PHARMACOKINETICS AND EFFECTS OF IM XYLAZINE-KETAMINE-BUTORPHANOL ALONE OR IN COMBINATION WITH ORAL SODIUM SALICYLATE IN THE DRINKING WATER ON THE STRESS RESPONSE ON HOLSTEIN CALVES FOLLOWING CONCURRENT CASTRATION AND DEHORNING

by

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Abstract

Objective—To determine the pharmacokinetic parameters of xylazine, ketamine, and butorphanol administered IM and sodium salicylate administered PO and to compare their effect on biomarkers of pain and distress following sham (Period 1) and actual (Period 2) castration and dehorning.

Animals—40 Holstein bull calves

Procedures—Calves weighing 108 to 235 kg received the following treatments prior to sham castration and dehorning (Period 1) and castration and dehorning (Period 2) (n=10 calves/group): (i) 0.9% saline solution IM (PLACEBO) (ii) sodium salicylate (SAL) supplied free-choice in water to provide concentrations from 2.5 to 5 mg/mL beginning 24 hours prior to Period 1 to 48 hours after Period 2; (iii) 0.025 mg/kg butorphanol, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine co-administered IM immediately prior to both periods (XKB); and (iv) a combination of treatments (ii) and (iii) (SAL + XKB). Plasma drug concentrations, average daily gain (ADG), chute exit speed, serum cortisol concentrations and electrodermal activity (EDA) were evaluated.

Results—ADG (0-13d) was significantly greater in the SAL and SAL + XKB groups. Calves receiving XKB had significantly slower chute exit speed in both periods. Serum cortisol concentrations were significantly increased in all groups during Period 2 compared to Period 1. However, XKB attenuated serum cortisol response for the first hour after castration and dehorning while oral salicylate significantly reduced cortisol from 1-6 hours. XKB administration significantly decreased EDA scores in both periods.

Conclusions and Clinical Relevance—Free-choice sodium salicylate decreases cortisol concentrations and reduced weight loss associated with castration and dehorning in calves.

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a. Draxxin, Pfizer, New York, NY.

b. Covexin 8, Schering Plough, Summit, NJ.

c. Bovi-shield Gold 4, Pfizer, New York, NY.

d. Ultra Boss Pour-on insecticide, Schering Plough, Summit, NJ.

e. Microsoft Excel, Microsoft Corp, Redmond, WA

f. Fisher Scientific, Pittsburgh, Penn.

g. Anased, Lloyd Lab, Shenandoah, IA.

h. Ketaset, Fort Dodge, Fort Dodge, IA.

i. Torbugesic, Fort Dodge, Fort Dodge, IA.

j. SireMaster, Ice Corp, Manhattan, Kan.

k. For-Most, Hawarden, IA.

l. Hospira, Inc, Lake Forest, Ill.

m. MILACATH, MILA International; Florence, Ken.

n. Baxter Health Care Corporation; Deerfield, Ill.

o. Stone Manufacturing and Supply Company Inc, Kansas City, Mo.

p. Stone Manufacturing and Supply Company Inc, Kansas City, Mo.

q. Farmtek Wireless rodeo electronic timing system, Farmtek, Inc, Wylie, Tex.

r. Kendall, Mansfield. Mass.

s. Public Health Information Systems, Inc, Dublin, OH.

t. Fisherbrand, Pittsburg, Penn.

u. Immulite 1000 Cortisol, DPS, Los Angeles, Calif.

v. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, Md.

w. API 4000, Applied Biosystems, Foster City, Calif.

x. Millipore Corporation, Billerica, Mass.

y. Waters XBridge Phenyl C18, 50 mm X 2.1 mm X 5 μm, Waters Corporation, Milford, Mass.

z. TDx, Abbott Laboratories, Abbott Park, Ill.

aa. WinNonlin, Pharsight Corporation, Cary, NC.

bb. SAS, version 9.1, Cary, NC.

cc. JMP 7.0.2, SAS Institute Inc, Cary, NC.

List of Abbreviations

NSAID	Non-steroidal anti-inflammatory drug
NMDA	N-methyl-D-aspartate
wks	Weeks
h	Hours
d	Days
C_{max}	Maximum plasma concentration
AUEC	Area under the effect curve
ADG	Average Daily Gain
EDA	Electrodermal Activity
SAL	Sodium salicylate
XKB	Xylazine, ketamine, and butorphanol
SAL + XKB	Xylazine, ketamine, butorphanol and Sodium Salicylate
T_{max}	Time to maximum plasma concentration
AUC	Area under the plasma cortisol concentration-time curve
λ_z	Slope of the terminal portion of the time-concentration curve
$T_{½\lambda z}$	Terminal elimination half-life time
Cl_F	Total body clearance per fraction of drug absorbed
Vz_F	Volume of distribution per fraction of drug absorbed
MRT	Mean residence time
AUMC	Area under the moment curve
LSM	Least square means
SEM	Standard error of the mean

FARAD	Food Animal Residue Avoidance Databank
ELDU	Extra-label drug use

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Dedication

First and foremost, I would like to dedicate this paper to my husband, Allen. His first role in my life was to serve as a mentor, and he continues to fulfill that role every day. His love, help, guidance, encouragement, and support have helped me persevere through each day.

Secondly, I would like to dedicate this to my parents, Tim and Claire. Since a young age they have instilled the importance of a strong work ethic shown through their own example. They've always provided endless love and support through each endeavor I undertake.

CHAPTER 1 - Literature Review

Cattle Welfare

The concept of animal welfare has been at the forefront of emerging issues facing agriculture today. The United Kingdom's Farm Animal Welfare Council has proposed that animal welfare consists of "5 freedoms." These freedoms include "1) freedom from hunger and thirst; 2) freedom from discomfort; 3) freedom from pain, injury, or disease; 4) freedom to express normal behavior; and 5) and freedom from fear and distress (FAWC 2009)". However, some of these freedoms have become challenging to provide to livestock with the increased pressure on producers to provide a plentiful supply of affordable food (Appleby 2005). Production agriculture has been forced to become increasingly efficient with space, time, resources, labor, and cost associated with raising and processing livestock.

