposed procedures did not influence calves’ pre-weaning risk of disease treatment or growth. Overall, there was limited evidence identified regarding an impact of tactile stimulation and umbilical disinfection, as they were applied in this study, on the welfare of pre-weaned Angus beef calves.

2.8. TABLES AND FIGURES

Table 1 - Description of the behavioural categories evaluated for the Reactivity Assessment During weighing (RDW).

Category	Definition	Variable
Leg Movement	The calf takes at least one step inside the cage, which could be stepping to the left, right, forward, or backward	Duration
Head Movement	Starting from a still position, the calf moves its head away from the neutral position to the right, or the left, or up, or down until the movement stops	Duration
Ear Movement	The calf moves its ears up, or down, backward or forward perpendicular to its head	Frequency
Presses Head Against the Cage	The calf pushes its face against the cage with pressure and maintains this behaviour until contact between the head and the cage ceases	Frequency
Tail Flicking	The calf moves its tail parallel to an imaginary vertical line between the pin bones to the right or left and returns the tail to this imaginary vertical line	Frequency
Jumping	The calf lifts its forelegs off the ground, elevating the front of the body. Movement is upwards, and hind legs may or may not also be lifted off the ground	Frequency
Stomping	The calf makes fast and continuous movements with any leg, not necessarily taking a step	Duration
Low Pitch Vocalization	The calf may or may not open its mouth but does not make movements contracting the stomach region while performing a short-duration vocalization	Frequency
High Pitch Vocalization	The calf may open its mouth and makes visible contraction movements of the stomach region while performing a long-duration vocalization	Frequency
Urination	The calf may or may not adopt a urination stance whereby it arches its entire body, and it is possible to see fresh urine appearing on the cage's floor. Female calves are seen to arch their tails	Present or Absent
Defaecation	The calf inclines its back and arches its tail, and it is possible to observe fresh feces falling on the cage floor	Present or Absent
Kneeling	Calf stands on its rear feet but rests on its carpal joints	Duration
Lying	Calf adopts lateral or sternal recumbent position. The ventral part of the calf’s body is in contact with the cage floor with the limbs stretched or tucked underneath its body	Duration
Standing	The animal stands upright with all four feet in contact with the ground	Duration Duration

Blind Spot (Head)	The calf places its head in a blind spot in the cage, making it impossible for the camera to capture the recording. The viewer is not able to see the following behaviours in the footage: head movement, ear movement, and pressing the head against the cage	
Blind Spot (Tail)	The calf places its tail in a blind spot in the cage, making it impossible for the camera to capture the recording. The viewer is not able to see the following behaviours in the footage: Tail Flicking	Duration

Table 2 - Description of the scores used to assess reactivity inside the squeeze chute (RSC) (Adapted from Ceballos et al., 2016; Grandin, 1993).

Traits	Scores	Descriptions
Movement (MOV)	1	No movements
	2	Few movements, for less than half of the observation time
	3	Frequent but not vigorous movements, for half of the observation time or more
	4	Constant and vigorous movements
	5	With constant and vigorous movements, the animal jumps and raises its forelimbs off the ground
Tension (TEN)	1	The animal did not exhibit sudden movements of the tail, head, and neck, no muscle tremors, and eye whites were not visible
	2	The animal exhibited few sudden movements of the tail, head, and neck, no muscle tremors, and eye whites may or may not have been visible
	3	The animal exhibited continuous and vigorous movements of the tail, head, and neck, no muscle tremors, and eye whites were visible
	4	The animal appeared paralyzed or freezing, muscle tremors were visible
Body posture (BC)	1	Standing: when the animal stands upright with all four feet in contact with the ground, still or moving
	2	Kneeling: when the animal stands on its rear feet but rests on its carpal joints
	3	Lying: when the ventral part of the animal's body is in contact with the ground
Kicking (KI)	1	When the animal does not exert a vigorous blow with its hind foot
	2	When the animal exerts a vigorous blow with its hind foot
Balking (BA)	1	Non-Balker: the calf entered voluntarily when the gate opened, without balking
	2	Balker: The handler places one or two hands on the rump and makes pressure to induce an animal to enter into the squeeze-chute

Table 3 - Description of study population by experimental group (tactile stimulation and application of umbilical antiseptic (TSUA), tactile stimulation and no application of umbilical antiseptic (TS), no tactile stimulation and application of umbilical antiseptic (UA), and no tactile stimulation and no application of umbilical antiseptic (C) and potential confounding factors (sex (male, female), pen (1, 2), stockperson pair (1 – 4), cow parity (2 – 13), and enrollment week (1, 2).

