

2017

## Effects of Timing of Local Anesthesia on Physiological Responses in Calves after Dehorning

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EFFECTS OF TIMING OF LOCAL ANESTHESIA ON PHYSIOLOGICAL RESPONSES IN  
CALVES AFTER DEHORNING

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science  
in  
The School of Animal Sciences

by  
Amanda Jane Mathias  
B.S., Oklahoma State University, 2014  
May 2017

## **ACKNOWLEDGEMENTS**

First I would like to thank Dr. Cathleen Williams for all of her support these past 3 years. Without her willingness to take on a student whom she had never met nor spoken to, as well as jumping feet first into a completely different research area, I would not be where I am today. To Dr. Don Thompson, I would like to thank for not only answering the many questions about hormone assays, but also for allowing me to spend many hours in his lab learning and measuring cortisol and ACTH. I would also like to thank Dr. Navarre and Dr. Welborn for both offering their advice and sharing their knowledge throughout these past three years. While there were difficulties we encountered, which no one could have imagined when I started out, my committee has made navigating them much easier. It was a privilege to work with and learn from people who are great at what they do, as well as being great mentors and friends.

I would also like to extend thanks to the student workers and research associates, as they made both of my research projects possible. To Ashley Dolejsiova, while I only worked with you for a short while, thank you for the lessons in pipetting, assays, helping plan logistics, and gather supplies. I learned many things from you, but the largest being to always have everything planned out ahead of time and more than enough supplies on hand. To the LSU Dairy Research Farm staff and the LSU Southeast Research Station staff, thank you for all of your work and help to make both of my projects possible.

A very large thank you to my fellow graduate students, Erin Oberhause, Brittany Foster, Michael McGee, and Carolyn Pham for their aid in data collection, help with assays, and support as both my friends and colleagues. Without all of you, I would not have been able to complete my projects.

A special thank you to my former lab mate and now research associate Steven Blair. You

helped me learn how to manage a research project. Your willingness to help out and all of your hard work made my career at LSU go extremely smooth. I cannot thank you enough. To my other former lab mate Marisol Orellana Rivas, thank you for your support and friendship.

I would also like to thank my Parents Brian and Margaret Mathias for their unwavering support and constant encouragement throughout my entire life. I could not have asked for a better set of cheerleaders and parents. Dr. Michelle Calvo-Lorenzo, you have been one of the best mentors, advisors, and friend anyone could ask for. Thank you for your constant willingness to offer support and advice, as well as pointing me west to Dr. Courtney Daigle. To Calvin and Cindy Hubbard, even though I have only known you for a short while, you have been a home away from home for me and I thank you for that. Lastly, thank you to Aaron Hubbard. You have been a constant rock for me these past two and a half years. Even though we may have been separated by distance this past year, I can always count on you to listen and offer unfailing support.

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## **ABSTRACT**

The American Veterinary Medical Association has stated the importance of minimizing pain associated with dehorning to limit the amount of distress and changes in the animal's behavior and physiological states (AVMA, 2014b). The following studies aim to determine the effect of timing of lidocaine administration on physiological responses in calves after dehorning. The first study compared cortisol and substance P levels in calves dehorned immediately after administration of lidocaine and meloxicam, calves dehorned 10 minutes after the administration of lidocaine and meloxicam, and calves that were administered lidocaine and meloxicam and not dehorned. It was concluded waiting 10 minutes after administration of lidocaine and meloxicam to dehorn calves provided no benefits, as it did not reduce plasma cortisol and substance P compared to other treatment groups. The second follow up study compared cortisol and adrenocorticotrophic hormone (ACTH) levels in calves dehorned immediately compared to calves dehorned 10 minutes after the administration of lidocaine alone, calves dehorned without lidocaine, and calves administered lidocaine but not dehorned. Waiting 10 minutes to dehorn calves after lidocaine administration may provide some benefits in reducing plasma ACTH and cortisol. Calves dehorned after a 10-minute waiting period had lower peak plasma ACTH levels and reached pretreatment levels for both plasma ACTH and cortisol quicker than the other treatment groups that were dehorned. However, differences observed in ACTH dissipate in 5 minutes and all calves reached baseline plasma cortisol levels within an hour of dehorning.

## **CHAPTER I INTRODUCTION**

Consumer interest in the welfare of livestock is at an all time high; especially pain associated with routine animal husbandry procedures such as dehorning (Croney and Anthony, 2011). The main purpose of dehorning is to increase the safety of handlers and other animals (Stafford and Mellor, 2011). While there are also economic benefits to dehorning, the procedure is considered to be painful (AVMA, 2014b).

In the United States, approximately four million calves are dehorned every year (Fraccaro et al., 2013). The number of beef calves born with horns in the United States has decreased to 12.4% in 2007, therefore the majority of calves being dehorned are dairy calves (Theurer et al., 2012). In 2007, it was reported 94% of dairy operations in the US still dehorned calves (Theurer et al., 2012). In a survey of 292 dairies 77% dehorned their calves using a hot iron, 18% removed the horns physically, and 5% used caustic paste (Bergman et al., 2014). That same survey also reported only 23% used a local anesthetic, non-steroidal anti-inflammatory drug (NSAID), or sedation at the time of dehorning (Bergman et al., 2014). However, in a survey of North-Central and North-Eastern United States dairies, only 12.4% of dairy owners reported using local anesthetics and only 1.8% used systemic analgesics at the time of dehorning (Coetzee et al., 2012).

The American Veterinary Medical Association (AVMA) (2014) has stated the importance of minimizing the pain associated with dehorning in order to limit the amount of distress and changes in the animal's behavior and physiological states (AVMA, 2014b). Research has shown the use of local anesthetics combined with an NSAID can diminish the onset of pain associated with dehorning (Heinrich et al., 2009; Stewart et al., 2009; Coetzee, 2011). Currently, there are no FDA approved drugs for pain relief during dehorning in cattle.



However, drugs can be used to provide pain relief, through extra label drug use (ELDU). The Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) stipulates ELDU is permitted under the supervision of a veterinarian when “the health of an animal is threatened, or suffering or death may result from failure to treat” and provided certain conditions are met (US FDA, 1994). The five conditions that have to be met are: (1) ELDU is allowed only by or under the supervision of a veterinarian, (2) ELDU is allowed only for FDA-approved animal and human drugs, (3) ELDU is only permitted when the health of the animal is threatened and not for production, (4) ELDU in feed is prohibited, and (5) ELDU is not permitted if it results in a violative drug residue in food intended for human consumption (US FDA, 1994).

Various methods have been used to measure the pain associated with dehorning. While there are many recognized indicators of pain, currently there are no validated assessments for scoring pain in cattle (Muir and Woolf, 2001). Physiological changes that can be measured in the blood include: plasma cortisol concentrations (Duffield, 2008), plasma Substance P (SP) concentrations (Coetzee et al., 2012), and adrenocorticotrophic hormone (ACTH) (Graf and Senn, 1999). Other measures that could be used to evaluate pain include: heart rate and respiratory rates (Heinrich et al., 2009; Stewart et al., 2009), ocular temperature, and behavioral changes such as ear flicks and head rubs (Faulkner and Weary, 2000; Heinrich et al., 2010).

Research has shown local anesthetics, like lidocaine, mitigate the acute pain response associated with dehorning in calves (Stafford and Mellor, 2011). Lidocaine is a class IB antidysrhythmic agent, and acts by blocking the sodium channels in nerves, preventing action potentials from being generated (Coetzee et al., 2012). Research has also shown lidocaine’s blocking effects last for ~90 minutes after injection. However, few studies have evaluated how quickly lidocaine’s effects take place. Most studies evaluating lidocaine’s effects on reducing the

amount of pain associated with dehorning waited 10-15 minutes after administering lidocaine to dehorn, thus ensuring lidocaine had taken effect (Heinrich et al., 2010; Stilwell et al., 2012; Glynn et al., 2013). A 2012 study by Fierheller and others looked at the onset, duration, and efficacy of four different methods of lidocaine administration to the area surrounding the horn buds. It was observed that lidocaine administered via cornual nerve block had an onset time of 30 seconds to 5 minutes (Fierheller et al., 2012). However, the calves were not dehorned in the study.

The purpose of the following studies was to determine the effect of timing of lidocaine administration on physiological responses in calves after dehorning. The objective of the first study was to determine if there was a difference in cortisol and substance-p levels over time in calves dehorned immediately after administration of lidocaine and meloxicam compared to calves dehorned 10 minutes after the administration of lidocaine and meloxicam. The follow up study's objective was to determine if there was a difference in cortisol and adrenocorticotrophic hormone levels over time in calves dehorned immediately after administration of lidocaine compared to calves dehorned 10 minutes after the administration of lidocaine and calves dehorned without the administration of lidocaine.

## **CHAPTER II REVIEW OF THE LITERATURE**

### **Animal Welfare**

In the United States, the public scrutiny of animal agriculture is higher than it has been historically (Croney and Anthony, 2011). Consumer concerns about livestock production range from the environmental impact and sustainability of industry practices, to food safety and security, and also farm animal welfare (Croney and Anthony, 2011). Since only 0.96% of the US population is vegan and 2.64% is vegetarian, over 96% of the US population is a consumer of meat (Prickett et al., 2010). The concern for the welfare of livestock is intensified further by having more easily accessible information available about animal welfare, debates over housing of livestock, and undercover videos showing animal abuse (Croney and Anthony, 2010; Croney et al., 2012). Research has shown consumers use animal welfare to gauge product characteristics, like safety and healthfulness (Harper and Makatouni, 2002). In a survey of 1,019 US households, 78% of respondents either agreed or strongly agreed “animals raised under higher standards of care will produce safer and better tasting meat” (Prickett et al., 2010).

Animal welfare is not necessarily a new term, however. Brambell made one of the first attempts to define animal welfare in 1965. His proposed definition of animal welfare stated, “welfare is a wide term that embraces the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the feelings of the animals that can be derived from the structure and functions and also from their behavior” (Brambell, 1974). In 1988, Broom defined animal welfare as “the welfare of the individual is its state as regards to its attempts to cope with its environment” (Broom, 1988). Since then, a myriad of definitions have followed. This is due to people having many different “moral philosophies and beliefs pertaining to animals and to the environment in general” (Winter et al., 1998).

Even with people's varied and diverse moral philosophies and beliefs regarding animal welfare, concerns about animal welfare focus on several broad questions. Three of those broad questions are: (1) is the animal functioning well, (2) is the animal feeling well, and (3) is the animal able to live a reasonably natural life (Fraser et al., 1997). Organizations typically use these three questions to outline their particular definition of animal welfare (von Keyserlingk et al., 2009). These three questions, it should be noted, may overlap each other. Consider lameness in a dairy cow (von Keyserlingk et al., 2009). Lameness causes the cow to be in pain, which speaks to the affective state of the animal or how the animal is feeling (von Keyserlingk et al., 2009). Lameness also negatively impacts milk production and reproduction, which speaks to the biological functioning of an animal (von Keyserlingk et al., 2009). Lastly, lameness causes reduced motility, which speaks to the animal's natural behavior (von Keyserlingk et al., 2009).

One concept of animal welfare has become the foundation of many welfare initiatives: The Five Freedoms. In 1965, the Brambell Committee outlined the Five Freedoms in their investigative report on the welfare of intensely housed livestock in the UK (Brambell, 1965; Croney and Anthony, 2011). The report stated:

“In principle, we disapprove of a degree of confinement of an animal which necessarily frustrates most of the major activities which make up its natural behavior and we do not consider such confinement or restraint permissible over a long period unless the other advantages conferred on the animal are likely to be very substantial. An animal should at least have sufficient freedom of movement to be able to without difficulty, to turn round, groom itself, get up, lie down, and stretch its limbs” (Brambell, 1965).

Thorpe (1965) focused on the topics of pain, discomfort, and stress as well as fright, anxiety, frustration, and apprehension in an appendix to the Brambell report (Brambell, 1965). Thorpe's

appendix, combined with the last line of the statement in the Brambell report, provides the framework for the Five Freedoms. The first version of the Five Freedoms stated animals should have:

- (1) freedom from thirst, hunger, and malnutrition;
- (2) freedom from discomfort;
- (3) freedom from pain, injury, and disease;
- (4) freedom to express normal behavior;
- (5) freedom from fear and distress (Brambell, 1965)

In 1992, the Farm Animal Welfare Council (UK) revised the Five Freedoms to include how they should be met (Kilgour, 2012). This version of the Five Freedoms states animals should have:

- (1) freedom from thirst and hunger— by ready access to fresh water and a diet to maintain full health and vigor;
- (2) freedom from discomfort— by providing an appropriate environment including shelter and comfortable resting area;
- (3) freedom from pain, injury, and disease— by prevention or rapid diagnosis and treatment;
- (4) freedom to express normal behavior— by providing sufficient space, proper facilities, and company of the animal's own kind;
- (5) freedom from fear and distress— by ensuring conditions and treatment which avoids mental suffering (Farm Animal Welfare Council, 2009).

The revised version of the Five Freedoms is still the cornerstone of the UK Farm Animal Welfare Committee (Kilgour, 2012).

Those involved in defining an acceptable quality of life for livestock based on the Five Freedoms must be aware that consumers might have different opinions on these definitions (Croney and Anthony, 2011). To a large extent both consumers and producers link animal welfare with the following: physical health, adequate amounts of food and drinking water, and sufficient heating and protection (Vanhonacker et al., 2008). Consumers also tend to be concerned about an animal's freedom to move and its ability to fulfill natural desires (Vanhonacker et al., 2008). Most consumers believe farmers meet the animal's needs for food, water, and shelter (Croney and Anthony, 2011). They are increasingly concerned that 'industrialized' livestock production does not give enough attention to the animal's quality of life (Croney and Anthony, 2011). A survey conducted in 2007 of 1,019 U.S. households found people in the US believe those animals raised on a "small" farm have better lives than animals raised on both "large" (57%) and "corporate" (69%) farms (Prickett et al., 2010).