There is increasing public interest in issues related to animal welfare. There has also been an increasing disconnect between the general public and common agricultural production practices and how and why such practices are performed. Public perception of animal welfare could have an impact on the governmental regulation of livestock management practices and shape current and future industry practices. This increased public concern is believed to originate partly from the change over from small farms producing most of the food to large vertically integrated agricultural schemes in which a much less significant part of the population (1.5% of the US) is engaged in production agriculture (Rollin 2004). Therefore the majority of the population lacks a general understanding of the work that goes into the management of livestock for the production of food. Rollin (2004) suggests other factors contributing to this disconnect are media focusing on animal related issues due to its every increasing popularity, the shift of focus to more ethically based issues in society, and the promotion of such issues by

philosophers, scientists, government, and celebrities. A nationwide telephone survey by Norwood (1997) studied public perception of animal welfare and reported several interesting results: 1) People believe the opportunity for animals to live "naturally" is more important than protection, shelter, and bedding 2) people believe livestock raised on small farms have a better quality of life than those raised on large or corporate farms 3) three quarters of people surveyed believe animals raised under higher welfare standards produce safer and better tasting meat and 4) people associate higher standards of care with increased food costs and 70% agree farmers should be compensated for higher welfare standards. This survey identified a discrepancy in what the public views as important to an animal versus what animal welfare experts believe is important. This was demonstrated by the observation that consumers preferred a "pasture production system" while welfare experts propose that shelter, comfortable temperatures, and protection from other animals are the most important considerations in ensuring animal welfare. In the future, retail restaurants and food labels in the grocery store may be tailored to address this public perception by developing claims on their products such as "animal compassionate," "food with integrity," "naturally raised," and "antibiotic-free."

On the other hand, producers would argue that the welfare of livestock has improved over the last several decades. Fulwider *et al.* (2008) reported in his survey that 77.9 % of producers thought the quality of their dairy cattle had improved due to the improved confinement housing which offers more ventilation, free access to food, water, and leisure, accessibility to nutritionists to test feed and balance rations to optimize production, and regular visits by veterinarians for routine care. Producers from this study noted disadvantages of today's dairy production practices include higher incidence of lameness or hoof problems and displaced abomasums, higher veterinary bills, added stress on livestock to increase production whether it be milk or meat, and reduced access to pasture. Another study by Heleski *et al.* (2004) found 84% of dairy and 87% of beef animal science faculty members thought dairy production systems employed appropriate animal welfare practices. On further questioning however, approximately 34% of faculty members agreed that castration without anesthetic was cause for concern. Furthermore, approximately 46% agreed that dehorning without local anesthetic warranted concern.

Available literature supports that most husbandry type procedures in livestock are practiced without the use of analgesia or anesthesia. Over the last decade however, there has been an increase in research focused on alleviating pain in livestock during such procedures using different analgesic and anesthetic drug regimens. However, pain management through use of pharmaceuticals has not been readily accepted among producers due to the burden of added cost, time, and assistance needed by veterinarians. Some of this new research is aimed at finding pain management protocols in livestock species during routine husbandry procedures that would be both economically viable and offer a production advantage such as increased average daily gain or reduced days off feed.

In the United States, the American Veterinary Medical Association (AVMA) developed guidelines for performing routine livestock practices including castration and dehorning. For example, the AVMA recommends the use of local anesthesia and the administration of analgesics to minimize or eliminate pain associated with castration (AVMA 2009). However it is ultimately up to the producer and/or veterinarian to choose how these procedures are performed and if analgesia or anesthesia is provided. In a survey of 189 bovine veterinarians conducted by Coetzee *et al*. (2010), only 22% of respondents reported administering local anesthetics and 21% reported administering systemic analgesics prior to castration.

Several countries have passed legislation regulating dehorning and castration practices. In New South Wales, it is illegal to castrate calves over 6 months of age unless under veterinary supervision (Irwin, 2004). In Sweden, under the 1992 Animal Rights Act, it is illegal to disbud by means of cauterization without local anesthesia and sedation (Bengtsson *et al.*, 1996). In the United Kingdom, under the Protection of Animals Act 1954/1964, anesthesia is not required if calves are disbudded under 1 week of age, however if amputation or cautery is performed, then local anesthesia must be provided (Kent *et al*., 1999). Additionally, if castration is performed after 8 weeks, a veterinarian must perform the procedure with the provision of anesthesia (Thuer *et al*., 2007). In Switzerland, as of 2001, bulls must be castrated under local or general anesthesia and the procedure must be carried out by a veterinarian (Thuer *et al*., 2007). In Canada, it is

recommended that disbudding and castration occurs within the first week of life (CVMA).

In the United States however, there are no such regulations. Studies have been conducted to determine how routine management practices are usually performed. A survey of dairies in Wisconsin, Minnesota, Indiana, Iowa, and New York in 2008 by Fulwider and others reported 34.5% of calves were dehorned by 8 weeks, 78.8% by 12 weeks, and 95% by 32 weeks. Most calves were dehorned by hot iron (67.3%) and the rest were dehorned by gouging (8.8%), paste (9.7%), saw (3.5%), or unknown (10.6%). In this survey, only 12.4% of dairy owners reported the use of anesthesia, and 1.8% used analgesia. For castration, a survey of members of the American Association of Bovine Practitioners and the Academy of Veterinary Consultants by Coetzee and others (2010) found surgical castration with a scalpel blade (57%) was the most commonly performed method, with removal of the testicals performed either by manually twisting testicles (44%) or the use of an emasculator (36%).

Canada has similar management practices as compared to the US. In a study by Hewson and others (2007), only 6.9% of beef calves and 18.7% of dairy calves were administered analgesia when undergoing castration at under 6 months of age, while 33.2 % of dairy calves and 19.9 % of beef cattle received analgesia if castrated over 6 months of age. In the same study, 90.2% of dairy cattle and 57.5% of beef cattle under 6 months received analgesia, while 84.8% of dairy cattle and 68.7 % of beef cattle over 6 months received analgesia. The most common analgesics used according to this survey were xylazine and lidocaine. In a survey conducted by Misch *et al.* (2007), out of 161 producers surveyed, only 9% used sedatives each time dehorning was performed. Only 18% provided local anesthesia to all of their calves during dehorning. Veterinarians used methods to relieve pain slightly more frequently: 65% used local anesthesia and 62% used sedatives. Reasoning provided by this survey for not using anesthesia or analgesia included cost, time, unfamiliarity with methods to relieve pain, or considering pain management unnecessary.

Models for Measuring Pain in Cattle

Pain has been defined as "the normal, predicted, physiologic response to an adverse chemical, mechanical, or thermal stimulus . . . associated with surgery, trauma, or acute illness" (Federation of State Medical Boards 1998). Pain can generally be divided into two broad categories: acute and chronic. Within each category, the type of pain can be further subdivided depending on the nature of the insult causing pain. Muir and Woolfe (2001) has classified castration and dehorning type procedures under inflammatory pain or clinical pain associated with intense or prolonged tissue damage. Currently there is no validated method for measuring pain in livestock. Therefore finding a method to reliably measure pain is critical to the development and approval of analgesic compounds for use in livestock. It is noteworthy that pain is one of the most difficult parameters to evaluate due to individual variability between animals, the inability of verbal communication between man and animals, and lack of consistent physiological or behavior measures for determining pain (Livingston, 2010, Van Reenen *et al.,* 2005).