Experimental Groups		**TSUA**	**TS**	**UA**	**C**	**P-value**
Total of Calves		30	30	30	30	
Sex	Bull	16	16	16	16	1
	Heifer	14	14	14	14	
Pen	1	10	14	13	15	0.59
	2	20	16	17	15	
Stockpeople Pair	1	13	16	11	15	0.95
	2	7	8	10	6	
	3	4	3	3	4	
	4	5	3	5	5	
Cows Parity	2	4	4	4	7	0.59
	3	0	1	1	1	
	4	2	1	1	0	
	5	9	6	6	8	
	6	1	2	2	1	
	7	7	6	6	8	
	8	2	5	5	1	
	9	2	1	1	0	
	10	0	2	2	1	
	11	1	1	1	2	
	13	0	0	0	1	
Enrollment Week	1 (May 6 – 12, 2022)	15	15	15	15	1
	2 (May 13 – 18, 2022)	15	15	15	15	

Table 4 - Terms representing each principal component generated from a Reactivity During Weighting (RDW) test of 120 beef calves at 8-24h after birth. Terms in bold represent loadings > 0.6.

Behaviour[a]	**PC 1**	**PC 2**	**PC 3**	**PC 4**
Head Movement	-0.12	**0.68**	-0.34	0.28
Ear Movement	-0.42	**0.62**	-0.08	-0.22
Standing	0.10	**0.62**	-0.29	-0.01
Low Pitch Vocalization	0.15	0.31	0.56	**0.61**
High Pitch Vocalization	0.17	0.29	**0.72**	-0.08
Presses Head Against the Cage	**0.72**	0.20	0.04	-0.42
Leg Movement	**0.88**	0.20	0.02	-0.20
Tail Flicking	**0.81**	-0.05	-0.13	0.11
Jumping	0.54	-0.18	-0.35	0.48
Percent of variance (%)	27.6	17.0	13.1	10.8

[a]Behaviours are described in the ethogram in Table 1.

Table 5 - Distributions of disease treatments and deaths among pre-weaned beef calves (n = 120) in four experimental groups (tactile stimulation and application of umbilical antiseptic (TSUA), tactile stimulation and no application of umbilical antiseptic (TS), no tactile stimulation and application of umbilical antiseptic (UA), and no tactile stimulation and no application of umbilical antiseptic (C).

Diseases	Number of calves treated	TSUA	TS	UA	C
Bovine Respiratory Disease	5	1	1	1	2
Castration Complication	1	-	-	-	1
Coccidiosis	3	-	1	1	1
Depressed	8	3	2	1	2
Ear Infection	2	-	-	1	1
Foot root	1	-	1	-	-
Neonatal Calf Diarrhea	4	-	2	-	2
Infectious Bovine Keratoconjunctivitis	41	13	10	13	5
Bloat (Death)	1	-	-	1	-
Ulcer (Death)	1	1	-	-	-

Table 6 - Treatments of neonatal calf diarrhea (NCD), Bovine Respiratory Disease (BRD), and either NCD or BRD (DBRD) by experimental group (tactile stimulation and application of umbilical antiseptic (TSUA), tactile stimulation and no application of umbilical antiseptic (TS), no tactile stimulation and application of umbilical antiseptic (UA), and no tactile stimulation and no application of umbilical antiseptic (C)).

Experimental Treatment Group	Calves were not treated	Calves Treated	p-value[a]
NCD			
TSUA	30	0	0.62
TS	28	2	
UA	30	0	
C	29	1	
BRD			
TSUA	29	1	1
TS	29	1	
UA	29	1	
C	29	1	
DBRD			
TSUA	29	1	0.83
TS	27	3	
UA	29	1	
C	28	2	

[a] P-values obtained after applied the Fisher Exact Test.

Figure 1 - A: Plots of individual calves' loadings of each experimental group in PC1 (x axis) and PC2 (y axis). With Tactile stimulation (WTS, blue circle) and no tactile stimulation (NTS, green triangle). B: Distribution plot of the behaviours of the Reactivity Assessment During Weighing (RDW) test in PC1 (x axis) and PC2 (y axis).