The same survey of US households also reported 45% of the people in the US believe "scientific measures of animal well-being should be used to determine how farm animals are treated" and 39% believed that moral and ethical considerations should be used when determining animal welfare (Prickett et al., 2010). This survey also reported 52% of respondents agreed with the statement "decisions about animal welfare should be left to experts, and should not be based on public opinion", and 40% indicated decisions should have public opinion included (Prickett et al., 2010).

Media sources sometimes depict neglect, mistreatment, or abuse of livestock which heightens consumers' concern about the welfare of farm animals (Croney and Anthony, 2011). When concerns are heightened and there is a failure to address these issues, the public can gain momentum to externally regulate animal agriculture (Rollin, 2004). A 2007 survey of US

households found 68% of people in the US believes “the government should take an active role in promoting farm animal welfare” and 75% stated they “would vote for a law in my state that would require farmers to treat their animals more humanely” (Prickett et al., 2010). A great example of this is California’s Proposition 2 ballot initiative from 2008, which passed with 63.4% of the vote (von Keyserlingk et al., 2009). This enacted California’s Prevention of Farm Animal Cruelty Act. This act prohibits the confinement of veal calves, laying hens, and swine for the majority of everyday in a manner that does not allow them to turn around freely, lie down, stand up, and fully extend their limbs. Opponents of this proposition argued battery cages were developed to promote bird health and egg quality by reducing exposure to feces, therefore promoting good biological functioning (von Keyserlingk et al., 2009). This was not a good argument, because the majority of Californians believed hens should be allowed enough space to perform natural behaviors (von Keyserlingk et al., 2009). Along with legislation passed in California, legislation has also passed in other states in the US. In 2009, Ohio voters passed Issue 2, which created the Livestock Care Standards Board to provide oversight of farm animal care practices (Croney and Anthony, 2011). Also, the majority of animal welfare legislation in the European Union (EU) and other countries around the world has focused on painful procedures and the ability of animals to have a larger degree of behavior expression (von Keyserlingk et al., 2009).

### **Legislation on Painful Procedures**

European laws and regulations concerning animal welfare are unique. The Council of Europe (CE) can specify minimum requirements for animal welfare that all of its member states have to follow. The European Union (EU) can also require member states to meet its minimum

requirements for animal welfare, and individual countries themselves can also create laws that either meet the minimum requirements or are even stricter.

The CE currently has 47 member states, some of which are not members of the EU. In the 1960's the CE started working on conventions covering animal welfare, which are binding on member states and non-member states who ratify them (Veissier et al., 2008). This was due to the belief "respect for animals was a common heritage of European countries closely linked to human dignity, and that harmonization between countries was necessary" (Veissier et al., 2008). The CE put together a group of experts on animal welfare, and it is this group who came up with the five European conventions on animal welfare (Veissier et al., 2008). The committee then went on to create recommendations for each species of livestock in subsequent years (Veissier et al., 2008). Conventions outline general principles, and recommendations are based on current scientific and technical knowledge (Veissier et al., 2008). In 1976 the CE adopted the Convention for the Protection of Animals kept for Farming Purposes which went into effect in 1978 (Council of Europe, 1976), and later amended in 1992 (Council of Europe, 1992). The recommendations for cattle were published in 1988 (Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes, 1988). It prohibits dehorning by any method except for surgical removal, with one exception (Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes, 1988). The exception allows for calves to be disbudded so dehorning can be avoided (Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes, 1988). These procedures must be performed under local or general anesthesia, except in calves less than 4 weeks of age disbudded by hot iron or caustic paste (Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes, 1988).



There is no EU legislation specific to cattle, but the EU did ratify the CE's Convention on the Protection of Animals kept for Farming Purposes and its Recommendations concerning cattle (Department for Environment Food & Rural Affairs, 1954). Therefore the previously stated recommendations for dehorning cattle made by the CE are also observed by the states in the EU as well, even if countries are not a part of the CE.

Some countries have laws that are stricter than the CE's recommendations for dehorning. In Austria if an animal is likely to experience severe pain, such as that caused by dehorning, the procedure must be carried out by a veterinarian after anesthesia has been established and there must also be post-operative treatment of pain as well (Austrian Federal Chancellery, 2004). Austria also prohibits the use of caustic paste or sticks as a method of dehorning (Austrian Federal Chancellery, 2004). In Switzerland dehorning is only allowed after local anesthetics are administered (Alsaad et al., 2014). Sweden also requires local anesthetics be used when dehorning calves of any age (Bengtsson et al., 1996). In the UK, under the Protection of Animals (Anesthetics) Act 1954, cattle cannot be dehorned without the use of an anesthetic except when calves are under one week of age and dehorned with caustic paste and caustic paste is only permitted during the first week of life (Department for Environment Food & Rural Affairs, 1954).

Other countries outside of Europe have also put laws in place to regulate the use of pain relief when dehorning cattle. Australia requires pain relief to be used when cattle are older than six months of age, or if they are older than twelve months of age when they enter the feed yard for the first time (Australian Animal Health Council, 2014). In New Zealand, pain relief must be used when cattle over nine months of age are dehorned (New Zealand National Welfare Advisory Committee, 2005). However in New Zealand, the recommended best practice is to

administer pain relief no matter the age of the animal at dehorning and that horns should be prevented from developing, or be removed, at a young age (New Zealand National Welfare Advisory Committee, 2005).

In the US, there is currently no legislation regarding the use of pain relief when dehorning cattle. Because there are no FDA approved drugs for pain relief in cattle, use of drugs to provide pain relief is considered to be extra label drug use (ELDU). The Animal Medicinal Drug Use Act of 1994 (AMDUCA) stipulates ELDU is permitted under the supervision of a veterinarian when “the health of an animal is threatened, or suffering or death may result from failure to treat” and provided certain conditions are met (US FDA, 1994). These conditions are: (1) ELDU is allowed only by or under the supervision of a veterinarian, (2) ELDU is allowed only for FDA-approved animal and human drugs, (3) ELDU is only permitted when the health of the animal is threatened and not for production, (4) ELDU in feed is prohibited, and (5) ELDU is not permitted if it results in a violative drug residue in food intended for human consumption (US FDA, 1994).

Although the use of drugs for pain relief during dehorning in the US is considered to be ELDU, researchers have studied different drugs effectiveness in alleviating pain associated with dehorning. This research has been conducted not only to find the most effective way to provide pain relief, but also in hopes of getting a drug approved by the FDA for the use of pain relief in cattle. However, the FDA Center for Veterinary Medicine has stated that to develop effectiveness data for a drug, validated pain assessments must be used for a drug to be labeled for pain relief in the proposed species (Glynn et al., 2013).

## **Dehorning**

Dehorning and disbudding are terms sometimes used interchangeably when referring to the removal of horn tissue. There is, however, a difference. Disbudding is removal of the horn bud prior it's attaching to the skull. Dehorning is the removal of a horn bud after it has attached to the skull. A horn bud is horn tissue that is 5-10 mm in length and is floating in the skin above the skull (Stafford and Mellor, 2005). As the animal ages, the horn bud will attach to the skull and gradually develop a diverticulum that connects with the frontal sinus of the skull (AVMA, 2014b). The age at which this happens varies from breed to breed in cattle and there can also be variation between individuals within breeds, however at approximately 2 months of age the horn bud will attach to the periosteum of the skull (AVMA, 2014b). For example, some beef breeds have horn buds that do not attach to the skull until much later in life compared to the dairy breeds (Stafford and Mellor, 2011).

Dehorning and disbudding are routine animal husbandry procedure used on beef and dairy farms around the world. According to the National Agricultural Statistics Service (NASS), dehorning is one of the most prevalent animal husbandry procedures on farms in the United States (US), with approximately four million calves dehorned every year (Fraccaro et al., 2013). On New Zealand and United Kingdom (UK) farms, more than one million calves are dehorned annually (Stafford and Mellor, 2005; Alsaad et al., 2014), and on Swiss farms, approximately two hundred and fifty thousand calves under three weeks of age are dehorned every year (Becker et al., 2012).

## **Reasons for Dehorning**

Safety and economics are the primary reasons producers dehorn their cattle. Safety is the foremost of the two reasons for dehorning. Horned cattle pose serious injury risks to stockmen

during many of the routine animal management practices, which include milking, hoof trimming, calving, moving cattle, and even processing, on beef and dairy farms. In a survey of 438 Finnish dairy producers, 70% indicated horned cattle have caused dangerous situations for handlers on their farms (Hokkanen et al., 2015). Dehorning also decreases the risk of injury to other cattle in the herd during aggressive interactions like competition at the feed bunk (AVMA, 2014b). There is also a decreased risk of injury to animals commonly used to work cattle, like horses and dogs.

There are monetary incentives at all stages of cattle production to dehorn calves. For example, increased amounts of space for cattle with horns are needed. During transportation, there has to be 5% more space allowed per head when there are animals in the herd with horns (Grandin, 1992). Horns are also costly to producers at the sale barn and at harvest. When horned cattle are sold at the sale barn, they are less valuable than cattle without horns (Stock et al., 2013). In Saskatchewan and British Columbia, there are horn levies of \$2 and \$10 respectively charged to producers when they are selling cattle that have horns at the sale barn (Stock et al., 2013). Processors in Canada lost on average \$0.06 per head in 2011, or \$192,535 in total, due to the extra labor costs associated with removing horns in both fed and non-fed cattle (Stock et al., 2013). Herds of cattle that have horns will have double the amount of carcass bruising compared to herds of hornless cattle (Millman, 2013). Bruising negatively impacts a carcass' value because the bruised portions will have to be trimmed away and will become waste.

### **Methods of Dehorning**

Producers may use multiple methods to dehorn calves. For calves whose horn buds have yet to attach, a hot iron and caustic paste are the most common methods used by producers. In a survey of 639 dairy farmers in Italy, 90.6% of respondents used a hot iron and the remaining 9% used caustic paste (Gottardo et al., 2011). A survey of Canadian producers reported 88.7% of the

respondents used a hot iron and only 6.1% used caustic paste (Vasseur et al., 2010). In the US, 69.1% of dairy heifer calves are dehorned using a hot iron at an average age of 7.6 weeks (USDA, 2009).

Hot iron dehorning works by applying a concave tip of an iron, heated to over 600°C, to the base of the horn bud for approximately 10 seconds. This causes the destruction of the horn bud and the horn generative tissue (Weaver et al., 2005). Hot iron dehorning is performed on calves when they are less than 8 weeks of age. This ensures the horn buds are between 5-10 mm of length and have yet to attach to the skull (Stafford and Mellor, 2005). When the horn buds grow longer than 5-10 mm, they are too big to fit in the concave tip of the iron and the destruction of the horn generative tissue is not ensured.

Caustic paste works through its active ingredients that chemically burn both the horn buds and the horn generative tissue. The active ingredients contained within caustic paste are calcium hydroxide and sodium hydroxide (Stafford and Mellor, 2011). These chemicals will continue to burn the tissues as long as they are present (Stafford and Mellor, 2011), and the paste will not dry out for about 24 hours (Villarroel, 2015). This leads to some safety concerns that producers should address when using caustic paste on their calves. If the calves are exposed to a water source like rain or sprinklers, the paste can run and spread to surrounding areas, like the eyes, causing unwanted damage. When calves have the paste applied and are being grouped with other calves, the other calves could try and lick the paste off which would cause themselves harm. If the calves are nursing, the paste could spread to the udder and cause harm to the cow. These concerns are why it is recommended when dehorning with caustic paste, calves must be kept dry and separate from dams and other calves for at least 24 hours (Villarroel, 2015). It is also recommended producers use caustic paste to dehorn their calves before they are over 2 days

of age (Villarroel, 2015). This is due to calves learning to scratch their head by rubbing it against something and learning to stand on three legs and scratch their head with the fourth once they are over 2 days old (Villarroel, 2015). Both of these actions have the potential to cause the paste to spread and cause unwanted damage.

For older calves, horns can be removed physically, and in the US this accounts for 44.5% of dehornings (USDA, 2009). If the horn buds are too big to fit into a hot iron, they can still be removed physically by making a cut at the base of the bud through the skin and removing both the buds and the horn generative tissue. If the horn bud has attached to the skull, then the cut still is made at the base of the horn, but the cut will go through bone as well as skin. There are several types of dehorning tools used to physically remove horns: Barnes type, scoop, and wire. Barnes type and scoop dehorning tools typically are used on calves that have horn buds too big to use the hot iron but have not attached to the skull. If they are used correctly, both the horn buds and the horn generative tissue will be removed. However, if not all the horn generative tissue is removed, the calf can still grow horns. Dehorning with wire is typically done on much older animals that have significant horn growth and it is necessary to cut through a significant amount of bone. Because physically dehorning calves involves cutting through tissue, hemorrhaging can occur. Therefore, to prevent significant blood loss, wounds from mechanical dehorning are also typically cauterized (Sylvester et al., 1998).