In livestock, acute pain is of concern due to the frequent need to perform routine procedures such as dehorning and castration. Several measures have been used to correlate with pain during castration and dehorning in cattle in the literature. These parameters have included: cortisol, substance P, interferon-γ, epinephrine and norepinephrine, average daily gain, heart rate, feed intake, eye temperature, chute exit speed, vocalization, and behavior scoring. Additionally in the present study, a novel device called the "Pain Gauge" was used to determine electrodermal activity across the nasal planum.

Activation of the sympathetic nervous system during painful stimuli causes several physiologic responses in an animal including increases in heart rate, dilation of the pupils and change in eye temperature, changes in peripheral blood flow, as well as changes in skin resistance (Molony, 1997, Stewart *et al*., 2010). This in turn can influence heart rate, distribution of blood and heat (as measured by thermography), and electrodermal activity (also known as skin conductance) (Molony, 1997, Graham, 1997). Epinephrine is difficult to measure in peripheral blood, and therefore is not routinely used as an indicator of changes in sympathetic tone (Minton *et al.,* 1994). The catecholamines have an extremely short half-life (1 to 2 minutes) rendering collection and analysis

extremely sensitive to the effects of processing. Furthermore these can be difficult to measure in serum due to low circulating concentrations, and assays for analysis are expensive (Hjemdahl, 1993, Stewart *et al*., 2010).

Monitoring changes in serum or salivary cortisol concentrations is one of the most common methods for measuring pain and/or distress reported in the literature (Stafford and Mellor, 2005a, Stafford and Mellor, 2005b). Cortisol is part of the neuroendocrine axis in which sensory stimuli, including stress and pain, activate the hypothalamo – pituitary system. This in turn causes the release of adrenocorticotropic hormone (ACTH) which stimulates the adrenal cortex to release cortisol among other components such as corticosterone, aldosterone, androgens, and catecholamines (Gayner and Muir, 2002). Corticosteroids are extremely stable under proper storage conditions (Stroud *et al*., 2007) and are relatively easily to measure making these a logical choice for pain studies in animals. Measuring cortisol concentrations has limitations including variability in serum levels due to natural diurnal changes, the possibility of outside stressors independent from pain activating the hypothalamic-pituitary-adrenal system, and individual variation of cortisol response to pain (Molony, 1997). An important consideration when using cortisol as a measure for pain is to minimize other causes of stress leading to falsely elevate cortisol concentrations. This could include eliminating repeated venipunctures, minimizing handling stress, and using and experimental control group during the study from which to compare cortisol levels.

Practical implications or secondary effects of pain would include parameters such as average daily gain and feed intake. Results have been equivocal as to whether castration or dehorning influences average daily gain and feed intake and furthermore, whether these effects are long lived enough to have an impact on final market weight. If pain relief strategies were to be implemented into production practices, an economic or production benefit would be important for producers.

Behavioral characteristics have been used in several studies to evaluate pain and can be classified into voluntary and involuntary changes in behavior. Involuntary postural changes could be hyperreflexia and increased muscle tone (Molony, 1997). Voluntary parameters may include stride length, posture, head position, head shaking and rubbing, ear flicks, tail flicks, kicking, biting at the affected area, rolling, rearing, and

foot stamping. Pitfalls of this method are the subjective nature of behavior scoring and individual variability in behaviors among animals.

For each parameter it is of the utmost importance to determine the validity and sensitivity of each for measuring pain.

Analgesic Use in Cattle

Some of the rationale supporting the wealth of new research focused on the use of different drug regimens during production practices can be attributed to the lack of a single FDA approved drug for the treatment and alleviation of pain in livestock (Smith *et al*., 2008). The FDA has set guidelines for industry in the development of an approved non-steroidal anti – inflammatory drug for effective analgesia in livestock. To gain approval, the prospective drug must include dosing regimens, animal safety data, field study data, pharamacokinetics, and label development (FDA 2006). Currently the only non-steroidal anti-inflammatory drug with a label in cattle is flunixin meglumine, for the "control of pyrexia associated with bovine respiratory disease and mastitis as well as for the control of inflammation associated with endotoxemia" (Smith *et al*., 2008).

Methods for providing analgesia and or anesthesia during castration and dehorning have included infusion of local anesthetics through the use of epidurals or local blocks, parenteral administration of α-agonists, opioids, N-methyl-D-aspartic (NMDA) antagonists, and/or the parenteral or oral use of non-steroidal anti-inflammatory drugs (NSAIDS). Some studies have used a multimodal approach to pain management in which combinations of local anesthetics and NSAIDs, opioids, or other sedatives have been used. Single-drug regimens for relieving pain are ineffective due to the several routes of activation of sensitizers and central mechanisms involved in modulating and amplifying pain (Muir *and Woolfe,* 2001). For example, it has been suggested by Duffield *et al.* (2008) that during dehorning calves greater than 4 weeks of age, the best approach to fully address pain experienced is to provide both local anesthesia through a cornual nerve block combined with systemic analgesia.

The mechanism of pain is complex and has many components; therefore, management through anesthetics and analgesics can be difficult. Damage to tissue

simulates activation of peripheral Aδ and unmyelinated C afferent nerve fibers to the dorsal horn of the spinal cord (Muir and Woolfe, 2001). These Aδ fibers have a low threshold for activation - transmitting noxious stimuli rapidly and are therefore primarily responsible for localized and acute pain occurring at the time of tissue insult. Unmylenated C fibers are responsible for prolonged transmission of pain and are associated with hyperalgesia and central sensitization more commonly associated with the second and more chronic stage of pain after an insult. This explains the biphasic nature of pain – initially, the noxious stimuli correlates with a brief, sharp, and localized pain which then transforms into a prolonged, dull, diffuse pain (Gottschalk and Smith, 2001). The second phase is correlated with increased hypersensitivity around peripheral nociceptors (Gottschalk *et al*., 2001). The neurotransmitter glutamate is released from peripheral fibers to activate 3 different receptors (NMDP, AMPA, and kainate) to transmit pain to the brain, stimulating acute nociception of the painful stimuli (Dingledine et *al.,* 1999). Hyperalgesia, or "wind-up pain" is caused by increased production of inflammatory cytokines which heighten the sensitivity and thus cause upregulation of transmission receptors on peripheral afferent neurons (Julius *et al.,* 2001). This eventually leads to central sensitization by the upregulation of NMDA receptors in the dorsal horn (Woolf et *al.,* 1991).

Analgesic drugs have different and possibly multiple sites of activity along the peripheral and central nervous system to provide pain relief. This physiology of pain is important for the development of proper therapy for pain relief during castration and dehorning.