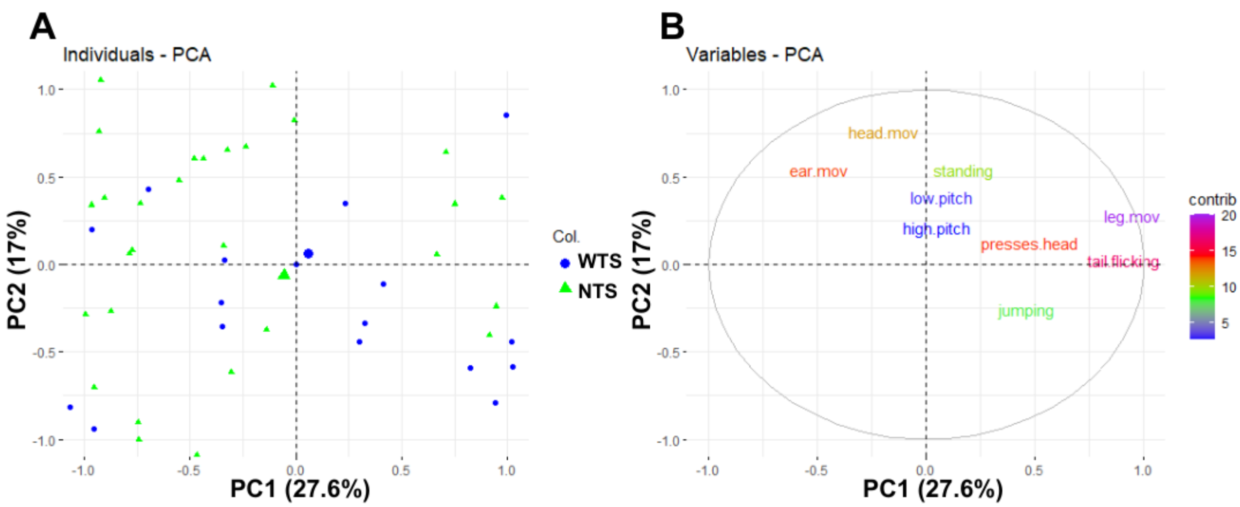
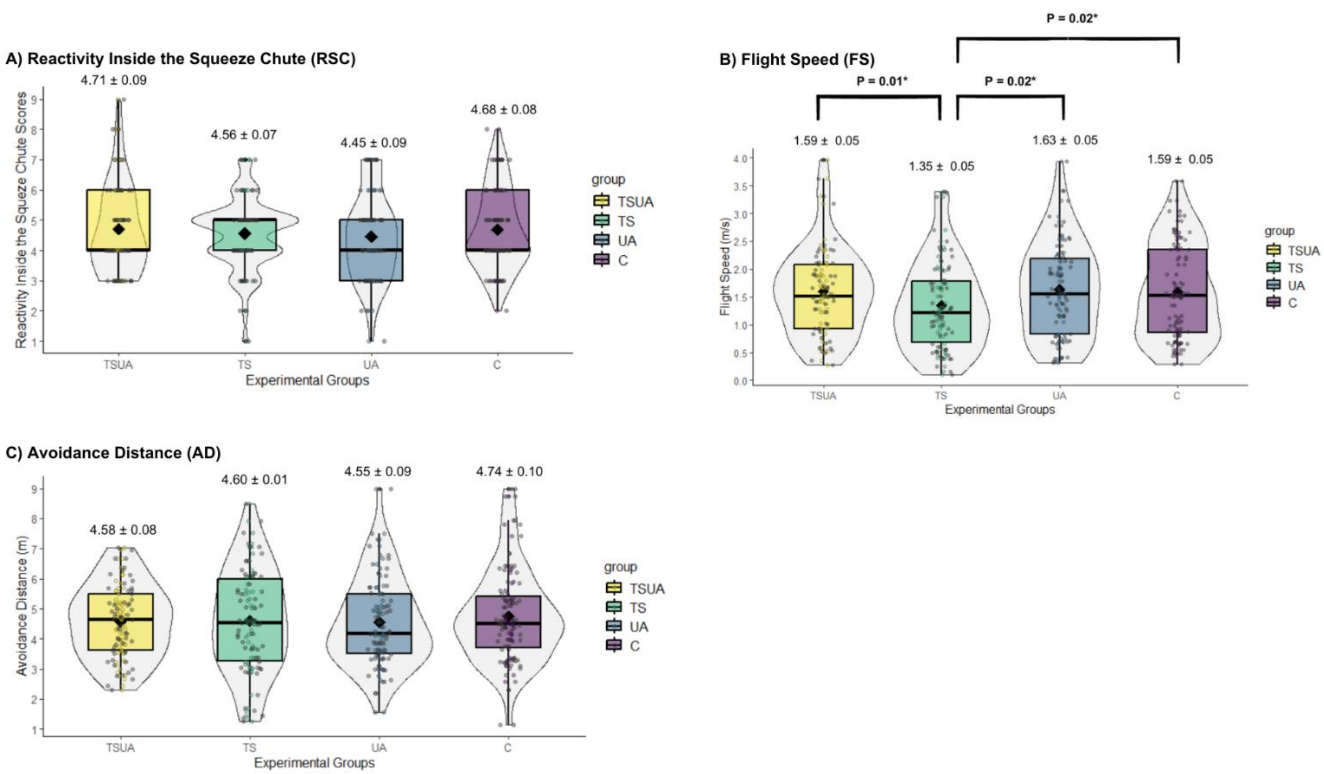