### **Pain Associated with Procedures**

Dehorning is considered by the American Veterinary Medical Association (AVMA) as a painful procedure (AVMA, 2014b; a). Research indicates heat cauterization of the wound will cause a reduction in plasma cortisol for 24 hours following dehorning (Stafford and Mellor, 2011). It is believed cauterization destroys nociceptors in the wound, thus keeping the nociceptor

impulse input below the pain threshold (Stafford and Mellor, 2011). The pain threshold is the point where the extent of the tissue damage or insult is perceived by the animal (Short, 1998).

Generally, it is recommended calves be dehorned less than two months of age for several reasons. First, early dehorning avoids having the horn buds attaching to the skull (AVMA, 2014b). Second, with less tissue to remove in younger animals, the exposed wounds and risk of infection is reduced compared to an older animal (Hulbert and Moisa, 2016). Last, it is also thought, due to the absence of behaviors indicative of pain, neonatal animals feel little or no pain when they are young (Anil et al., 2005). It has been claimed pain is transmitted through mature myelinated nerves rather than immature unmyelinated nerves, like those found in newborn humans, who are believed to be unable to feel pain (Lee, 2002). Assuming a calf's nervous system functions similar to an infant human's nervous system, a calf would not experience much, if any, pain from dehorning. However, it has also been stated neonatal animals and young animals could experience pain more acutely than older animals (Mellor and Gregory, 2003). This is due to a report that the spinal cord sensory nerve cells in both humans and rats are more reactive in neonates than older individuals (Fitzgerald et al., 1988; Fitzgerald et al., 1989; Fitzgerald, 1991; 1995). Also, histological examinations of the horn bud and the area adjacent to the horn bud in calves from birth until 4 months of age did not show any apparent differences in the density of cutaneous innervation (Graf and Senn, 1999).

Research indicates following an acute stressor, a hypothalamic-pituitary-adrenal axis (HPA) that is functioning will display a short duration but high intensity increase in circulating glucocorticoids (GC), such as cortisol (Moberg and Mench, 2000). It has been shown after a known stressor, like castration, older calves will have a greater peak in cortisol and lose more weight than younger calves (Stafford and Mellor, 2011). This would make it logical to conclude

castration would be more painful in older animals than younger ones. Since young calves still have short duration and high intensity increase in cortisol after castration, it still may indicate the HPA axis is well developed and their pain level could be comparable to that of older calves since there is a smaller wound (Moberg and Mench, 2000).

## **Pain**

Pain is difficult to define because it is subjective to the person's perception of pain at the time they are defining it. The simplest definition for pain is if an animal is given analgesics, a form of pain relief, and the animal responds positively then the animal was indeed in pain (Gibson and Paterson, 1985). The International Association for the Study of Pain (IASP) defined pain in 1979 as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (International Association for the Study of Pain, 1979). These two definitions, while both are simplistic in nature, are very different from each other. The first definition makes it so the only time a person could conclude an animal is in pain is if they have positive response to pain relief after the administration of analgesics. The second definition by the IASP considers things like the emotional experiences, as well as that an animal could be in pain even if there is not any damage to tissue. Molony put forward a longer definition in 1997 which states "pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal's physiology and behavior to reduce or avoid the damage, [and] to reduce likelihood of reoccurrence to promote recovery" (Vinuela-Fernandez et al., 2007). This definition takes into account the measures scientists use to assess pain indirectly, while also accounting for the factors of emotional experiences and potential damage like the IASP definition.

Pain itself only can be assessed through indirect means. A major barrier to evaluating pain



in animals is we cannot talk to the animal and ask them to rank their pain on a level of 1 to 10, like is done as standard procedure in evaluating pain in humans.

A person's perception of pain not only impacts their definition of pain, but also their estimation of how painful something may be. Veterinarians are crucial when it comes to providing pain relief to animals, both in the US and abroad. However, they all do not perceive the animal's level of pain in the same way. Their estimation of pain levels varies from veterinarian to veterinarian, much like the definition of pain varies from person to person. Veterinarians' estimation of pain levels has been found to differ with gender, nationality, and even religious beliefs (Livingston, 2002). Several studies have shown female veterinarians will rate pain levels of animals greater than their male counterparts (Wikman et al., 2013). There are also differences in estimation of pain levels between younger and older veterinarians. Not only do younger veterinarians rate pain higher than their older colleagues, they also treat pain more often as well (Wikman et al., 2013). The differences in the estimation of pain levels are not just limited to veterinarians. This occurs in human medicine as well. Students in medical school will rate chronic conditions as more painful than older doctors (Wikman et al., 2013). Also, as medical students progress through school their empathy levels decline over time (Wikman et al., 2013).

With veterinarians and human medical professionals unable to agree on how painful a condition or procedure may be, it is expected producers also would have varying estimations of how painful dehorning may be. This is true not only of US producers, but also producers around the world. A 2006 survey of US dairy producers revealed 40% of the respondents believe disbudding causes moderate pain and 9.7% believe it causes severe pain (Hoe and Ruegg, 2006). However, in that same survey 43% believe disbudding causes little to no pain at all (Hoe and

Ruegg, 2006). In other countries, most of the producers agree dehorning does cause pain, but there is disagreement regarding the amount of pain caused by dehorning and how long the pain lasts. In a survey conducted by Hokkanen et al. (2015) in 2010 of 438 dairy producers in Finland, only 5% of respondents estimate disbudding without pain medication causes only mild pain, 25% believe it causes moderate pain, and 70% believe it causes severe pain (Hokkanen et al., 2015). Finnish producers who always had a veterinarian use pain medication when dehorning their calves also believe the pain from dehorning is more severe than those producers who sometimes used a veterinarian or never used a veterinarian when dehorning their calves (Hokkanen et al., 2015). Also, 33% of the Finnish dairy farmers believe the pain caused by dehorning could last for at least 3 days (Hokkanen et al., 2015). In contrast to the Finnish producers, 48% of Italian producers believe the pain caused by dehorning only lasts a few minutes, while 43% believe it lasts less than 6 hours (Gottardo et al., 2011). These surveys highlight how hard it is to identify, understand, and even rate the severity of something as complex as pain.

### **How Pain Works**

There are two different types of pain, acute and chronic. Acute pain, or physiological pain, is well localized, temporary, and is critical to normal defense mechanisms (Muir and Woolf, 2001). It occurs when there is nominal or no tissue damage. Chronic pain, or clinical pain, occurs when excessively intense or prolonged stimuli cause tissue damage resulting in extended discomfort and abnormal sensitivity (Muir and Woolf, 2001). Chronic pain can be the result of inflammation associated with tissue damage, central or peripheral nerve injury, and even normally innocuous stimuli (Muir and Woolf, 2001).

Acute and chronic pain are seen with dehorning. The acute pain response is seen at the time of dehorning. Therefore the acute pain response to dehorning is the result of damaging the

horn tissue and the ensuing chemical changes to the damaged tissue. The chronic pain response seen with dehorning is typically lower in intensity than the acute pain and is a result of inflammation (Muir and Woolf, 2001).

Pain begins with nociception. Nociception is a multi-step process that starts with the detection of noxious stimuli (mechanical, chemical, or thermal) by nociceptors (Hudspith, 2016; Steeds, 2016). Nociceptors are receptors located in the free nerve endings of nerve fibers and activated specifically by pain (Steeds, 2016). The nociceptors then convert the noxious stimuli into an electrophysiological neural signal, also known as transduction, and transmit it from the periphery to the central nervous system nociceptors (Hudspith, 2016; Steeds, 2016). Therefore nociception is a neural process involving the detection, transduction, and transmission of a noxious stimulus to the brain (Steeds, 2016). Pain, however, is the perception of the noxious stimulus (Muir and Woolf, 2001).

There are two types of nociceptors involved with the transduction and transmission of pain, A $\delta$  fibre and C-fibre nociceptors. A $\delta$  fibre nociceptors are finely myelinated and can be categorized by their stimulation threshold (Hudspith, 2016). Type I high-threshold mechanoreceptors (HTMR) are activated by heat ( $>50^{\circ}\text{C}$ ) and noxious mechanical stimuli (Hudspith, 2016). Type I HTMRs will become more sensitive with injury and are also responsible for the first sensation of pain to a mechanical stimulus (Hudspith, 2016). Type II HTMRs have significantly lower thermal thresholds ( $45^{\circ}\text{C}$ ) but notably higher mechanical thresholds. Type II HTMRs are responsible for the first sensations of pain in response to heat (Hudspith, 2016).

The C-fibre nociceptors mediate the dull aching sensation associated with acute pain (1). The C-fibre nociceptors are unmyelinated and polymodal (Hudspith, 2016; Steeds, 2016). They

are responsive to an array of stimuli that includes: noxious thermal stimuli ( $>45^{\circ}\text{C}$ ), noxious mechanical stimuli, and noxious chemical stimuli (Hudspith, 2016).

Tissue damage and inflammation can also lead to a reduction in the activation threshold of the nociceptors. This is due to A $\delta$  fibre and C-fibre nociceptor activation thresholds also being dependent upon their extracellular environments (Muir and Woolf, 2001; Hudspith, 2016).

Damaged cells will release  $\text{H}^{+}$ ,  $\text{K}^{+}$ , ATP, and induce enzymes, including proteases, cyclooxygenase-2, and nitric oxide synthase (Muir and Woolf, 2001; Hudspith, 2016). Tissue damage also triggers an inundation of inflammatory cells such as macrophages, neutrophils, lymphocytes, and mast cells (Muir and Woolf, 2001; Hudspith, 2016). The inflammatory cells release chemical mediators, which includes: bradykinin,  $\text{H}^{+}$ , ATP, purines, prostaglandins, leukotrienes, cytokines, and growth factors (Muir and Woolf, 2001; Hudspith, 2016). The damaged cell contents, inflammatory cells, and chemical mediators not only activate the nociceptors, but may also work collectively to reduce the activation threshold of the HTMRs to noxious stimuli (Muir and Woolf, 2001; Hudspith, 2016).

The activation threshold of the A $\delta$  fibre and C-fibre nociceptors can also be lowered through the activation of the sympathetic division of the autonomic nervous system (ANS) (Seth and de Gray, 2016). A noxious stimulus, like tissue damage, will activate the sympathetic nervous system (SNS). This results in epinephrine and norepinephrine being released as part of the ‘fight or flight’ response. As a result, the activation threshold of peripheral nerves will lessen and they become more sensitive to stimulation (Seth and de Gray, 2016).

Once the activation threshold of the nociceptors has been reached, the nociceptors are ‘activated’. The nociceptors will then transduce, or encode, the duration, intensity, location, and

quality of the noxious stimulus into an electrophysiological neural signal in the form of an action potential (Muir and Woolf, 2001; Hudspith, 2016).

The action potentials will travel along the afferent axons to the dorsal horn of the spinal cord (Vinuela-Fernandez et al., 2007). The dorsal horn of the spinal cord has a laminar structure and where nociceptors terminate is dependent upon their structural and pharmacological characteristics (Hudspith, 2016).

Input from the A $\delta$  and C fibres causes glutamate to be released (Muir and Woolf, 2001). Glutamate will then bind to  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainite, and Nmethyl-D-aspartate (NMDA) ligand-gated sodium and calcium channels (Muir and Woolf, 2001). AMPA receptor ion channel activation causes depolarization of the membrane, which produces fast excitatory postsynaptic potentials focused by local and descending inhibitory neurons (Muir and Woolf, 2001). These fast excitatory postsynaptic potentials are carried predominately by the A $\delta$  sensory nerve fibers (Muir and Woolf, 2001). They signal the onset, duration, and location of the noxious stimulus (Muir and Woolf, 2001). The fast excitatory postsynaptic potentials are also responsible for the well-localized and temporary sharp, stinging, and stabbing pain (Muir and Woolf, 2001). When there is intense thermal or mechanical stimulation, the amount of glutamate released will be increased along with the amount of substance p (SP) released from the afferent sensory nerve terminals (Muir and Woolf, 2001). This increases release of glutamate and SP which facilitates and prolongs the release of intracellular calcium and generates plateau potentials by activation of the voltage-gated calcium channels and non-specific cation channels (Muir and Woolf, 2001). The size of these events is proportionate to the intensity of the noxious stimulus (Muir and Woolf, 2001). It is also responsible for the NMDA receptors being activated, when they are typically blocked by a

magnesium ion (Muir and Woolf, 2001). This generates a prolonged postsynaptic depolarizing response, allowing pain to remain long after the noxious stimulus is removed (Muir and Woolf, 2001).

Somatic sensory information is projected to the reticular formation of the brain stem and surrounding nuclei before converging in the thalamus (Muir and Woolf, 2001). There is not really one part of the brain which processes these projections (Hudspith, 2016). The brain processes these signals in a very synchronized manner (Hudspith, 2016). The ventral posteriorlateral thalamic nucleus output is processed to somatosensory cortex combined with polysynaptic activation of areas of the brain involved in emotion (anterior cingulate and insular cortex) and fear or anxiety (amygdala and limbic structures) (Muir and Woolf, 2001). This provides the perception of pain.

### **Measuring Pain in Cattle**

Pain assessment in calves is difficult. However, pain caused by animal husbandry procedures, like dehorning, causes quantifiable changes. Physiological, neuroendocrine, immune, and behavioral changes are observed in response to painful procedures. Neuroendocrine changes are among the most common of measurements used by researchers. Quantification of these changes is how pain is assessed in calves. One parameter on its own may not be a good indicator of pain, however, when several parameters are combined they may be able to accurately assess pain.

Catecholamines released due to the activation of the SNS play a role in facilitating some of the changes that can be quantified after calves are dehorned. The catecholamines, epinephrine and norepinephrine, promote energy mobilization, blood vessel dilation, increased muscle contractility, alter cardiac output, as well as altering respiratory rate (Stewart et al., 2010).