Recent studies in pain management have not only focused on the type of analgesia provided, but also the importance of timing of administration in relation to controlling pain. Pre-emptive analgesia is the practice of administering analgesics or local anesthesia before the onset of tissue damage to reduce the analgesic requirement to manage pain after the insult (Nolan, 2001). One of the most practical and effective applications of such therapy is during surgical procedures in which the time of onset for a noxious stimuli is known (McQuay, 1992). Depending on the surgical procedure, multimodal therapy addressing several sites along the pain pathway may be needed to prevent central

sensitization (Kehlet and Dahl, 1993). The application of such practices in an agricultural system may be difficult unless clinical and economic benefits can be proven.

Anesthetic drugs such as lidocaine, mepivicaine, and bupivicaine have been used extensively to provide regional anesthesia castration and dehorning in studies reported in the literature. These drugs are useful in targeting peripheral sensory neurons involved in nociception by blocking sodium channels and thus preventing depolarization of afferent nerves (Vinuela – Fernandez *et al.*, 2007). During dehorning, 5 – 10 mL of local anesthetic is deposited between the lateral canthus of the eye and the base of the horn along the zygomatic process in order to block innervations of the cornual branch of the zygomaticotemporal nerve (Edmondson, 2008). Duffield *et al*. (2008) has described the procedure best performed when depositing 5 mL of 2% lidocaine at the point 1/3 the distance from the lateral canthus of the eye to the horn, with most of the anesthetic deposited in a fan shape below the frontal crest and depositing around 1 mL as the needle is withdrawn. Likewise during castration, nerve blocks involving the spermatic cord and surrounding structures have been described and performed. A disadvantage of this technique is the duration of action of lidocaine is only 60 to 120 minutes (Lumb and Jones, 2007) and therefore only provides temporary pain relief. For example, in a study by Petrie *et al.* (1996), lidocaine cornual nerve blocks abolished cortisol response immediately after scoop disbudding and for 2 hours thereafter. However, after the nerve block wore off at 2.5 hours, cortisol concentrations remained significantly elevated until 7.5 hours after disbudding. Pharmacokinetic studies of lidocaine in blood serum investigated by Sellers *et al.* (2009) found that after administration of 100 mL (3.5 mg/kg) in 573 kg cows, the C_{max} was 572 ng/mL, T_{max} was 0.521 hours, $T_{1/2}$ was 4.19 hours, and AUC was 1,348 ng · hr/mL, and the last measurable time in serum was at 8.5 hours. These numbers were prolonged for measurement in milk: T_{max} of 1.75 hours and last measurable time at 32.5 hours. This study suggests that the estimated milk withdrawal time (based on the calculation of 10 times the $T_{1/2}$) should be 80 hours or 4 days, which was four times greater than suggestions by FARAD. Another disadvantage of local anesthetic use is the technical skill required to perform such blocks and a time delay between administration and maximum anesthetic effect. Therefore effective and

practical application in typical production systems, especially those involving beef cattle, is unlikely.

Another method for providing anesthesia during castration is through the use of caudal epidural injections. Lidocaine, alpha-2 agonists, ketamine, and opioids are typically deposited in the sacrococcygeal (high caudal epidural) space (Edmondson *et al*., 2007). Disadvantages to these procedures are the technical skill and training needed to perform them, the added cost of a veterinarian to perform such technical procedures, risk of infection by introducing bacteria to the epidural space, and affects of the drugs on locomotion and possibility of recumbency.

The use of α-agonists, especially xylazine, has become a popular choice for standing sedation in cattle (Lin and Riddell, 2003). Xylazine exhibits potent sedative, analgesic, and muscle relaxant effects and cattle have been found to be 10 times more sensitive to these effects than horses (Abrahamsen *et al.,* 2008). Xylazine acts by binding to α-2 receptors in the central nervous system in the dorsal horn of the spinal cord. This binding leads to central nervous system depression promoting mild sedation and/or recumbency (depending on the dose), decreased sympathetic tone, and simulation of noradrenaline which acts on inhibitory pathways leading to decreased transmission of nociception in the dorsal horn of the spinal cord (Stilwell *et al.,* 2010). The use of xylazine in cattle has some pharmacokinetic data in the published literature. For example in a study by Lin and Riddell (2003), effects of xylazine and detomidine with or without butorphanol were studied in dairy cattle. Both drugs significantly decreased heart and respiratory rate with the duration of sedation being 49.0 ± 12.7 minutes for xylazine and 47.0 ± 8.1 minutes for detomidine (as determined by behavioral scoring). Garcia-Villar *et al.* (1981) administered 0.2 mg/kg of xylazine by intravenous and intramuscular routes and determined the pharmacokinetic parameters of each. The elimination half-life was 36 minutes, elimination rate constant was 0.022 min^{-1}, clearance was 42 $ml \cdot kg^{-1} \cdot min^{-1}$, and V_d of 1.944(L/kg) after intravenous administration. However, the pharmacokinetics of xylazine after intramuscular administration were not able to be determined because xylazine could not be detected in bovine plasma at this dosage. Another study by Bayer *et al.* (1975) evaluated tissue residues after 0.33 mg/kg IM injection of C-radiolabelled xylazine found residues to be <0.04 p.p.m. after 72 hours at the injection site. Xylazine

has a recommended 4 day slaughter withdrawal interval and 24 hour milk withdrawal interval as suggested by FARAD (Haskell *et al*., 2003). Disadvantages of this drug is the short duration of action (providing sedation and analgesia only for a few hours (Nolan *et al*., 2001)) and the varying level of sedation provided depending on the demeanor of the animal (Abrahamsen *et al.*, 2008).

A common N-methyl-D-aspartate (NMDA) antagonist used in cattle is ketamine. During painful stimuli, glutamate, a neurotransmitter, acts on NMDA receptors in the central nervous system leading to sensitization and hyperalgesia (Lamont, 2008). The physiological action of ketamine is both centrally and peripherally, blocking predominatly NMDA receptor sites but has other sites of action including opioid, AMPA, GABA-A, and KA receptors (Pozzi *et al.*, 2006). By blocking such receptors, ketamine blocks pain by providing analgesia and preventing central sensitization (McCartney *et al.* 2009). The analgesic effects of ketamine are preported to occur at subanesthetic doses (Abrahamsen *et al.*, 2008). A study using 0.05 mg/kg xylazine and 0.1 mg/kg of ketamine in 4 to 6 month bull calves found the following pharmacokinetic parameters for ketamine and norketamine: volume at steady state was 389.87 ± 108.73 ml/kg, volume of the central compartment of 132.82 ± 68.23 mL/kg total clearance of 24.97 ± 4.46 mL/min/kg (Gehring *et al*., 2008). Ketamine, at a dose of 10 mg/kg given intramuscularly, has a meat withdrawal time of 3 days and a milk withdrawal time of 48 hours (Craigmill *et al.*, 1997).