Figure 2 - A: Comparison of the Reactivity Inside the Squeeze Chute measurements (RSC) among experimental groups (tactile stimulation and application of umbilical antiseptic (TSUA), tactile stimulation and no application of umbilical antiseptic (TS), no tactile stimulation and application of umbilical antiseptic (UA), and no tactile stimulation and no application of umbilical antiseptic (C); B: Comparison of mean Flight Speed (FS) among experimental groups; and C: Comparison of the mean Avoidance Distance between the experimental groups.



*Indicates statistical difference when comparing groups. The groups mean ± SE with the crude values is listed for the three graphics.

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2.10. APPENDICES

Appendix A. The distributions for Reactivity Inside the Squeeze Chute (RSC), Flight Speed (FS), and Avoidance Distance (AD).

Figure A.1. Reactivity inside the squeeze chute (RSC) distributions overtime (between event 2 and 3).

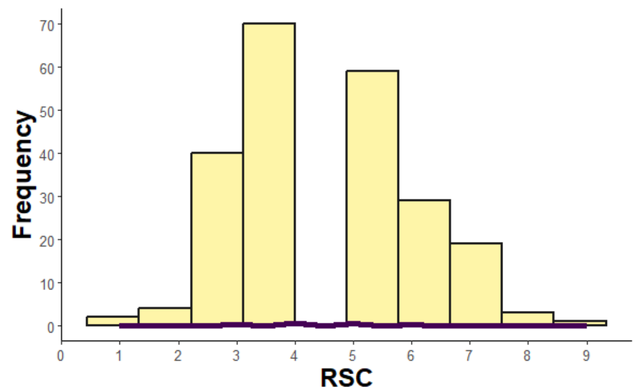


Figure A.2. Flight Speed (FS) distributions overtime (between event 2 and 3).

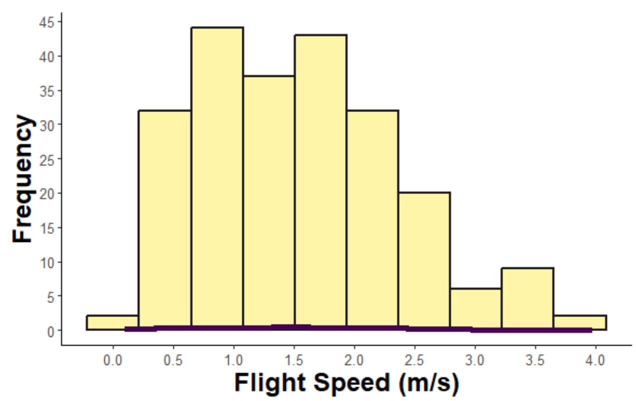
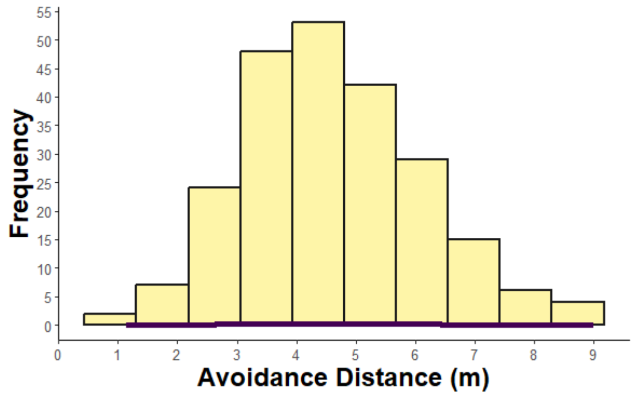


Figure A.3. Avoidance Distance (AD) distributions overtime (between event 2 and 3).