Catecholamine concentrations have been observed to be increased in response to branding, isolation, simulated transport, and dehorning in cattle (Stewart et al., 2010).

Neuroendocrine changes are quantified through measuring changes in hormone or neurotransmitter concentrations in the blood plasma. Along with affecting heart rate and blood flow, the catecholamines released due to the activation of the SNS also activate the hypothalamic-pituitary-adrenal (HPA) axis (Hadley and Levine, 2007). Catecholamines trigger the release of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) from neurons located in the paraventricular nucleus of the hypothalamus (Hadley and Levine, 2007). CRH and AVP will act synergistically to stimulate the release of adrenalcorticotrophin hormone (ACTH) from the anterior pituitary (Hadley and Levine, 2007). ACTH stimulates the secretion of glucocorticoids from the adrenal cortex (Hadley and Levine, 2007). Cortisol is the primary glucocorticoid released in humans and cattle (Sylvester, 2002). Cortisol's effects are mainly catabolic (Hadley and Levine, 2007). It stimulates gluconeogenesis, inhibits energy storage, and also suppresses several anabolic processes including growth, immune, and inflammation systems (Hadley and Levine, 2007). Activity of the HPA axis is regulated by a negative feedback loop. Cortisol provides negative feedback on the higher brain centers, hypothalamus, and pituitary to decrease the secretion of CRH, ACTH, and ultimately cortisol (Hadley and Levine, 2007).

Traditionally cortisol has been the primary measure of pain, since the cortisol response has a slow onset, is persistent, and easily measured (Stewart et al., 2010). Research has found cortisol is a good indicator of the acute and chronic pain responses associated with dehorning. The acute pain response is marked by a rapid increase in plasma cortisol that peaks within several minutes (Duffield, 2008). After plasma cortisol peaks, it declines to a plateau still above baseline and persists at this level for several hours, which is the chronic pain response to

inflammation (Duffield, 2008). When dehorning is performed with a cornual nerve block, cortisol does not increase indicating cortisol is primarily a pain related response and not a response to another stressor, such as handling (Heinrich et al., 2010).

Unlike cortisol, there has not been a large amount of research that has measured the ACTH response to dehorning. Graff and Senn (1999) observed a large increase in plasma ACTH concentrations that peaked within 5 minutes and remained elevated for 20 minutes following cautery dehorning (Graf and Senn, 1999).

Substance-P (SP) is a neuropeptide that is released from the afferent sensory nerve terminals in the dorsal horn of the spinal cord (Muir and Woolf, 2001). It is involved with regulation of the nociceptive neurons and can be stimulated by a noxious stimulus (Muir and Woolf, 2001). It is a new measure that may be more specific to pain. In a castration study of 10 calves, a significant increase in plasma SP concentrations in castrated calves compared to control calves was observed (Coetzee, 2011). In the same study, cortisol was also found to be not significant between the castrated and control calves (Coetzee, 2011). This suggests SP is only associated with nociception and not other stressors, like handling (Coetzee, 2011). In a dehorning study of 12 calves, SP concentrations were found to be reduced by an estimated 50% in calves that received meloxicam prior to dehorning compared to calves that received a placebo (Coetzee et al., 2012). Increases in SP concentrations in the study conducted by Coetzee et al. (2012) were also found to correspond with lower log plasma meloxicam concentrations (Coetzee et al., 2012).

### **Pain Prevention**

The most common method of reducing the acute pain response in calves to dehorning is the use of local anesthetics. Local anesthetics work by blocking the voltage gated sodium



channels in the nerves, preventing the generation and propagation of action potentials (Coetzee et al., 2012). This makes the peripheral portions of the nociceptive pathways nonfunctional, but does not impact the CNS (Coetzee et al., 2012).

Lidocaine is the most commonly used local anesthetic when calves are dehorned. It can be administered in a variety of ways including cornual nerve block, ring block, topical, and jet administration. The cornual nerve block technique is the one that is most commonly used (Fierheller et al., 2012). This is due to ring blocks requiring multiple injection sites, making it more difficult to perform and time consuming (Fierheller et al., 2012). Also topical and jet administrations are both novel techniques rarely used due to variability in effectiveness (Fierheller et al., 2012). To achieve a cornual nerve block, lidocaine is injected midway between the horn and the lateral canthus of the eye, ventral to the frontal crest (Fierheller et al., 2012). Lidocaine is able to maintain a cornual nerve block for 60-90 minutes (Fierheller et al., 2012). In calves dehorned after they had been administered lidocaine via cornual nerve block, there is a significant reduction in the plasma cortisol concentrations compared to calves dehorned without local anesthetics (Stafford and Mellor, 2011; Sutherland et al., 2013). However, once the lidocaine dissipates, cortisol levels increase to levels similar to calves dehorned without lidocaine (Graf and Senn, 1999; Faulkner and Weary, 2000; Stafford and Mellor, 2011).

Lidocaine also decreases the ACTH response in calves. Graff and Senn (1999) observed plasma ACTH to be significantly higher in calves dehorned without lidocaine versus calves dehorned after the administration of lidocaine (Graf and Senn, 1999). They found plasma ACTH levels to be significantly different from its peak at 5 minutes to 20 minutes post dehorning (Graf and Senn, 1999). Therefore, lidocaine does effectively reduce the acute pain response associated with dehorning, but it does not mitigate any of the chronic pain associated with inflammation.

However, when a non-steroidal anti-inflammatory drug, e.g. meloxicam, is used in combination with lidocaine, plasma cortisol levels were lower in calves for 6 hours post dehorning (Heinrich et al., 2009; Allen et al., 2013). Heart rates and respiratory rates were also found to be lower in calves given meloxicam for ~24 hours after dehorning (Heinrich et al., 2009).

### **Conclusions**

While lidocaine is effective at diminishing the acute pain response to dehorning in calves, there is still a lingering question. Most research has focused on the duration of the cornual nerve block produced by lidocaine and how it affects any of the parameters used to measure pain. There has been very little research evaluating how quick the onset is of a lidocaine cornual nerve block.

Studies that have administered lidocaine via cornual nerve block typically wait at least 10-15 minutes to ensure that it has taken effect (Heinrich et al., 2010; Stilwell et al., 2012; Glynn et al., 2013). Fierheller and others conducted a study in 2012 using 8 bull calves to determine the onset, duration, and efficacy of four different methods of administering lidocaine (Fierheller et al., 2012). Using a peripheral nerve stimulator they found the cornual nerve block to have an onset time range of 30 seconds to 5 minutes (Fierheller et al., 2012). They did not, however, dehorn the calves. Therefore, more research is needed looking at the timing of the administration of a lidocaine cornual nerve block and when the calves are dehorned to assess the onset of the cornual nerve block in a clinical setting.

## **CHAPTER III STUDY I**

### **Materials and Methods**

This study was conducted in December of 2014 and January of 2015 at the LSU Dairy Science Research and Teaching Farm in Baton Rouge, Louisiana. The Louisiana State University Agricultural Center Institutional Animal Care and Use Committee approved all procedures for this experiment.

#### **Animal Husbandry and Housing**

Twenty-four intact Holstein bull calves were obtained from the LSU Dairy Science Research and Teaching Farm. Calves were approximately 12-15 weeks of age at dehorning. Housing consisted of 2 identical one-acre pens. The pens consisted of a concrete pad, 136 ft<sup>2</sup>, covered by a tin roof, and grass paddocks. Calves were randomly assigned to pens. Calves were fed 2.26 kg of calf grower diet (Kentwood Co-Op, Kentwood, LA) per day. The diet consisted of rolled corn, soybean hulls, corn gluten feed, dried distiller's grains, soybean meal, cottonseed meal, molasses, and a vitamin/mineral mix. The diet was 90% dry matter, with 21% crude protein, 81% total digestible nutrients, 5% crude fat, 33% neutral detergent fiber, and 15% acid detergent fiber. Water and grass hay were offered ad libitum via troughs and hay feeders, respectively.

#### **Study Procedure**

The study design was a randomized complete block design with 4 periods. Calves were approximately 12-15 weeks of age at the time of this study commencement and were blocked by weight obtained at 12 weeks of age. Each period, calves were randomly assigned to one of 3 treatments (n=8).

## **Catheterization**

Approximately 4 h prior to dehorning, calves were restrained with a halter for catheter placement and to clip the hair around the horns. The area of the jugular vein was clipped and disinfected using povidone iodine and 70% isopropyl alcohol swabs. Prior to catheter placement, 0.5 mL of 2% lidocaine hydrochloride (Lidocaine HCL 2% (20 mg/ml) VetOne, Boise, ID) was injected subcutaneously over the jugular vein to provide analgesia. Next a 14 gauge, 3.5-inch catheter (MILA International, Inc., Erlanger, KY) was inserted and a high flow catheter extension (MILA International, Inc., Erlanger, KY) attached. Catheters were sutured in place and patency was maintained with 5 mL of heparin saline flush (4 U/mL). Catheters remained patent a total of 28 hours and were removed after the 24 h blood sample was collected.

## **Dehorning**

After randomization, calves were assigned to one of three treatment groups: anesthesia with sham dehorning (CON); anesthesia followed by immediate dehorning (ANET\_0); and anesthesia with a 10 minute delay prior to dehorning (ANET\_10). Approximately 15 minutes before dehorning, calves were restrained with a rope halter. All calves received gelatin capsules containing meloxicam (Meloxicam Tablets USP 15 mg, Unichem Pharmaceuticals USA Inc., Rochelle Park, NJ), an NSAID, at a dose of 1 mg/kg of body weight immediately prior to the administration of lidocaine. Calves were also administered 5 mL of 2% lidocaine hydrochloride (Lidocaine HCL 2% (20 mg/ml) VetOne, Boise, ID) via cornual nerve block as previously described (Stock et al., 2013). Barnes dehorners were used to amputate each horn and electro-cautery was used to control any hemorrhages. Calves were administered local anesthesia and NSAID immediately prior to sham dehorning, dehorning, or the 10 minute delay prior to

dehorning. To maintain consistency the same veterinarian performed all nerve blocks, meloxicam administration, and dehorning.

### **Sample Collection**

Blood samples were collected using the aforementioned catheters and catheter extensions at baseline (approximately 10 min prior to anesthesia administration), immediately prior to anesthesia administration (0 min), and at 10, 20, 30, 40, 50, 60, 90, 120, 240, 360, 480, 720, and 1,440 min after dehorning relative to lidocaine and meloxicam administration. Catheter and catheter extension patency was maintained using 5mL of heparin saline flush before and after blood was drawn and 3mL of blood was discarded before the sample was taken. The samples were collected while the calves were haltered and tied with access to water and hay. Blood was collected in 15 mL aliquots and divided between K<sub>3</sub> EDTA (Monoject, Mansfield, MA) for substance P, and lithium heparin tubes (BD Vacutainer, Franklin Lakes, NJ) for cortisol. Blood collection tubes for substance P contained the protease inhibitor, benzamidine hydrochloride (Benzamidine Hydrochloride Hydrate, Sigma-Aldrich, St. Louis, MO). A 20mM solution of benzamidine was prepared in water and 250  $\mu$ L was added to each tube for a final concentration of 1 mM benzamidine in whole blood to act as a protease inhibitor. All samples were kept on ice prior to centrifugation at 2000g for 20 min. Prior to analysis, plasma for cortisol was harvested and stored at -20°C and plasma for substance P was harvested and stored at -80°C.

### **Sample Analysis**

Plasma cortisol concentrations were determined using radioimmuno assay kits (ImmuChem™ Cortisol Coated Tube RIA Kit, Orangeburg, NY). Analysis of SP was performed by enzyme-linked immunosorbent assay as previously validated (Coetzee et al., 2008).

## **Statistical Analysis**

The GLM procedure of SAS® (Version 9.4, SAS Inst. Inc., Cary, NC) was used to analyze the data as a repeated measures analysis of variance. Response variables included cortisol and substance P. Fixed effects in the model included: treatment, time, and treatment by time interaction. The random effect was calf within treatment. The plasma cortisol concentration measurement was log transformed and baseline corrected to stabilize the distribution variances. The log cortisol results were back-transformed by taking the antilogarithm of least-squares means and presented as geometric means. When overall differences were detected, post hoc analyses were conducted with pairwise t-test comparisons of least-squares means. Values reported are least-squares means. Statistical significance was determined at  $P < 0.05$  and tendencies were declared at  $P < 0.10$ .

## **Results and Discussion**

### **Plasma Cortisol Concentration**

Least squares means plasma cortisol concentrations graphed versus time are shown in Figure 1 and Figure 2. Geometric mean cortisol concentrations after treatment compared against time are shown in Figure 3 and Figure 4.

There was an effect of time on mean cortisol concentrations after dehorning ( $P < 0.0001$ ). Mean plasma cortisol concentrations in CON calves peaked 10 minutes after treatment (10.643 ng/mL), and returned to baseline levels by 30 minutes. In the ANET\_10 calves, mean plasma cortisol concentrations peaked at 20 minutes after treatment (15.757 ng/mL) and returned to baseline by 60 minutes after treatment. The mean concentrations of ANET\_0 rose to a peak at 20 minutes after treatment (10.073 ng/mL), and then decreased back to baseline levels by 50 minutes post treatment. Similar patterns in plasma cortisol were reported by Henirch et al. (2009)

and Allen et al. (2013) when calves were dehorned after being administered lidocaine and meloxicam.