Opioids are a class of analgesics exhibiting effects on several targets along the nociceptive pathway including the dorsal horn of the spinal cord, the thalamocortical structures, and descending antinociceptive pathways (Lamont, 2008). Activation of opioid receptors decreases the release of excitatory transmitters such as substance P from primary afferent neurons leading to inhibition of nociceptive transmission. Secondly, binding to opioid receptors causes enhanced potassium efflux leading to hyperpolarization of post synaptic afferent neurons causing inhibition of ascending pathways (Lamont, 2008). Butorphanol is one of the only opioids that have been described in cattle during painful procedures. Butorphanol is a mixed agonist antagonist, exerting its actions at Kappa receptors and antagonizing μ receptors (Lumb *et al.*, 1996). Most commonly it is combined with another form of analgesia to potentiate its affects.

For example, in a study by Dodman *et al.* (1992), calves undergoing a laparotomy were provided 0.02 mg/kg IV xylazine with or without the use of 0.05 or 0.07 mg/kg butorphanol IV. It was found that calves administered butorphanol, especially at higher doses, responded less to cutaneous needle-pricks and forceps pinches and fewer cattle needed supplemental local anesthetic once the procedure began.

While opioids and NMDA receptor antagonists offer potent analgesia, a major disadvantage is they are designated by the U.S. Drug Enforcement Agency as Schedule 3 drugs, and therefore would be restricted to use only by a licensed veterinarian (DEA 2010). The added regulatory issues associated with these drugs also make their use in the production scheme more costly and time consuming to the producer.

Several non-steroidal anti-inflammatory drugs have been used in castration and dehorning studies due to low cost, ease of administration, and wide availability. NSAIDS act by inhibiting cyclo-oxygenase activity which in turn prevents the liberation of prostaglandins and other mediators of inflammation (Anderson and Muir., 2005). Two forms of COX exist: COX-1 which is constitutively expressed and COX-2 which is induced and plays a role in the generation of inflammation and hyperalgesia (Nolan *et al.*, 2001). Prostaglandins play a role in lowering the activation threshold for afferent neurons and increasing their sensitivity to inflammatory mediators such as bradykinin. This can lead to a phenomenon known as hyperalgesia (Muir and Woolfe, 2001). Non-steroidal inflammatory drugs also exert effects on TxA_2, PGE_2, LTB_4, β-glucuronidase, and bradykinin (Lees *et al.*, 2004).

Presently, flunixin meglumine is the only non-steroidal anti-inflammatory drug with an FDA approved label in cattle. Other NSAIDS used in the literature include ketoprofen, carprofen, aspirin, phenylbutazone, and meloxicam. Effects of the mentioned NSAIDs on pain biomarkers are described below. The pharmacokinetics of ketoprofen has been investigated and found after administration of 3 mg/kg, calves experienced a short elimination half-life of 0.42 hours, volume of distribution of 0.2 to 0.22 L/kg, and high clearance of 0.32 to 0.33 L/kg/h (Landoni *et al.*, 1995). The pharmacodynamics of the drug were also evaluated in this study and found to significantly inhibit production of serum TxB_2 for 12 hours, PGE_2 for 24 hours, and bradykinin for 25 hours after administration however did not significantly change LTB_4

concentrations. The drug was also found to be a non-selective inhibitor for COX-1 and COX-2.

Aspirin is a non-selective COX inhibitor. A study by Myers *et al.*, (2008) looked at the *in vitro* effects of aspirin on concentrations of PGE_2, bradykinin, tumor necrosis factor α (TNFα), and COX - 2 production in cattle to determine its anti-inflammatory effects. This study found that a 300 µM concentration of aspirin in culture media significantly decreased PGE_2 production as compared to control and that aspirin significantly decreased bradykinin and TNFα production. Its use of aspirin in the literature however during pain studies in cattle is deficient. The only study to date examining possible analgesic effects of aspirin in cattle was by Coetzee in 2007. This study is mentioned more in detail under the castration section. A previous study by Gingerich *et al.* (1975), found a single IV dose of 50 mg/kg of 20% sodium salicylate to have the following pharmacokinetic parameters: $t_{1/2}$ of 0.54 ± 0.04 hrs, V_d or 0.24 L/kg. In the same cattle, 50 mg/kg and 100 mg/kg were given orally as a single dose and the absorption $t_{1/2}$ was 2.91 hours, the elimination $t_{1/2}$ was 3.70 hours, C_{max} was 2 to 4 hours, and the bioavailability was 70%. In the same study, multiple dosing therapy was initiated with the oral administration of 50 mg/kg or 100 mg/kg administered at 12 hour intervals for 5 consecutive days. After the 9^{th} dosing, serum concentrations were found to range from 11 to 25 µg/mL (50 mg/kg dose) and 45 to 65 µg/kg (100 mg/kg dose). Another pharmacokinetics study by Whittem *et al.* (1996) found after an IV bolus of 26 mg/kg of salicylate, the $T_{1/2}$ was 30.8 hours, the V_d was 199.5 mL/kg, the clearance was 263.9 ml/h · kg, MRT was 48.8 minutes, k_{el} was 1.35 hr^{-1}, and AUC was 106.0 mg · h/L. Another study by Bertoni *et al.* (2004) used acetyl-salicylate as an anti-inflammatory to prevent anorexia, reduction of milk yield and reproductive traits during stress associated with the transition period. Intramuscular injections of acetyl-salicylate of 15 g/d for the first 3 days and 7.5 g/day for the next two days was associated with higher milk yield and better fertility traits, however was also associated with higher frequency of metritis. Coetzee *et al.* (2007) found the following pharmacokinetic parameters of sodium salicylate: C_{max} of 41.34 ± 2.01 µg/mL after (100 mg/kg PO administration), T_{max} of 2.08 ± 0.49 hours, a $T_{1/2}$ of 4.31 ± 0.42 hours, bioavailability of 61.05 ± 0.02 %. FARAD suggests a 24 hour meat and milk withdrawal time for salicylate (Smith *et al.*, 2008).

As mentioned in the previous section, current pain management strategies are shifting to a multi-modal approach. This rational has stemmed from the synergism observed from combinations of analgesics, a decreased dose needed when combining analgesics, and consequently a decreased risk for adverse side effects (Lamont, 2008). For example, combined with xylazine and butorphanol, a low dose ketamine is purported to provide standing sedation in cattle (Abrahamsen, 2008). Coined the "ket-stun" or "5 – 10 – 20 technique", this standing sedation is provided when subanesthetic doses of ketamine are combined with a chemical restraint technique, typically butorphanol and xylazine (Abrahamsen, 2008).