Appendix B. Distribution for Average Daily Gain

Figure B.1. Average Daily Gain between event 1 and 2 (kg/day) distribution

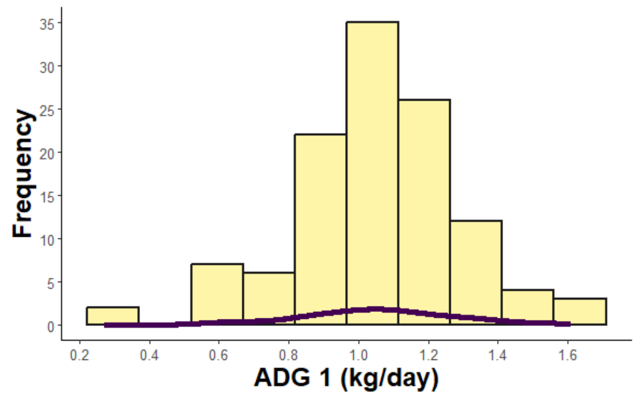
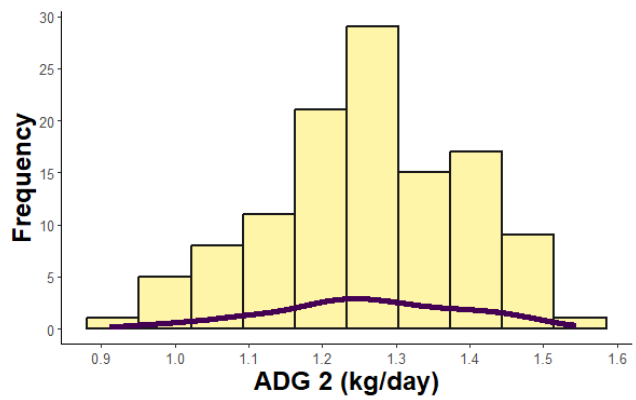


Figure B.2. Average Daily Gain between event 2 and 3 (kg/day) distribution.



CHAPTER 3 – Summary, Limitations, and Future Implications

The objective of this thesis was to evaluate the impacts of the application on neonatal beef calves of tactile stimulation and umbilical antiseptic on pre-weaned beef calves' welfare. This was assessed using three animal welfare indicators: behaviour, focusing on calves' reactivity when interacting with humans in handling facilities; health, assessing the potential reduction of pre-weaning disease treatment; and productivity, identifying the effects on calves' average daily gain.

3.1. SUMMARY OF RESULTS

Data collection was carried out at W.A. Ranches at the University of Calgary, located in Alberta, Canada. A total of 120 calves (65 males and 55 females), randomly divided into a 2x2 factorial design, were enrolled into one of four experimental groups: TSUA - calves with tactile stimulation and application of umbilical antiseptic (Bulls = 16, and Heifers = 14); TS - calves with tactile stimulation but no application of umbilical antiseptic (Bulls = 16, and Heifers = 14); UA - calves without tactile stimulation but with application of umbilical antiseptic (Bulls = 16, and Heifers = 14), and C – control calves without tactile stimulation or application of umbilical antiseptic (Bulls = 17, and Heifers = 13). Data collection occurred during three handling events: E1 - event 1, within 24 hours of birth; E2 – event 2, near the time of spring processing (average age = 40d); and E3 – event 3, near the time of weaning (average age = 158d). The welfare of calves was assessed using three indicators: behaviour, health, and productivity outcomes. For the behaviour indicators, different calves' reactivity traits were evaluated: Reactivity Assessment During Weighing (RDW), Reactivity Inside the Squeeze Chute (RSC), Flight Speed (FS), and Avoidance Distance (AD). The RDW was performed just after calves underwent tactile stimulation (WTS, n=60) or not (NTS, n=60) at E1, while the other traits were measured at E2 and E3. Occurrence of treatment for disease and death from birth to pre-weaning were recorded as indicators of health. Lastly, calves' average daily gain was used as a productivity indicator.

Of all animal welfare indicators evaluated, only FS was influenced by experimental group. Calves from TS group left the squeeze chute more slowly compared with other groups, indicating that calves that underwent just tactile stimulation were less reactive upon exiting a restrained environment. However, a previous study contradicts this result, as it demonstrated no differences in flight speed between dairy heifers that did or did not receive brushing (Silva et al., 2017). The FS did not differ among the other experimental groups, which may be because the application of iodine caused a stinging sensation that resulted in those calves having a negative experience that influenced their behaviour (Ceballos et al., 2018; Hemsworth and Coleman, 2011; Waiblinger et al., 2006).