At 20 minutes after treatment, calves of the ANET\_10 treatment group had greater ( $P=0.05$ ) cortisol concentrations than calves in the CON treatment group. At 30 minutes after treatment, cortisol concentrations of calves in the ANET\_10 group tended ( $P=0.0553$ ) to be greater than the cortisol concentration of calves of the CON group. Forty minutes post treatments, plasma cortisol concentrations of ANET\_10 ( $P=0.075$ ) and ANET\_0 ( $P=0.06$ ) both tended to be greater than CON calves. At 120 minutes post treatment cortisol concentrations of calves of the CON treatment group were greater than calves in the ANET\_0 treatment group ( $P=0.0065$ ).

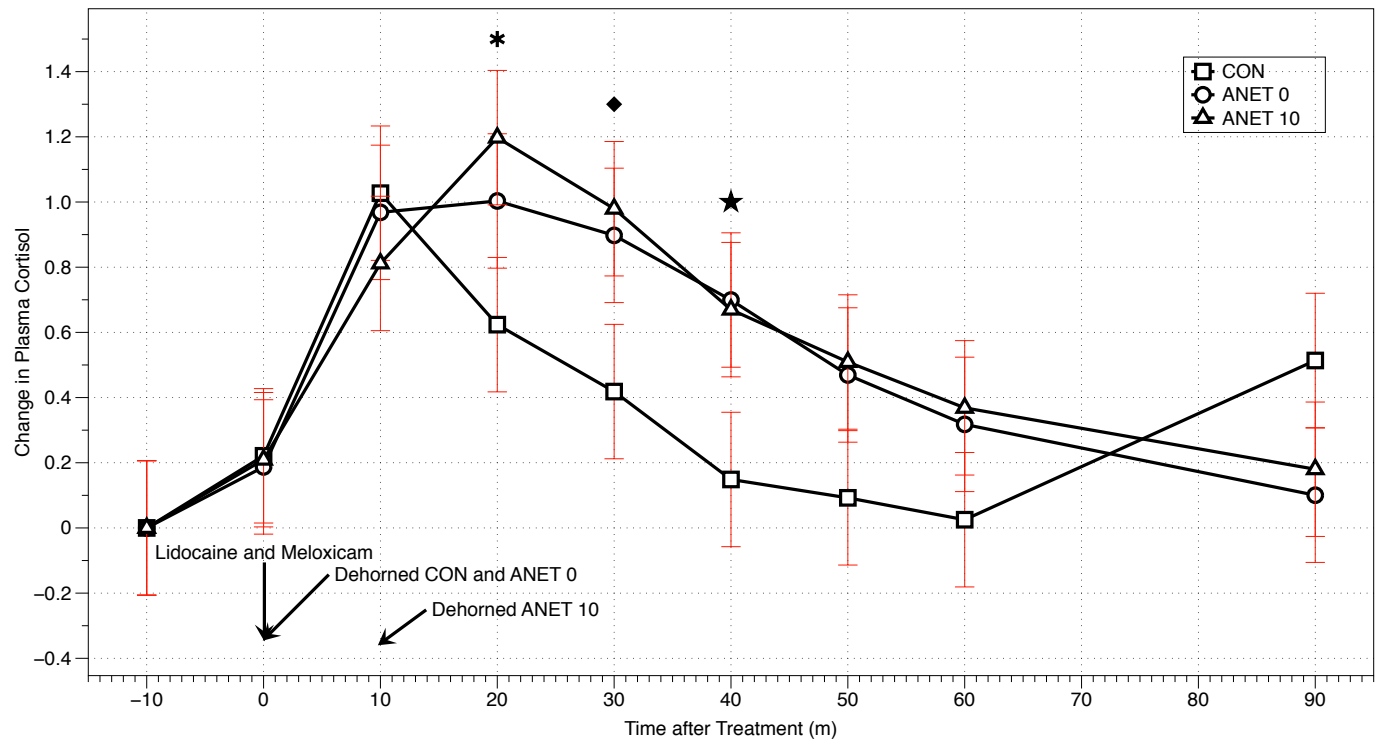
Since administration of lidocaine and meloxicam being designated as time point 0, to evaluate the impact of the treatment on plasma cortisol relative to dehorning, ANET\_10 must be compared against the other treatment groups at different sample times (e.g. 10 m after dehorning for ANET\_10 is the 20 m sample, but for ANET\_0 and CON it is the 10 m sample). When comparing the treatments relative to the time of dehorning, unlike the results above, there are no differences in plasma cortisol levels seen between ANET\_10 calves and CON calves at any point after dehorning. Also, there were no observed differences seen between ANET\_10 and ANET\_0 at any time point.

In both ANET\_0 and ANET\_10, plasma cortisol concentrations were elevated above pretreatment levels for at least 40 minutes post-dehorning. This indicates dehorning caused pain and distress in the calves, even with the administration of lidocaine and meloxicam. The difference in plasma cortisol concentrations observed in CON calves and ANET\_0 calves at 120 minutes may be the result of human error or random chance. The lack of difference observed

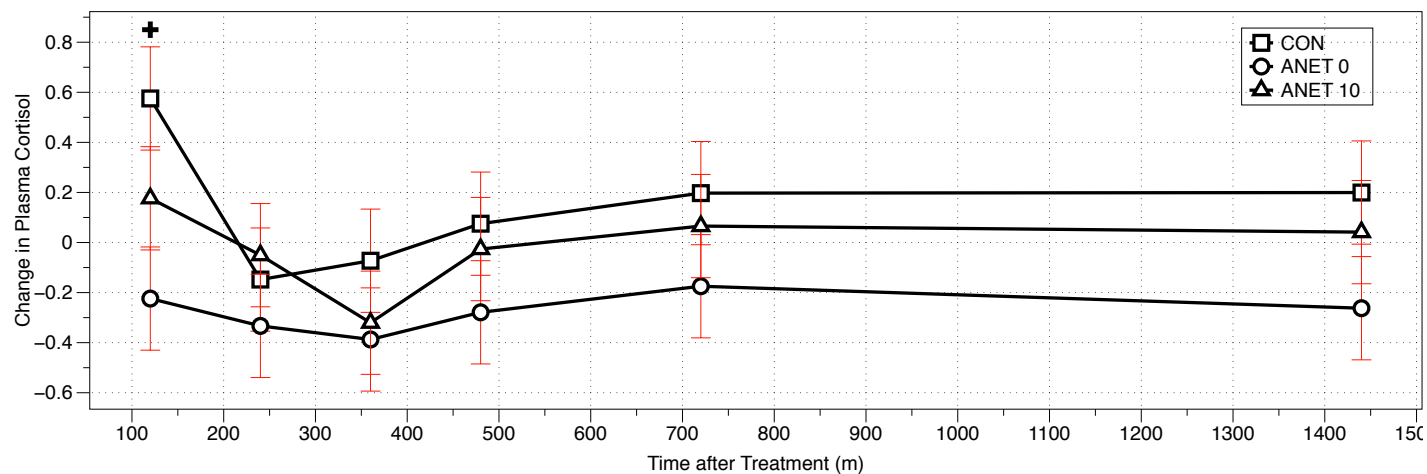
between ANET\_0 and ANET\_10 may be attributed to the use of lidocaine and meloxicam. An increase in plasma cortisol concentrations in calves dehorned with only local anesthesia has been previously reported at 1.5 hours post dehorning (Stafford and Mellor, 2005; Heinrich et al., 2009). However in a study conducted by Allen et al. (2013), there were no observed differences in plasma cortisol concentrations of calves administered meloxicam either 10 minutes or 12 hours prior to dehorning along with the administration of lidocaine 10 minutes prior to dehorning. The results reported by Allen et al. (2013) support the theory that the combined use of lidocaine and meloxicam may have contributed to the similarity in plasma cortisol concentrations in ANET\_0 and ANET\_10.

After the calves were dehorned with barnes dehorner the wounds were cauterized. This may have also attributed to the lack of difference seen in plasma cortisol concentrations between ANET\_0 and ANET\_10 treatment groups. The wounds were cauterized to reduce hemorrhage, but Sylvester et al. (1998) reported cauterization of wounds after dehorning marginally reduced the plasma cortisol response compared to calves dehorned without wound cauterization. When a local anesthetic is used prior to dehorning and the wounds are cauterized after dehorning, it has been found the plasma cortisol concentration will be diminished for 24 hours after dehorning (Sylvester et al., 1998). It is thought the local anesthesia blocks the pain caused by the removal of the horns and cautery, while the cautery destroys enough nociceptors in the wound to keep the nociceptor impulse below a calf's pain threshold after local anesthesia wears off (Stafford and Mellor, 2011).

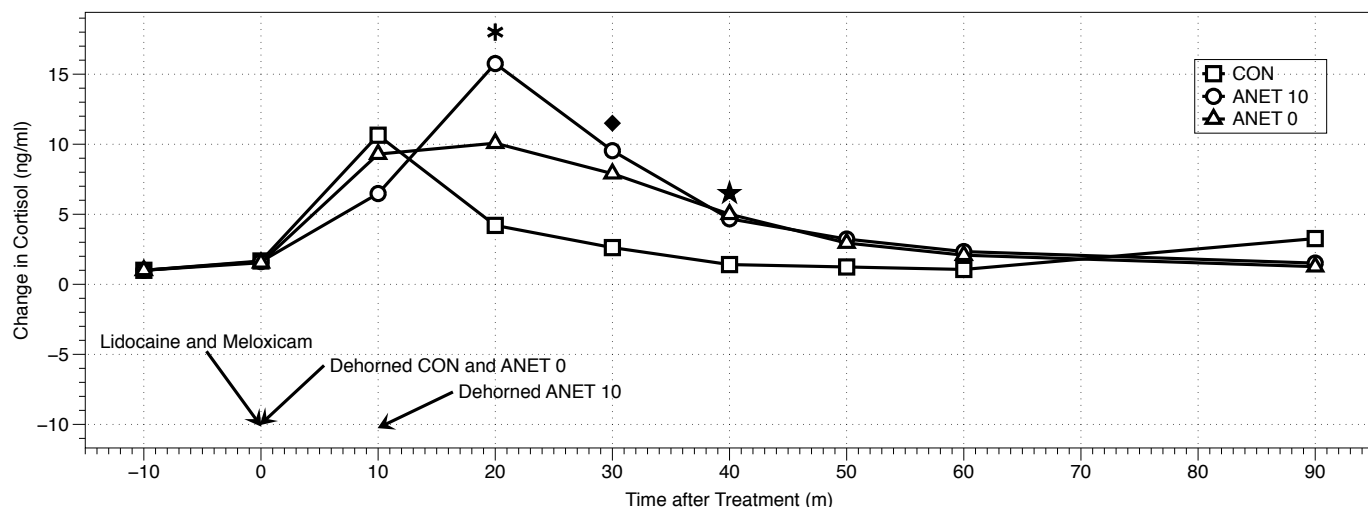




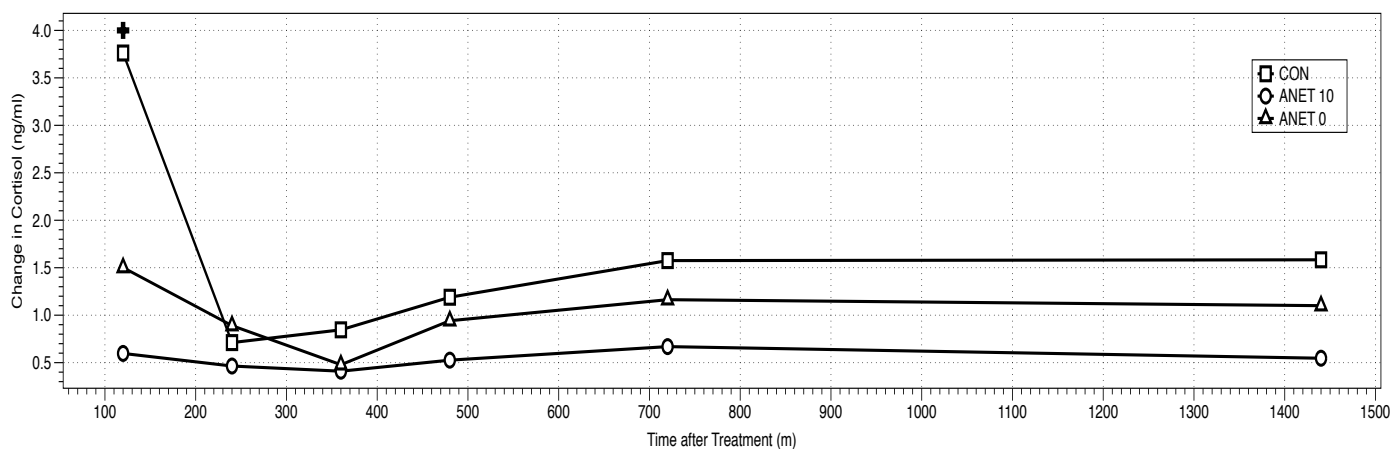
**Figure 1.** Least squares mean  $\pm$  SEM change of plasma cortisol concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). \*: ANET\_10 > CON ( $P < 0.05$ ); ◆: ANET\_10 > CON ( $P < 0.10$ ); ★: ANET\_10 and ANET\_0 > CON ( $P < 0.10$ )



**Figure 2.** Least squares mean  $\pm$  SEM change of plasma cortisol concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). +: CON > ANET\_0 ( $P < 0.01$ )



**Figure 3.** Geometric mean change in plasma cortisol concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). \*: ANET\_10 > CON ( $P < 0.05$ ); ♦: ANET\_10 > CON ( $P < 0.10$ ); ★: ANET\_10 and ANET\_0 > CON ( $P < 0.10$ )