It should be noted the use of these drugs in this manner would at the current time be considered extra-label. Under the Animal Medicinal Drug Use Clarification Act. Requirements for use of drugs in an extra-label manner include the following: 1) must be used under the supervision of a veterinarian 2) the drug must be FDA approved in humans or animals 3) use must be for therapeutic purposes 4) the drug must not be given in the feed 5) the drug must not leave a violative residue and 5) the drug must not be prohibited from use. In addition in food animals, extra-label drug use may only occur if a drug does not already exist with a label for the intended use, an extended withdrawal time has been established and enforced, a careful diagnosis or condition has been made by the veterinarian, and the identity of the animal must be maintained.

Castration in Cattle

Castration of calves is performed for a variety of reasons including: elimination of breeding, reduction in aggressive behavior, improved safety for handlers, decreased incidence of dark-cutting beef, and the production of higher quality grade meat (AVMA 2009). There are several methods of castration including surgical (newberry knife, scalpel blade, emasculator), burdizzo, application of bands or rubber rings, chemical, and immunocastration. Different countries tend to employ some methods over others. For example, a survey in New Zealand by Stafford *et al.* (2000) found out of 2,825 farmers, 85% used rubber ring, 18% used surgical castration, and <1% used the clamp method. In a survey by Coetzee *et al.* (2010) of bovine veterinarians, the most common methods

used in the United States were surgical castration with a scalpel blade (57%), followed by manually twisting (44%), or the use of an emasculator (36%). The same survey found that around 70% of the responding veterinarians usually perform the castrations if calves weigh over 270 kilograms while over 80% of producers perform the castrations if calves are less than 90 kilograms. In the United Kingdom, Kent *et al.* (1996) reported that 43% of farmers used burdizzo, 39% used surgical castration, 32 % used rubber rings, and 10% used more than one method of castration. The use of local anesthetic was 4%, 6%, and 35% respectively for each method. There remains debate over pain experienced by banding versus other forms of castrations.

Research on pain caused by castration, specifically measured by cortisol concentrations and ADG, has been examined extensively (Table 1). Pain associated with castration is believed to be manifested by certain behaviors including kicking of the hind legs, tail swishing, hoof stamping, head turning, restlessness, abnormal posture, decreased food intake, reduced activity, and increased recumbency (AVMA 2009). Other biomarkers that have been used to evaluate pain have included serum cortisol, substance P, inflammatory mediators and cytokines such as interferon – γ, white blood cells, acute-phase proteins, adrenocorticotropic hormone, heart rate, electrodermal activity, vocalization, and chute exit speed.

Some studies have examined the effects of age on the pain response during castration of calves. A covariate for the amount of pain experienced by the calf depending on age is scrotal circumference which usually increases with age. A study by King *et al.* (1991) looked at the effects of castration on two different age groups of cattle: 78 days and 167 days. This study found at 3 hours post castration, cortisol raised to 71.7 nmol/L, 49.7 nmol/L, and 53.4 nmol/L for surgical, burdizzo, and control castration for 78 day old calves, respectively. For 167 day old calves, cortisol raised to 122.5 nmol/L, 106.5 nmol/L, 66.5 nmol/L for surgical, burdizzo, and control castration, respectively. While the C_{max} for 167 day old calves was higher than 78 day old calves, no statistical data was compared across age groups. Another study by Robertson *et al.* (1994) between 6, 21, and 42 day old calves found that cortisol C_{max} was significantly higher in 6 and 42 day old calves than 21 day old calves and the plasma cortisol concentrations remained higher for a longer period of time in 42 day old calves. It was also found that in all age

groups, surgical castration elicited the greatest peak cortisol response in all three age groups which was significantly higher than rubber ring, burdizzo, and control.

Other studies have compared methods of castration on cortisol concentrations. A study by Molony *et al.* (1995) using 1 week old Ayrshire bull calves found surgical castration produced the greatest rise in cortisol concentrations (approximately 100 nmol/L) as compared to burdizzo, rubber ring, and a combination of rubber ring and burdizzo castration. By 24 minutes, cortisol response in calves undergoing surgical and burdizzo castration were significantly higher than the other treatment groups and by 132 minutes, there was no significant difference between any of the treatment groups, including un-castrated controls. A study by King *et al.* (1991) compared the effects of surgical versus burdizzo castration on the cortisol response experienced by 167 day old (5.5 month old) calves. This study found at 3 hours post castration, surgical castration elevated serum cortisol levels to 122.5 ± 11.6 nmol/L which was not significantly different from burdizzo castration which elevated levels to 106.5 ± 22.9 nmol/L. However by 6 hours, surgical castration (75.9 ± 14.9 nmol/L) was significantly greater than burdizzo castration (42.2 ± 11.3 nmol/L). A study by Fisher *et al.* (1996) comparing similar castration methods found that surgical castration in 5.5 month Friesian bulls without anesthesia invoked a significantly higher spike in cortisol (126.9 nmol/L) than burdizzo castration (86.6 nmol/L) at 0 to 2 hours post castration and for cortisol C_{max}. However this difference was short-lived and by 2 to 6 hours after castration, there was no significant difference between treatment groups.

Another study by Fisher *et al.* (2001) compared surgical to banding castration in 14 month old bull calves. This study found no significant difference in cortisol between the two methods when cortisol concentrations were measured from 1 to 14 days thereafter. However in a study by Chase *et al*. (1995), 20 to 22 month old bull calves castrated surgically with a Newberry knife experienced a significantly higher cortisol response immediately after castration (54.6 nmol/L) as compared to banded calves (42.5 nmol/L). Pang *et al.* (2006) reported that banding in 5.5 month old Holstein/Friesian bull calves produced a significantly higher integrated cortisol response (147.6 ± 11.0 nmol/L· h) than burdizzo castration (92.2 ± 11.3 nmol/L· h) from 0 to 2 hours. The same study examined the effects of 1.4 mg/kg carprofen given IV 20 minutes prior to banding and

reported a significantly lower integrated cortisol response at 2 to 6 and 6 to 12 hours, in carprofen treated banded calves (85.8 ± 13.5 nmol/L · h and 72.6 ± 11.8 nmol/L · h, respectively) as compared to untreated banded calves (102.4 ± 12.8 nmol/L· h and 110.6 ± 11.2 nmol/L· h respectively). However a significant difference was not observed between carprofen treated, burdizzo castrated calves and untreated burdizzo castrated calves. Calves treated with carprofen and undergoing burdizzo castration demonstrated the lowest C_{max} for serum cortisol. This was significantly lower than untreated burdizzo calves, untreated banded calves, and carprofen treated banded calves. Chemical castration versus surgical castration was investigated by Cohen *et al.* (1990). The AUC for cortisol from 0 to 6 days was significantly greater in surgically castrated calves. C_{max} was 64.0 ± 1.8 nmol/L at 6 hours for surgically castrated calves and 46.4 ± 1.4 nmol/L at 3 hours for chemically castrated calves.