During early calf processing at E1, no associations were detected between the experimental groups and any of the principal component (PC) generated via principal component analysis of the RDW test. This may be due to the context in which the experimental protocols were applied (i.e., immediately after the calves were caught, inserted in the cage, and separated from their dams). This separation may have resulted in separation distress (Flower and Weary, 2001; Hudson and Mullord, 1977; Veissier et al., 1989; Weary and Chua, 2000), eliminating any possible effects of the interventions on calf reactivity.

There was an association between the stockpeople pair and the behaviours of head movement, ear movement, and standing (PC2). Calves handled by Pair 1 exhibited more of the behaviours from PC2 than those handled by Pair 3. Other studies with cattle and horses (de Passillé et al., 1996; Schmidek et al., 2020) have reported that the animals presented these behaviours more after positive interactions with humans. Furthermore, subjective observations in the field suggested that Pair 3 handled calves less carefully than other pairs of stockpeople. In contrast, Pair 1 were perceived to take greater care and were gentler with the calves. It may be that these handling practices influenced the associated behavioural outcomes, although this was not explicitly evaluated in this study.

There was an association between calves' birth weight and the behaviours of pressing the head against the cage, leg movement, and tail flicking (PC1), with lighter calves exhibiting higher PC1 values than heavier ones. It has been demonstrated previously that calf size is associated with their vigour at birth (Comerford et al., 1987; Murray and Leslie, 2013); when they are larger, birth can be prolonged or more difficult. This leads to the hypothesis that heavier calves may have experienced birth-related issues affecting their vigour, which may have influenced their behaviour at this time. However, all calves enrolled in this study were unassisted at delivery, so any birth-related issues likely would have been minor and did not require human intervention. An alternative explanation could be the cage size that may have influenced behaviours exhibited by calves. Whalin et al. (2021) indicates that the size of the environment, like a pen, can influence how animals express their behaviours. While it is not possible to say that the relative size of smaller versus larger calves restricted their ability to display many assessed behaviours in the cage, the confinement in a comparatively small area might have had varied effects on calves of different birth weights. As such, this observed association may have been due to unmeasured factors or was a spurious finding.

The RSC and AD did not differ among groups. The RSC evaluated calf reactivity inside a restrained environment. One plausible interpretation for this outcome is that the RSC test might not be ideal for evaluating the reactivity of very young calves. Differences in behaviour were found using this test in adult animals (Ceballos et al., 2018; Macedo et al., 2011; Sant'Anna et al., 2013), which typically already have their personalities formed (Cabrera et al., 2021). It has been suggested that the temperament of calves only becomes established only after 6 months of age (Dennison, 1985). It could be that because young calves' personality is still being established, the applicability of RSC test in this context may be limited.

Another potential explanation for this finding regarding both RSC and AD could be the limited duration of tactile stimulation (i.e., 2 minutes) applied to the calves, just once, within their first 24 hours of life. This study aimed to find practical procedures that beef producers could apply in their existing routines, so more than 2 minutes or several handlings of calves would not have been practical. It is plausible that only 2 minutes of tactile stimulation once in their life at a time when something else stressful was happening (i.e., being separated from their dams and being tagged and vaccinated) was not enough to create a good memory with humans, so it did not affect their reactivity in the future. Other studies that have reported effects of tactile stimulation on cattle behavioural responses applied this practice for a prolonged period, more than once, at times that were not associated with other negative interactions (Probst et al., 2012; Windschnurer et al., 2009). Therefore, the intervention applied in this study might have not been sufficient to affect calves' behavioural responses, as represented by the RSC and AD results. Additionally, this study had an external factor that may also have influenced this result. There was an infectious bovine keratoconjunctivitis outbreak in this group of calves. This condition required close monitoring by stockpeople to identify and treat affected calves. The visits required the stockperson to walk around the entire group daily for several days. This neutral interaction with the group may have resulted in a habituation process to human presence (Aubé et al., 2023; Sharma and Phillips, 2019; Ujita et al., 2021), which could have masked any expected effect of the treatments on calves' behaviour.