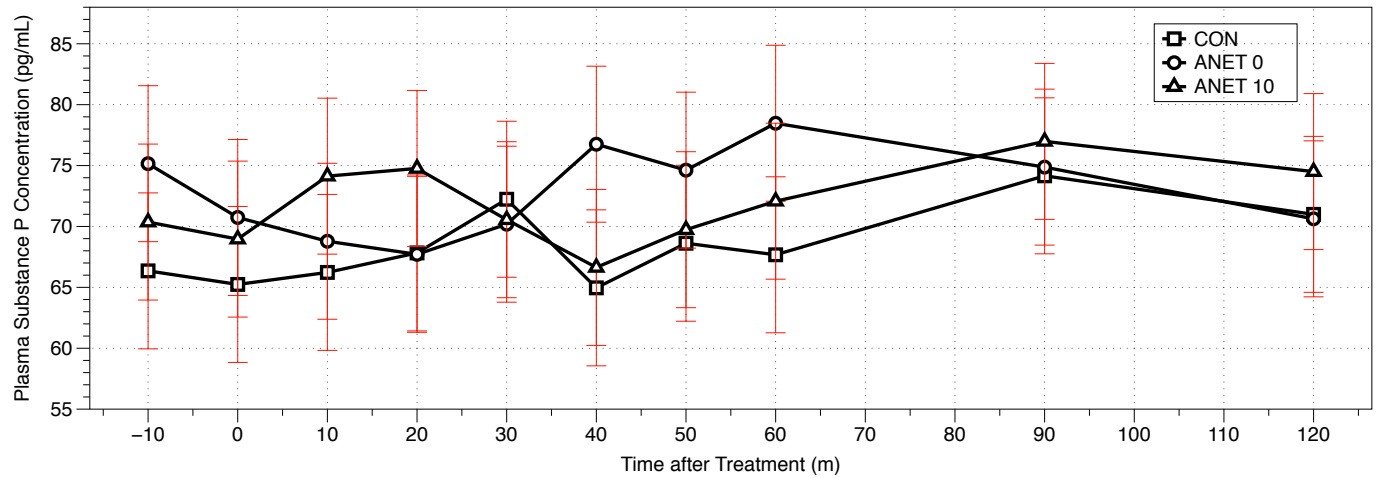
**Figure 4.** Geometric mean change in plasma cortisol concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). +: CON > ANET\_0 ( $P < 0.01$ )

### Substance P Concentration

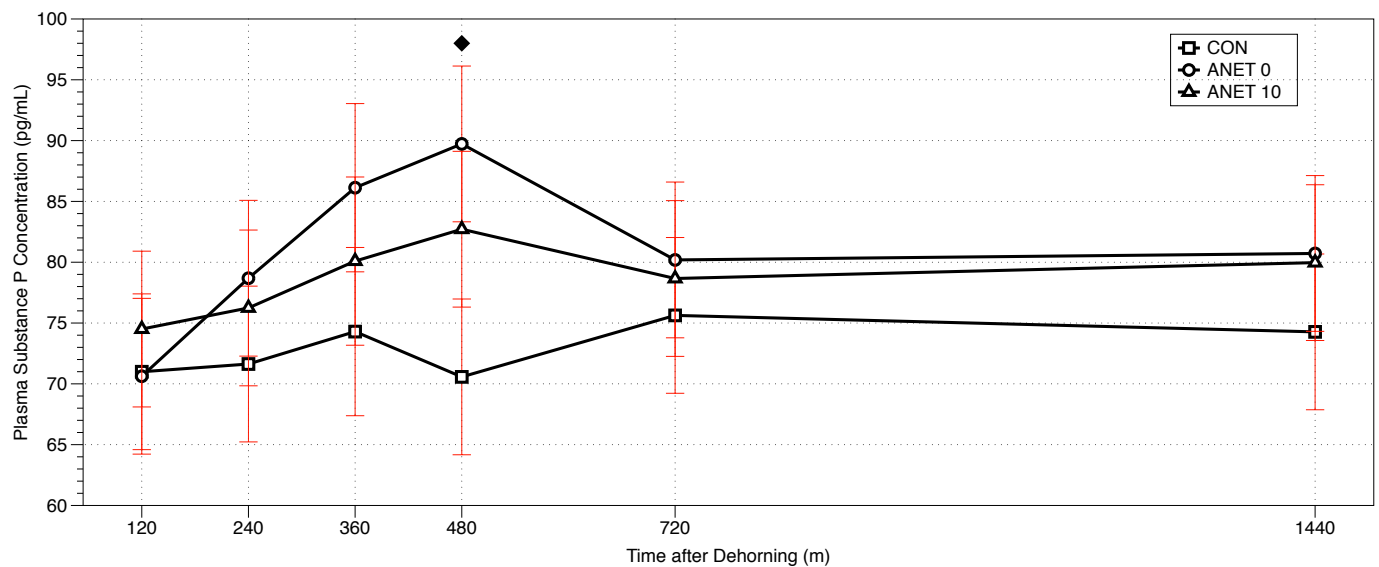
Least squares means of SP concentrations graphed versus time for each treatment are illustrated in Figure 5 and Figure 6.

There was evidence of an effect of treatment on SP concentrations after dehorning ( $P=0.0190$ ). At 480 m post treatment, calves of the ANET\_0 group had greater substance P concentrations than calves in the control group ( $P=0.0297$ ). This difference is likely due to some irregularity due to human error or random chance.

Substance P is a neuropeptide released from the dorsal horn of the spinal cord, is involved with the regulation of nociceptive neurons, and can be stimulated by a noxious stimulus (Muir and Woolf, 2001). Coetzee et al. (2008) reported elevated plasma SP concentrations in castrated calves when compared with calves that were sham castrated, even though the groups had similar plasma cortisol concentrations. In 2012, the same research group reported an intravenous injection of meloxicam significantly reduced plasma SP concentrations in calves after dehorning compared to calves that received a placebo (Coetzee et al., 2012). Allen et al. (2013) also observed no difference between treatment groups in Holstein bull calves given a cornual nerve block in the same manner and either meloxicam 12 h prior, immediately after dehorning, or given a placebo. However, Allen et al. (2013) did observe differences in mean plasma SP concentrations at 120 h post dehorning between calves given analgesia and those not given analgesia ( $p=0.039$ ). Observations reported by Allen et al. (2013) support the theory that the difference at 480 m post treatment between ANET\_0 and CON calves was likely due to some irregularity or random chance. Also, the present study contributes proof to support the hypothesis that meloxicam suppresses plasma SP concentrations after dehorning.



**Figure 5.** Least squares means  $\pm$  SEM plasma Substance P concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). Time of lidocaine administration was designated at time 0.



**Figure 6.** Least Squares Means  $\pm$  SEM plasma Substance P concentrations in Holstein bull calves dehorned (n=8 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), and a control group that were not dehorned (CON). Time of lidocaine administration was designated at time 0. ♦: ANET\_0 > CON (P < 0.05)

## **Conclusions**

Overall the data from this study suggest there are no benefits to waiting 10 minutes after the administration of lidocaine and meloxicam to dehorn calves. Calves dehorned after the 10-minute waiting period had similar plasma cortisol concentrations and SP concentrations compared to calves dehorned immediately after the administration of lidocaine and meloxicam and to calves that were not dehorned but administered lidocaine and meloxicam. This finding is significant because no previous study has reported the effects of the time of lidocaine and meloxicam administration relative to dehorning on these outcomes.

## **CHAPTER IV STUDY II**

### **Materials and Methods**

This study was conducted in October of 2016 through December of 2016 at the LSU AgCenter Southeast Research Station in Franklinton, Louisiana. The Louisiana State University Agricultural Center Institutional Animal Care and Use Committee approved all procedures for this experiment.

#### **Animal Husbandry and Housing**

Twenty-four Holstein heifer calves, approximately 6-8 weeks of age at dehorning, were used for this project. Housing consisted of individual calf hutches measuring 2.5m<sup>2</sup> with a 2.8m<sup>2</sup> wire enclosure on grass. Calves were fed milk replacer (20% CP, 20% Fat; Kentwood Co-Op, Kentwood, LA) twice daily mixed at 15% solid, providing 2.28 kg of dry matter per day. Calves were also offered water, calf starter, and grass hay ad libitum. The calf starter consisted of cracked corn, soybean meal, rolled oats, chopped alfalfa hay, molasses, and a vitamin/mineral mix. The calf starter was 85% dry matter, with 23% crude protein, and 79% total digestible nutrients.

#### **Study Procedure**

The study design was a complete randomized design with 6 periods. Calves were approximately 6-8 weeks of age at the time of this study commencement. Each period consisted of 4 calves assigned, using randomly drawn numbers, to one of 4 treatments (n=1 calf/treatment/period).

#### **Catheterization**

Approximately 2 h prior to dehorning for each period, calves were restrained with a halter for catheter placement and to clip the hair around the horns. A 14 gauge, 3.5-inch catheter

(MILA International, Inc., Erlanger, KY) was inserted and a high flow catheter extension (MILA International, Inc., Erlanger, KY) attached. Catheters were sutured in place and patency was maintained with 5 mL of heparin saline flush (4 U/mL). Catheters remained patent a total of 5 hours and were removed after the 2 h blood sample was collected.

## **Dehorning**

After randomization, calves were assigned to one of four treatment groups: anesthesia without dehorning (CON); dehorning without anesthesia (NO\_ANET); anesthesia followed by immediate dehorning (ANET\_0); and anesthesia with a 10 minute delay prior to dehorning (ANET\_10). Approximately 3 hours before dehorning, calves were restrained with a rope halter. Calves in CON, ANET\_0, and ANET\_10 received 5 mL of 2% lidocaine hydrochloride (Lidocaine HCL 2% (20 mg/ml) VetOne, Boise, ID) for each horn bud as previously described (Stock et al., 2013). Calves in NO\_ANET received 0.9% saline in the same manner as those calves that received lidocaine. Horn buds were then removed via hot iron, as previously described (Heinrich et al., 2010). Calves were administered local anesthesia and NSAID immediately prior to sham dehorning, dehorning, or the 10 minute delay prior to dehorning. To maintain consistency the same veterinarian performed all nerve blocks and dehornings. After the 2h sample was collected, calves received gelatin capsules containing meloxicam (Meloxicam Tablets USP 15 mg, Unichem Pharmaceuticals USA Inc., Rochelle Park, NJ), an NSAID, at a dose of 1 mg/kg of body weight. To maintain consistency the same veterinarian performed all nerve blocks and dehorning.

## **Sample Collection**

Blood samples were collected approximately 10 min prior to treatment administration (-10 min), immediately prior to anesthesia administration (0 min), and at 1, 2, 3, 4, 5, 10, 15, 20,

25, 30, 40, 50, 60, 90, and 120 after dehorning. Catheter and catheter extension patency was maintained using 5mL of heparin saline flush before and after blood was drawn and 3mL of blood was discarded before the sample was taken. Samples were collected while the calves were haltered and tied. Blood was collected in 12 mL aliquots and divided between lithium heparin tubes (BD Vacutainer, Franklin Lakes, NJ) for cortisol, and K<sub>3</sub> EDTA tubes (Monoject, Mansfield, MA) for ACTH. ACTH blood collection tubes contained the trypsin inhibitor, Aprotinin (Aprotinin from Bovine Lung Solution, Sigma-Aldrich, St. Louis, MO). Each blood tube had 166 µL of Aprotinin added to it to achieve 0.5 Trypsin Inhibiting Units (TIU) per mL of blood. All samples were kept on ice prior to centrifugation at 2000g for 20 minutes. Prior to analysis, plasma for cortisol analyses was harvested and stored at -20°C, and plasma for ACTH was harvested and stored at -80°C.

### **Sample Analysis**

Serum cortisol concentrations were determined using radioimmuno assay kits (ImmuChem™ Cortisol Coated Tube RIA Kit, Orangeburg, NY). Serum ACTH concentrations were determined using radioimmuno assay kits (ImmuChem™ Double Antibody ACTH <sup>125</sup>I RIA Kit, Solon, OH).

### **Statistical Analysis**

The mixed procedure of SAS® (Version 9.4, SAS Inst. Inc., Cary, NC) was used to analyze the data as a repeated measures analysis of variance. Normality was achieved for serum cortisol and adrenocorticotrophic hormone concentrations and by a log transformation. Treatment, time, and treatment\*time were fixed effects, and calf(treatment) was the random effect. When overall differences were detected, post hoc analyses were conducted with pairwise t test comparisons of least-squares means. Values reported are least-squares means. The log cortisol



results were back-transformed by taking the antilogarithm of least-squares means and presented as geometric means. Statistical significance was declared at  $P < 0.05$  and tendencies were reported at  $P < 0.10$ .

## **Results and Discussion**

### **Plasma Cortisol Concentration**

Least Squares Means for plasma cortisol concentrations graphed against time are summarized in Figure 7. Figure 8 shows the geometric mean plasma cortisol concentrations post dehorning graphed against time.