A study by Stafford and Mellor (2002) examined both the method of castration on cortisol response (rubber ring, banding, surgical, emasculator, and clamp) and the effect of analgesic treatment. This study compared control castrated calves with calves castrated by the different methods using 3 mL of 2% lidocaine as a local block or 3 mg/kg IV ketoprofen, 20 minutes prior to castration in addition to the local block. The AUC for untreated calves was higher for ring, banding, and surgical when compared to control calves for the first 4.5 hours. The AUC for calves treated with a lidocaine block and castrated surgically or by emasculator were significantly higher than control, ring, clamping, and banding techniques from 0 to 4.5 hours. There was no significant difference in cortisol concentrations among all calves treated with ketoprofen and lidocaine and all castration methods for the first 4.5 hours. For ring and banding methods, treatment with lidocaine and combination of lidocaine and ketoprofen significantly attenuated cortisol responses. Lidocaine alone did not significantly attenuate cortisol response associated with surgical castration. However the combination of lidocaine with ketoprofen did attenuate plasma cortisol response. There were no differences between treatment groups in calves castrated by emasculator.

A common method for evaluating pain associated with castration is the measurement of serum cortisol concentrations for a period of time after castration. Several studies have measured parameters such as individual cortisol concentrations at

designated time points post castration, C_{max}, T_{max}, and AUC and examined the effect of analgesic drug administration on these parameters. A study by Mellor (1992) using the rubber ring method of castration in calves within the first seven days of life found no differences between time points for plasma cortisol concentrations in calves castrated by application of rubber rings and calves handled and not castrated for 240 minutes post event. A study by Fisher *et al.* (1997) found that surgical castration significantly elevated cortisol C_{max} (118.9 nmol/L) when compared to uncastrated calves (35.6 nmol/L) and caused a significantly higher AUC from 0 to 12 hours for castrated (480.6 nmol/L · hr) as compared to uncastrated calves (170.5 nmol/L· hr). A study by Coetzee *et al*. (2008) found no significant difference in cortisol C_{max} and AUC between calves surgically castrated (128.80 ± 9.06 nmol/L, 137.87 ± 6.11 h · nmol/L) and simulated castration (136.58 ± 31.94 nmol/L, 144.50 ± 39.98 h · nmol/L)

Other studies evaluated differences in cortisol concentration between different analgesic and anesthetic treatment regimens. Nerve blocks are commonly performed during castration to provide anesthesia. For example, a study by Stewart *et al.* (2010) with 4 month old Friesian calves found local anesthesia with 5 mL of 2% lidocaine injected into each testicle and surrounding tissues significantly curbed elevations in cortisol response in calves compared with no treatment or surgical castration. Thuer *et al.* (2007) evaluated cortisol response at time periods, AUC, and C_{max} for burdizzo castration with and without 10 mL of 2% lidocaine injected into the spermatic cord and subcutaneously as well as for rubber ring castration with or without the same anesthesia. This study found calves left untreated or castrated by burdizzo had significantly higher cortisol concentrations at 20 minutes and a significantly higher AUC from 0 – 1 hours and C_{max} as compared to calves treated with local anesthesia. The study also found that calves left untreated and then castrated by rubber ring and a significantly greater cortisol concentrations from baseline at 1.5 hours and 4 hours after castration, however this was not significantly higher than calves treated with local anesthesia. A study by Boesch *et al.* (2008) found calves receiving a lidocaine or bupivicaine block involving the spermatic cord and surrounding structures had a significantly lower AUC from 0 to 11 hours than calves receiving no anesthesia. Lidocaine had a significantly lower AUC than bupivicaine for this time period.

In other studies, other forms of analgesia are provided such as opioids, NSAIDs, and/or α-2 agonists. For example, a study by Faulkner *et al.* (1992) using 268, 6 – 9 month old crossbred Friesian bull calves found no difference in serum cortisol concentrations between calves treated with 0.07 mg/kg butorphanol and 0.02 mg/kg xylazine IV, 90 seconds prior to castration (56.5 nmol/L) versus no analgesia (62.8 nmol/L) at 3 days after the event. However by day 7, untreated calves had a significantly higher cortisol concentration (62.5 nmol/L) than those treated (48.9 nmol/L) and those not castrated (51.6 nmol/L). A study by Ting *et al.* (2003b), in 50, 11 month old Holstein x Friesian bull calves surgically castrated found that calves untreated experienced a significantly higher cortisol concentration (40.65 nmol/L) and AUC (324 nmol/L · h) than calves treated with 3 mg/kg ketoprofen IV 20 minutes prior, `1.5 mg/kg ketoprofen IV 20 minutes and immediately prior, and 1.5 mg/kg ketoprofen IV 20 minutes and immediately prior and 3 mg/kg ketoprofen 24 hours post castration. Coetzee *et al.* (2007) examined the effects of 50 mg/kg of sodium salicylate given IV immediately prior to castration versus 50 mg/kg acetylsalicylic acid given orally immediately prior to castration by a newberry knife and Henderson castration tool on cortisol. This study found that calves given oral salicylate experienced a higher C_{max} as compared to control calves and that calves administered salicylate IV actually had a significantly lower C_{max} when compared to the oral route.

Other studies have evaluated the multimodal approach to providing pain relief to calves. For example, Early and Crowe (2002) found that 9 mL of 2% lidocaine injected to each testis 20 min prior to surgical castration failed to reduce the AUC for serum cortisol response (360.9 ± 41.9 nmol/L·h^{-1}) as compared to surgical castration without treatment (485.9 ± 76.4 nmol/L·h^{-1}) in 5.5 month old Friesian bull calves. However, treatment with 3 mg/kg ketoprofen IV 20 min prior to castration and ketoprofen in addition to the previously mentioned local anesthesia did significantly lower the serum AUC for cortisol (215.5 ± 38.3 nmol/L·h^{-1} and 324.5 ± 54.5 nmol/L·h^{-1}, respectively). In that same study, the C_{max} for cortisol was highest in untreated surgical castration (126.4 ± 17.0 nmol/L) versus ketoprofen (68.2 ± 14.1 nmol/L), local anesthesia (60.9 ± 7.42 nmol/L), or combination of ketoprofen and local anesthesia (79.5 ± 1.1 nmol/L). Marti *et al.* (2010) found that injection of 3 mL of 2% lidocaine into each testis and 2 mL in the