Calves' reactivity increased from E2 to E3, as demonstrated by FS. In contrast, RSC decreased between the two events. Usually, cattle reactivity decreases from one event to another (Curley Jr et al., 2006; Hall et al., 2011, Sant'Anna et al., 2019). The calves were approximately 40 days of age the first time that FS was measured and about 160 days old the second time, with a clear difference in weight and size. It might be that the increase in FS was due to the natural growth of the animals, as the older and bigger calves get, their capacity to leave the chute more quickly would increase. Conversely, average for RSC only decreased by 0.06 points from the first to the second event, and in general, calves had low reactivity. It is likely this small change in the relatively calm calves did not have any relevant biological significance.

Regarding health and productivity indicators, neither tactile stimulation nor the application of umbilical antiseptic influenced the risk of animals being treated for neonatal calf diarrhea (NCD) or bovine respiratory disease (BRD), nor did it impact their average dairy gain (ADG). The study sample size calculation was done with a greater focus on behavioural indicators and the logistics of the farm (i.e., number of animals that could be held in the same pasture). Thus, each experimental group had 30 calves, which could have influenced the result for these other outcomes. The limited number of animals treated in this study resulted in a lack of power to identify statistical differences among experimental groups. After a post-hoc size calculation based on the results from the present study, it would be necessary to include approximately ~600 calves to have sufficient power to determine statistical significance of a difference of the magnitude observed in health indicator and ~400 in productivity indicator.

The influence of tactile stimulation and application of umbilical antiseptic on calves' welfare, particularly beef calves, remains a relatively understudied area. A positive effect has been reported in Brazil with one-minute tactile stimulation during processing with calves at about 3 days of age on the ADG and emotional expression of beef Nelore calves (Cerezo, 2023). The effects observed in this study may have been related to a broader effect, not just the tactile stimulation and use of umbilical antiseptic. The farm where this study was performed had already incorporated tactile stimulation into their routine within a larger package of animal welfare-related practices called “each calf matters”. This training involved explaining management practices from birth to weaning of calves following management concepts with a focus on calf welfare. This training encompassed concepts on how to handle animals in the corral while respecting the calves' behaviour and management practices that could generate positive and neutral relationship between livestock farmers and animals. It has been demonstrated that this kind of training aimed at improving skills and knowledge can improve stockpeople's attitudes and behaviours towards animals (Ceballos et al., 2018, Hemsworth and Coleman, 2011). Attitudes precede behaviours, and when people have better attitudes toward animals, their behaviour is better. The stockpeople from the Brazilian study performed all the handling and interventions themselves. Anecdotally, stockpeople subsequently mentioned things like, "I started seeing calves differently since the farm started implementing this practice", and "now I care more about the calves, I pay more attention to them, and they like when I give them the massage". In other words, the "massage" per se is not the only factor that influence calves' responses in that context, but also the change that implementing these practices made on the people. One could hypothesize that when low-stress handling has already been implemented and the stockpeople already have positive attitudes toward animals, additional practices such as tactile stimulation may have a higher probability of generating a positive memory in the calves than in scenarios when calves are generally more stressed or fearful. In the present study, the researcher performed all the treatments and the stockpeople who were in charge of the calves did not have the opportunity to undergo any changes in attitude related to the experience of giving a massage to the calves.

Overall, this study demonstrated limited evidence that tactile stimulation and umbilical disinfection, as they were applied in this study, impacted the welfare of pre-weaned crossbred Angus beef calves. The impact of tactile stimulation and umbilical disinfection on welfare indicators assessed in this study must be interpreted with caution, considering the context of this study and specific intervention protocol. In this case, the application of these practices alone may not have been effective without being integrated into a broad systematic change of implementing more animal welfare-related practices.

3.2. STUDY LIMITATIONS

There are some limitations of the study that must be recognized. As mentioned before, the initially determined sample size was primarily focused on behavioural indicators and logistics at the farm. The sample size had less consideration given to the health and productivity indicators. Thirty calves per group did not represent appropriate statistical power to identify significant variations in health and productivity indicators, and the post-hoc size calculation reinforced this, as it would have been necessary to include 400-600 calves to demonstrate an effect of the magnitude observed in this study on health or productivity.

Another limitation that could have influenced the results is that the time and the quality of the interaction with calves when catching them from the ground to being inserted inside the cage to apply the experimental treatment was not assessed. The process of picking up the calves may have varied in time and the number of attempts required to capture them, consequently the physical effort that calves may have done. The stress experienced by the calves during this procedure could have influenced their behaviour. There are studies demonstrating the impact of stresfull practices of handlings in animals behaviour (Hemsworth et al., 1987; Grandin, 1997; Hemsworth et al., 2000; Hemsworth et al., 2011). Future research should address this limitation by performing a more standardized handling procedure.