There was evidence of a time effect on cortisol levels after dehorning ( $P < 0.0001$ ). Mean plasma cortisol concentrations in CON calves initially peaked at 4 minutes after dehorning (3.07 ng/mL) with another peak at 90 minutes after dehorning (3.2 ng/mL), however neither peak was greater than pretreatment levels. Plasma cortisol concentrations in CON calves also never increased above pre-treatment levels at any time point. In the ANET\_0 calves, mean plasma cortisol concentrations peaked at 25 minutes after dehorning (8.97 ng/mL). ANET\_0 mean plasma cortisol concentrations tended to be above baseline values at 3 minutes after dehorning ( $P = 0.0751$ ), greater from 4 to 40 minutes, and tended to be above baseline values from 50 to 60 minutes ( $P = 0.0613$  and  $P = 0.0839$  respectively) before reaching pretreatment values at 90 minutes. The mean plasma cortisol concentrations of NO\_ANET peaked by 25 minutes (8.25 ng/mL). Like the ANET\_0 treatment group, NO\_ANET mean plasma cortisol concentrations had a tendency to be above baseline at 3 minutes ( $P = 0.0702$ ), greater from 4 to 40 minutes, decreased to marginal significance at 50 and 60 minutes ( $P = 0.0547$  and  $P = 0.0743$ ), before reaching pre-treatment levels at 90 minutes. Mean plasma cortisol concentrations in the ANET\_10 treatment group peaked at 10 minutes (7.05 ng/mL). Cortisol concentrations for the ANET\_10 treatment

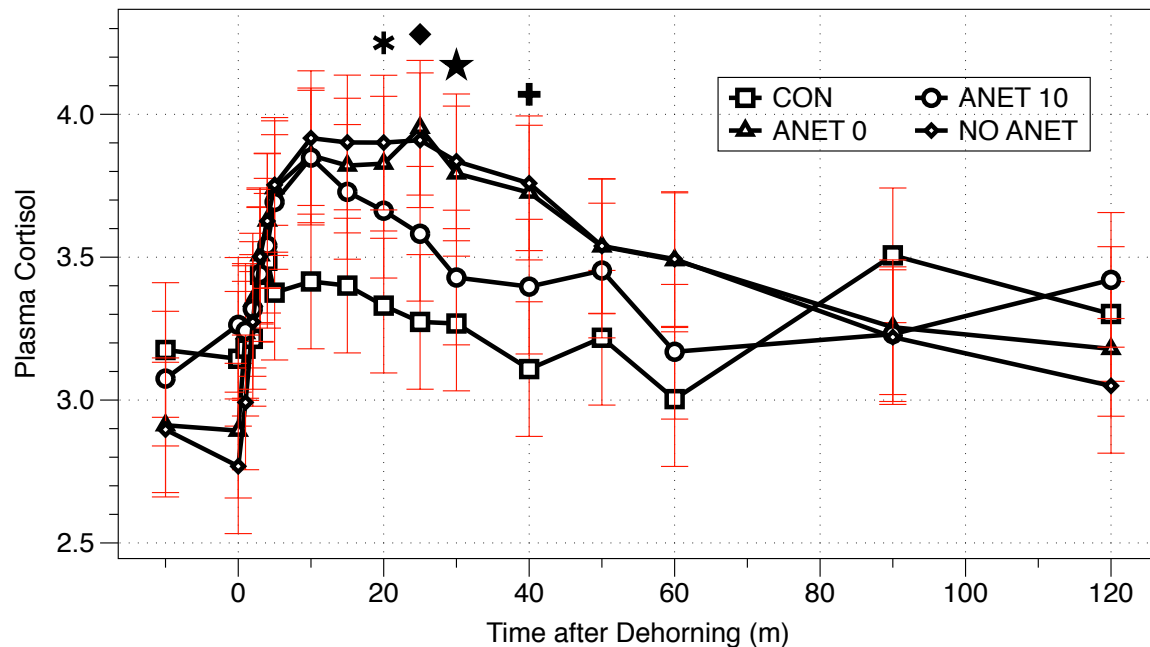
group tended to be above pretreatment values at 5 minutes ( $P=0.0645$ ), greater at 10 minutes ( $P=0.0209$ ), and tended to be above pretreatment levels from 15 to 20 minutes, before reaching pretreatment levels at 25 minutes. Similar patterns in plasma cortisol were reported by Graf and Senn (1999) and Petrie et al. (1996) after calves were dehorned following the administration of lidocaine.

At 20 minutes after dehorning, calves in CON tended to have lower plasma cortisol concentrations than the calves in the group dehorned ANET\_0 ( $P=0.0878$ ). Twenty-five minutes post dehorning, calves in the CON treatment group had lower plasma cortisol concentrations than calves in the ANET\_0 treatment group ( $P=0.0422$ ) and tended to have lower cortisol concentrations than calves in the NO\_ANET group ( $P=0.0572$ ). At 30 minutes post dehorning, CON calves tended to have lower cortisol levels than calves in the NO\_ANET treatment group ( $P=0.0894$ ). Forty minutes post dehorning, calves in the CON treatment group had tended to have lower plasma cortisol concentrations than calves in the treatment groups ANET\_0 ( $P=0.0647$ ) and NO\_ANET ( $P=0.0518$ ).

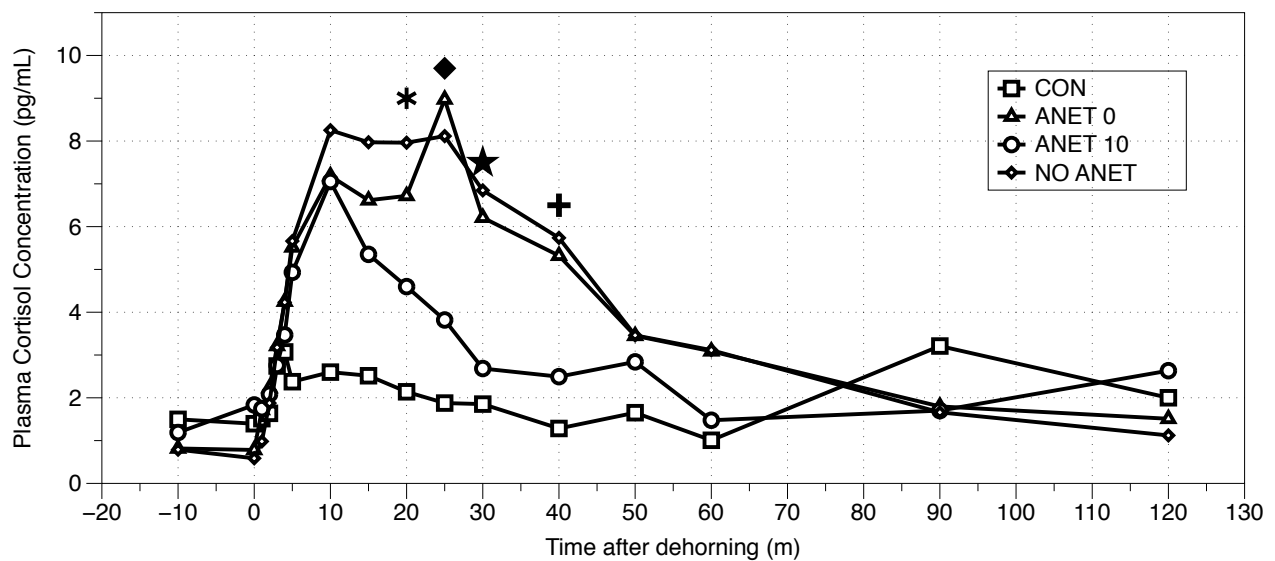
Regardless of treatment, plasma cortisol levels were increased above pretreatment levels in all dehorning treatments. This indicates dehorning caused pain and distress, even with the administration of lidocaine. The lack of difference observed between the ANET\_10 treatment group and the ANET\_0 and NO\_ANET treatment groups during the 30 minutes post dehorning indicates no benefit to waiting 10 minutes after the administration of lidocaine to dehorn calves. Also, there were no differences in peak plasma cortisol levels between any dehorning treatments. These findings could be attributed to the method of dehorning. It has been reported cautery dehorning will cause a small total plasma cortisol response that peaks by 30 minutes and returns to pretreatment values by 2 hours (Petrie et al., 1996). Petrie et al. (1996) also reported a small

transient increase in plasma cortisol concentrations when local anesthetics were administered, but concentrations returned to pretreatment levels by 60 minutes post dehorning.

Previous research also showed no differences in plasma cortisol concentrations after dehorning between calves that were administered local anesthesia and those that were not (Boandl et al., 1989; Petrie et al., 1996). These results suggest no difference in the pain and distress in calves dehorned by a hot iron with or without local anesthesia. However, as theorized by Stafford and Mellor (2011), the local anesthesia may not have been effective in all calves due to error in the placement or administration of the lidocaine in the studies conducted by Bondal et al. (1989) and Petrie et al. (1996). In the present study, as well as the studies conducted by Boandl et al. (1989) and Petrie et al. (1999), effective anesthesia was also not tested with needle pricking the area surrounding the horns. Therefore the calves in the treatment groups that received local anesthesia prior to dehorning may not have received effective anesthesia. This would possibly have caused the plasma cortisol concentrations of calves administered lidocaine immediately before dehorning to be similar to calves dehorned without the administration of lidocaine.



**Figure 7.** Least squares mean  $\pm$  SEM plasma cortisol concentrations in Holstein heifer calves dehorned ( $n=6$  calves/group) with lidocaine administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), a control group that were not dehorned (CON), and a group that were dehorned without lidocaine (NO\_ANET). \*: ANET\_0 > CON ( $P < 0.10$ ); ♦: ANET\_0 > CON ( $P < 0.05$ ), NO\_ANET > CON ( $P < 0.10$ ); ★: NO\_ANET > CON ( $P < 0.10$ ); + ANET\_0 and NO\_ANET > CON ( $P < 0.10$ )



**Figure 8.** Geometric mean cortisol concentrations in Holstein heifer calves dehorned ( $n=6$  calves/group) with lidocaine administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), a control group that were not dehorned (CON), and a group that were dehorned without lidocaine (NO\_ANET). \*: ANET\_0 > CON ( $P < 0.10$ ); ♦: ANET\_0 > CON ( $P < 0.05$ ), NO\_ANET > CON ( $P < 0.10$ ); ★: NO\_ANET > CON ( $P < 0.10$ ); + ANET\_0 and NO\_ANET > CON ( $P < 0.10$ )

## **Adrenocorticotrophic Hormone Concentration**

Least Squares Means for plasma ACTH concentrations graphed against time are summarized in Figure 10. Figure 11 shows the geometric mean plasma ACTH concentrations post dehorning graphed against time for all treatment groups.

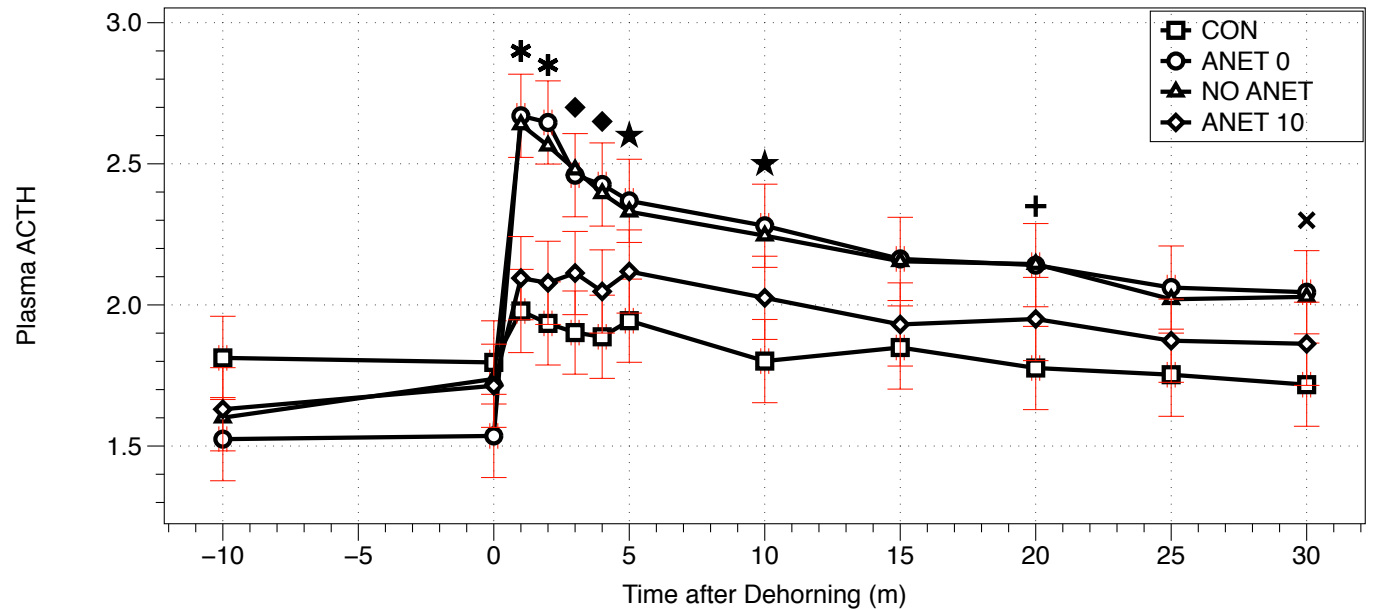
There was an effect of time on plasma ACTH concentrations after dehorning ( $P < 0.0001$ ). Mean plasma ACTH concentrations peaked 1 minute post dehorning for calves of the CON (95.16 pg/mL), ANET\_0 (467.84 pg/mL), and NO\_ANET (435.81 pg/mL) treatment groups. Plasma ACTH concentrations peaked 5 minutes after dehorning ANET\_10 (131.34 pg/mL). Calves in the ANET\_0 and NO\_ANET had greater peak plasma ACTH concentrations than CON calves ( $P = 0.0003$  and  $P = 0.0006$ , respectively). Also peak plasma ACTH concentration in ANET\_10 calves was lower than both ANET\_0 ( $P = 0.0041$ ) and NO\_ANET ( $P = 0.0067$ ). Peak plasma ACTH concentrations for the ANET\_0 and NO\_ANET treatment groups were also greater than pretreatment levels ( $P < 0.0001$ ). The ANET\_10 and CON peak ACTH levels never increased above pretreatment levels ( $P = 0.0109$  and  $P = 0.383$  respectively).

Plasma ACTH concentrations in CON calves never increased above pretreatment values at any time point. In the ANET\_0 calves and NO\_ANET calves, plasma ACTH concentrations were significantly greater than baseline from 1 minute post-dehorning to 30 minutes. Plasma ACTH concentrations for ANET\_10 increased above pretreatment values from 1 minute to 10 minutes before decreasing to pretreatment levels by 15 minutes. However, at 20 minutes plasma ACTH concentrations in ANET\_10 calves tended to increase ( $P = 0.0940$ ) before decreasing back to pretreatment levels by 25 minutes.

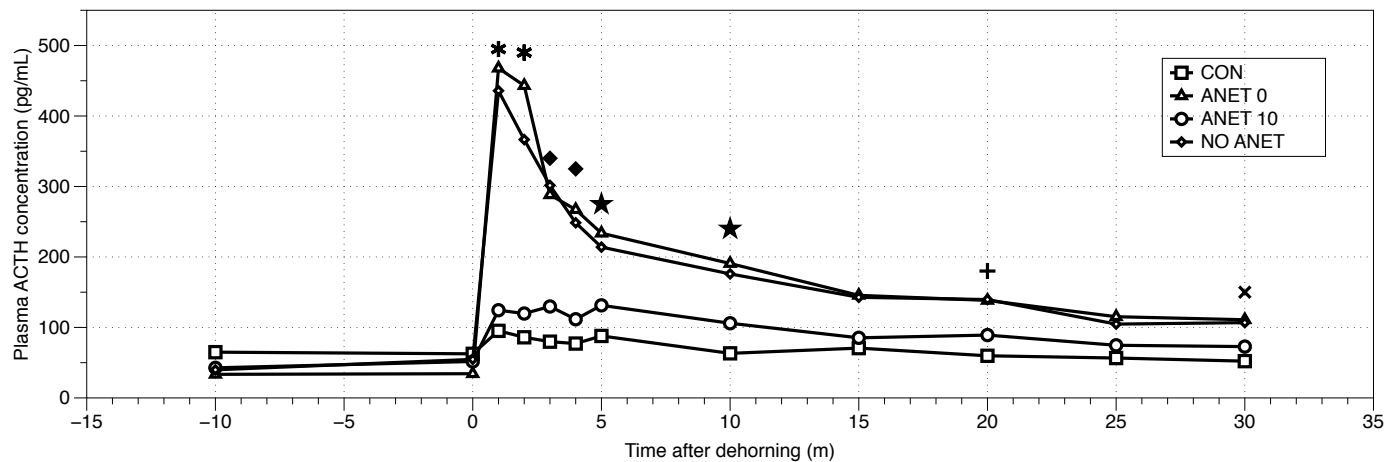
At 1 and 2 minutes post dehorning, CON and ANET\_10 calves had lower plasma ACTH concentrations than ANET\_0 ( $P=0.0003$  and  $P=0.0043$ , respectively) and NO\_ANET ( $P=0.0006$  and  $P=0.0068$ , respectively) calves. At 3 and 4 minutes post dehorning, CON calves had lower plasma ACTH levels than ANET\_0 ( $P=0.0037$  and  $P=0.0050$ , respectively) and NO\_ANET ( $P=0.0027$  and  $P=0.0081$ , respectively). Also at 3 and 4 minutes, ANET\_10 calves tended to have lower plasma ACTH levels than ANET\_0 ( $P=0.0836$  and  $P=0.0587$ , respectively) and NO\_ANET ( $P=0.0677$  and  $P=0.0828$ , respectively). At 5 and 10 minutes post dehorning, ACTH concentrations were lower in CON calves than those in ANET\_0 ( $P=0.0265$  and  $P=0.0124$ , respectively) and NO\_ANET ( $P=0.0435$  and  $P=0.0204$ , respectively). At 20 minutes post dehorning, calves in the CON treatment group tended to have lower plasma ACTH concentrations than ANET\_0 ( $P=0.0566$ ) and NO\_ANET ( $P=0.0542$ ). At 30 minutes post dehorning, calves in the CON treatment group had tended to have lower plasma ACTH concentrations than calves in the ANET\_0 ( $P=0.0864$ ).

The data of this study show regardless of treatment, ACTH concentrations will increase when calves are dehorned, thus indicating that dehorning causes pain and distress even when lidocaine is administered. The plasma ACTH concentrations of the CON treatment group failing to rise above pretreatment levels could be indicative that handling and lidocaine injections did not cause any transient stress or pain. This may be attributed to lidocaine's anesthetic effect. Graf and Senn (1999) observed when calves are injected with saline, there was a significant increase in plasma concentrations of vasopressin and an even more noticeable increase in ACTH and cortisol 10 minutes after the injection. In the same study, they also observed the injection of a local anesthetic did not cause an increase in vasopressin, ACTH, or cortisol (Graf and Senn, 1999).

The differences in plasma ACTH levels seen between the ANET\_10 treatment group and both ANET\_0 and NO\_ANET treatment groups indicates that waiting to dehorn calves 10 minutes after the administration of lidocaine may reduce plasma ACTH levels immediately following dehorning. The peak cortisol level for the ANET\_10 treatment group was also significantly lower than the ANET\_0 ( $P= 0.0041$ ) and NO\_ANET ( $P= 0.0067$ ) treatment groups, which also supports this hypothesis. However, the difference observed in plasma ACTH between the ANET\_10 treatment group and the ANET\_0 and NO\_ANET treatment groups dissipated by 5 minutes after dehorning. This indicates the benefits of waiting 10 minutes may be short lived and may not be beneficial for longer periods of time after dehorning. Although, the ANET\_10 treatment group plasma ACTH levels declined to pretreatment levels 15 minutes after dehorning, the plasma ACTH levels of the ANET\_0 and NO\_ANET treatment groups never decreased back to baseline values during the 30 minute sample collection period. The quicker decline to pretreatment values by the ANET\_10 treatment group, could be attributed to the lower peak ACTH level than both ANET\_0 and NO\_ANET treatment groups.



**Figure 9.** Least squares mean  $\pm$  SEM plasma ACTH concentrations in Holstein heifer calves dehorned (n=6 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), a control group that were not dehorned (CON), and a group dehorned without lidocaine (NO\_ANET). \*: ANET\_0 and NO\_ANET > CON and ANET\_10 ( $P < 0.01$ ); ♦: ANET\_0 and NO\_ANET > CON ( $P < 0.001$ ), ANET\_0 and NO\_ANET > ANET\_10 ( $P < 0.10$ ); ★: ANET\_0 and NO\_ANET > CON ( $P < 0.05$ ); +: ANET\_0 and NO\_ANET > CON ( $P < 0.10$ ); × ANET\_0 and NO\_ANET > CON ( $P < 0.10$ )



**Figure 10.** Geometric mean plasma ACTH concentrations in Holstein heifer calves dehorned (n=6 calves/group) with lidocaine and meloxicam administration either at the time of dehorning (ANET\_0), 10 minutes prior to dehorning (ANET\_10), a control group that were not dehorned (CON), and a group dehorned without lidocaine (NO\_ANET). \*: ANET\_0 and NO\_ANET > CON and ANET\_10 ( $P < 0.01$ ); ♦: ANET\_0 and NO\_ANET > CON ( $P < 0.001$ ), ANET\_0 and NO\_ANET > ANET\_10 ( $P < 0.10$ ); ★: ANET\_0 and NO\_ANET > CON ( $P < 0.05$ ); +: ANET\_0 and NO\_ANET > CON ( $P < 0.10$ ); × ANET\_0 and NO\_ANET > CON ( $P < 0.10$ )



## **Conclusions**

Based on the results of this study, it is inconclusive as to whether or not there is a benefit to waiting 10 minutes after the administration of lidocaine to dehorn calves. Calves dehorned after a 10-minute waiting period had a significantly lower peak in ACTH than ANET\_0 and NO\_ANET and reached baseline levels for both plasma ACTH and cortisol concentrations before the ANET\_0 and NO\_ANET treatment groups. However, the observed differences in plasma ACTH concentrations dissipate within 5 minutes, and plasma cortisol concentrations in all calves returned to pretreatment levels within an hour of dehorning. These findings are significant because no previous research has reported the effects of time of lidocaine administration on ACTH and cortisol after cautery dehorning.

## CHAPTER V SUMMARY AND CONCLUSIONS

There is a significant amount of research indicating dehorning is painful, as well as research showing there are techniques that can be used to successfully manage the pain. However, many producers still do not consider the use of pain management necessary. The American Veterinary Medical Association emphasizes the importance of minimizing the pain associated with dehorning in order to limit the amount of distress and changes in the animal's behavior and physiological states (AVMA, 2014b). Currently there are no FDA approved drugs for pain relief in the US, due to the lack of validated assessments for scoring pain in cattle (Muir and Woolf, 2001). Several biomarkers of pain have been suggested including: plasma cortisol concentrations (Duffield, 2008), substance p (SP) concentrations (Coetzee et al., 2012), and plasma adrenocorticotrophic hormone (ACTH) concentrations (Graf and Senn, 1999).

Although there have been various methods of local anesthesia studied, lidocaine has become the most commonly used local anesthetic to prevent pain associated with dehorning. This is due to its quick onset (2-5 m), intermediate duration (~90 m), and inexpensive cost (Coetzee et al., 2012). However, most studies which evaluated lidocaine's effects on reducing the amount of pain associated with dehorning waited 10-15 minutes after its administration to dehorn the calves, ensuring the lidocaine had taken effect (Heinrich et al., 2009; Stilwell et al., 2012; Allen et al., 2013; Glynn et al., 2013). A 2012 study by Fierheller and others looked at the onset, duration, and efficacy of four different methods of lidocaine administration to the area surrounding the horn buds. It was observed that lidocaine administered via cornual nerve block had an onset time of 30

seconds to 5 minutes (Fierheller et al., 2012). However, the calves were not dehorned in the study.

The study in Chapter 2 compared cortisol and substance P levels in calves dehorned immediately after administration of lidocaine and meloxicam (ANET\_0), calves dehorned 10 minutes after the administration of lidocaine and meloxicam (ANET\_10), and calves that were administered lidocaine and meloxicam and sham dehorned (CON). Plasma cortisol concentrations in both ANET\_0 and ANET\_10 calves, unlike CON calves, were elevated above pretreatment levels for at least 40 minutes post-dehorning. Also, there was no difference observed in ANET\_0 and ANET\_10 plasma cortisol concentrations at any time point. Substance P was found to be greater in calves in ANET\_0 than CON calves only at 480 minutes. Like plasma cortisol concentrations, there were also no differences seen between ANET\_0 and ANET\_10 plasma SP concentrations. This suggests there is no difference in effectiveness of lidocaine and meloxicam administered either immediately prior to dehorning or 10 minutes prior to dehorning. Therefore it was concluded waiting 10 minutes after administration of lidocaine and meloxicam to dehorn calves provided no benefits, as it did not reduce plasma cortisol and SP concentrations compared to other treatments.

The study in Chapter 3 compared cortisol and adrenocorticotrophic hormone (ACTH) levels in calves dehorned immediately after administration of lidocaine (ANET\_0), calves dehorned 10 minutes after the administration of lidocaine alone (ANET\_10), calves dehorned without lidocaine (NO\_ANET), and calves administered lidocaine and sham dehorned (CON). Calves in ANET\_0 and NO\_ANET plasma cortisol concentrations peaked by 25 minutes after dehorning and were elevated above pretreatment levels until 90 minutes post dehorning. Calves

in ANET\_10 plasma cortisol concentrations peaked at 10 minutes post dehorning and reached pretreatment levels by 25 minutes after dehorning. There were no differences in plasma cortisol concentrations observed between ANET\_10, ANET\_0 and NO\_ANET at any time point. However, ANET\_0 and NO\_ANET had greater plasma cortisol concentrations and tended to have greater plasma cortisol concentrations than CON from 20 to 40 minutes post dehorning, while plasma cortisol concentrations of calves in ANET\_10 did not differ from CON calves at any time point. Plasma ACTH concentrations peaked at 1 minute post dehorning in calves in CON, ANET\_0, and NO\_ANET, but not until 5 minutes after dehorning for ANET\_10. Calves in ANET\_0 and NO\_ANET also had greater peak plasma ACTH concentrations than CON and ANET\_10. Plasma ACTH concentrations reached baseline values by 25 minutes after dehorning, while concentrations for NO\_ANET and ANET\_0 did not reach baseline by 30 minutes after dehorning. From 1-4 minutes post dehorning, plasma ACTH concentrations were either greater or tended to be greater in ANET\_0 and NO\_ANET calves than ANET\_10 or CON calves. These results suggest it may be beneficial to wait after the administration of lidocaine to dehorn calves. However, differences observed in ACTH dissipate in 5 minutes and all calves reached baseline plasma cortisol levels within an hour of dehorning.

In conclusion, it is unclear as to whether or no waiting to dehorn calves offers any benefits in the reduction of pain associated with dehorning. The studies in Chapter 2 and 3 are, to the author's knowledge, the first studies to evaluate the timing of lidocaine administration on pain responses in calves after dehorning. Further research is needed to determine if waiting to dehorning calves after the administration of lidocaine is beneficial in reducing pain associated with dehorning in calves.

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