Another potential limitation was that the implementation of tactile stimulation and the application of umbilical antiseptic were performed in isolation, without including these practices in conjunction with other animal welfare-related practices, as mentioned above. In addition, they were applied by the researcher in charge of this study and not by the stockpeople handling the animals every day. There was a similar study performed for three consecutive years in a cow-calf operation in Alberta, Canada (Desiree Gellatly, personal communication), which reported benefits on newborn calves that received tactile stimulation at 1-day of age. Calves that received tactile stimulation required 20% fewer treatments for illnesses in the first 30-days of age over the 3 years of study and were 21 kg heavier at weaning (~220 days of age) in year 1 of the study compared to non-tactile stimulated ones (Desiree Gellatly, personal communication). In this study, it was reported that only two people were responsible for handling the calves during the processing time.

However, it is important to highlight that this study was not published still and the results should be used carefully. Therefore, both received a comprehensive training on animal welfare-related practices such as low-stress handling similar to the one previously described in Brazil. The practice of tactile stimulation combined with the other low-stress handling practices performed by the producer may have contributed to their outcomes. However, that study has not undergone peer-review and extensive details are not available to assess the external validity of the results. This report and the study from Brazil may help to reinforce the hypotheses generated from the present study. Tactile stimulation was similarly applied for a short period of time only once on the animal's first day of life; however, the effects may have been evident because it was applied into a broader system of low-stress handling practices.

3.3. CONTRIBUTIONS TO NEW KNOWLEDGE AND FUTURE STUDIES

This thesis was the first scientific study to incorporate both tactile stimulation and umbilical antiseptic application into a protocol within a beef cow-calf operation in western Canada and evaluated the impacts of these interventions on three animal welfare indicators: behaviour, health, and productivity.

One significant adjustment for future investigations on this topic could involve re-evaluating the context of the farm where tactile stimulation is being applied. Longer stimulation protocols in dairy cattle studies have demonstrated benefits on different welfare indicators, suggesting that only a 2-minute duration may not be sufficient to elicit measurable effects. However, it is necessary to think about the context of the beef cow-calf operation, which does not offer many opportunities of interaction with the calves and when this happens, it is for a short time. However, based on the Brazilian study (Cerezo, 2023), and Desiree Gellatly (personal communication) results, a single, short-duration massage early in calves' life may be enough to influence health and productivity indicators if it is applied by stockpeople and within a more systematic change that includes training in low-stress handlings practices and other animal welfare-related concepts. It may be necessary to align all management practices during calf processing in relation to animal welfare, especially focussing on low-stress cattle handling practices. Training stockpeople to apply tactile stimulation themselves may be a powerful way of reinforcing a bond of respect between stockpeople and calves, consequently improving their attitudes toward animals. Therefore, future research should explore human-animal relationship in terms of human attitudes and behaviours.

Based on the findings of this study, under the specific conditions it was performed, limited evidence was demonstrated of the effect of the interventions on beef calves' welfare, with only an effect on one reactivity test (i.e. flight speed). Thus, the application of the proposed procedures should be carefully considered within a contextual framework rather than in isolation. Additionally, the use of reactivity tests in Angus beef cattle should consider the age of the animal, as the effectiveness of these tests have been reported in adult subjects and may not the same as in younger ones.

Another consideration about the application of umbilical antiseptic is that while it holds the potential to reduce treatments for umbilical infections and associated diseases in the dairy industry, it may not be universally necessary but rather selectively employed based on actual environmental needs. It is important to note the beef cattle environmental conditions in western Canada context significantly differs from the intensive dairy farm conditions, as the environmental challenges in terms of pathogens, cleanliness, etc., are lower and beef calves' experiences lower rates of animals treated for these issues (Pearson et al., 2019; Waldner et al., 2022).

The current investigation described that the general responses of pre-weaned crossbred Angus calves submitted to tactile stimulation and umbilical disinfection early in their lives needs deeper comprehension related to the context in which these interventions are applied. By delving into individual differences, a future study could examine how distinct personality traits and other external factors (e.g., related to the people implementing them) contribute to how these calves perceive these interventions and how they affect their welfare. This understanding may enhance our comprehension of the complex interaction of individual calves' responses and provide valuable insights to promote animal welfare practices and husbandry strategies in agricultural settings.

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