spermatic chord as well as an IM dose of 3 mg/kg of flunixin meglumine 20 minutes prior to application of bands in 3 month old Holstein calves actually experienced a significantly lower mean elevation in serum cortisol (5.6 ± 1.56 nmol/L) as compared to calves remaining intact (13.2 ± 1.56 nmol/L) from 30 to 180 after application. The AUC from 0 to 180 minutes was higher ($P = 0.06$) as well for calves remaining intact as compared to those castrated. Ting and others (2003b) found that administration of either 3 mg/kg of ketoprofen IV 20 minutes prior to castration or a lidocaine block of the spermatic cord 20 minutes prior to castration, or a caudal epidural with 0.05 mg/kg of xylazine and 0.4 mg/kg of 2% lidocaine 10 minutes prior to burdizzo castration in 13 month old calves significantly reduced the peak serum cortisol response as compared to untreated burdizzo castrated calves. The same study also found that calves treated with ketoprofen alone had the greatest effect in attenuating the cortisol response ($P < 0.05$) following castration among all treated and untreated castrated calves from 2 to 12 hours and total area under the curve after castration. By 3 days there were no significant differences between treatment groups. A study by Stillwell *et al.* (2008) found that the use of a caudal epidural with 2% lidocaine plus a subcutaneous injection in the neck of 5 mL (1.4 mg/kg) carprofen 5 minutes before castration procedures had a lower serum cortisol response at 6, 24, and 48 hours as compared to untreated calves and those calves receiving an epidural of 2% lidocaine alone. The study also found that substituting flunixin for carprofen given in the same manner with a caudal epidural also significantly lowered cortisol response as compared to untreated calves at 6 hours.

Dehorning in Cattle

Dehorning or disbudding in cattle is performed for a variety of reasons including: safety for handling, decreased incidence of carcass wastage due to bruising, less feeding trough space needed, decreased risk of injury to other cattle, increased value of the animal, and fewer aggressive behaviors exhibited (AVMA 2010). Disbudding is a method of removing horns in calves up to around 8 weeks old when horn buds are 5 – 10 mm long and can be removed via a heated disbudding iron (Stafford *et al.*, 2004). Once

horns grow longer, they must be removed by amputation. There are several different methods of performing this including manual amputation (barnes, keystone, gauges, saws, gigli wire), hot iron (buddex, rhineheart, Portasol), and the application of caustic paste (Duffield, 2008).

Some studies have examined the effects of dehorning on cortisol response. For example, a study by Schwartzkopf-Genswein *et al.* (2005) using 26 to 59 day old Holstein calves evaluated the cortisol response to dehorning over a period of 3 consecutive days. Cortisol response was measured in calves that were not dehorned, sham dehorned, and then dehorned by hot iron without the addition of analgesia or anesthesia. The study found that elevations in cortisol were significantly higher from 0 to 30 minutes after dehorning as compared to between both 60 to 240 minutes and 24 to 48 hours. Additionally, from 0 to 60 minutes, cortisol response was greater for calves dehorned as compared to sham dehorning or no dehorning. Another study by Laden *et al.* (1985) looked at the effects of electric dehorning in 18 Holstein calves at 8 weeks of age on cortisol response. The study found calves dehorned at 8 weeks of age had significantly higher cortisol response at 5, 15, 30, and 60 minutes post dehorning than calves not dehorned.

Some studies focus on effects of dehorning on pain responses between different ages of calves. As a covariate in one study by Milligan *et al.*, (2004), serum cortisol concentrations prior to dehorning and then at 3 and 6 hours post dehorning were adjusted based on calf age (range of 2 days to 2 weeks old). It was found that older calves had significantly lower serum cortisol concentrations immediately before ($P < 0.01$), 3 hours after ($P < 0.05$), and 6 hours after ($P < 0.01$) dehorning.

Since several methods of dehorning exist, some of the literature focuses on differences between techniques used to remove horns based on relative changes in biomarkers for pain. A review of dehorning by Stafford and Mellor (2005b) ranked the severity of different methods of dehorning with the least severe being dehorning after local anesthetic, xylazine, and/or NSAID administration and the most severe being amputation dehorning with wound cautery. Disbudding ranked in the middle of all the procedures. A study from Wohlt *et al.* (1994) with 3 to 4 week old Holstein calves compared Buddex and cautery dehorning. This study found no significant difference in

C_{max} between Buddex (57.1 nmol/L) and cautery (60.4 nmol/L) methods. A study using scoop versus cautery dehorning by Petrie *et al.* (1995) using 6 to 8 week old Friesian calves found scoop dehorning without the provision of anesthesia or analgesia produced a significantly higher cortisol AUC from -70 minutes to 2 hours post procedure as compared to treated and untreated sham dehorning and treated and untreated cautery dehorning. The study also found no significant difference in the cortisol AUC from 2.5 to 9 hours post procedure between calves treated with a cornual nerve block using 3 mL of 2% lidocaine administered 20 minutes prior (14,024.0 ± 1,206.4 nmol/L·min) and then scoop dehorned and calves untreated and then scoop dehorned (9,110.2 ± 2467.6 nmol/L·min). Furthermore, the calves in these two treatment groups had a significantly higher cortisol AUC during 2.5 to 9 hours as compared to calves sham dehorned (3,332.9 ± 1247.1 nmol/L·min), cautery dehorned (4,723.4 ± 935.3 nmol/L·min), and calves receiving a cornual nerve block and then sham (3,404.6 ± 935.3 nmol/L·min) or cautery (5,799.4 ± 1,528.5 nmol/L·min) dehorned. Another study by Sylvester *et al.* (1998b) compared the differences in cortisol concentrations in calves dehorned by 4 different methods of dehorning: barnes scoop dehorning, guillotine shears, a butcher's saw, and embryotomy wire. This study found no differences among treatment groups during the 36 hours post dehorning for cortisol, except for calves dehorned by guillotine shears had a significantly lower cortisol at 2 to 2.5 hours post procedure. The cortisol C_{max} and integrated cortisol response was not statistically different among treatment groups.

A study by McMeekan *et al.* (1997) selected a technique and then investigated differences in cortisol response to variations in performing the technique of scoop dehorning. Shallow scoop dehorning versus deep scoop dehorning was performed in 30, 14 to 16 week old Friesian calves and no significant difference was found between rises in cortisol concentrations or the integrated cortisol response from 0.25 hours after dehorning to 5 hours after dehorning. The only difference noted was cortisol concentrations in calves undergoing shallow scoop dehorning returned to control values by 8 hours while deep scoop dehorning calves returned by 6 hours. Another study by Sutherland *et al.* (2002a) studied the effects of scoop dehorning versus scoop dehorning with cautery, both with and without the addition of local anesthesia. This study found calves undergoing dehorning had significant elevations in cortisol from control calves

from 0.5 hours to 6 hours and then again at 13 to 15 hours. Interestingly, however while local anesthesia with lidocaine and bupivicaine 15 minutes prior to procedures and then again at1 hour and 45 minutes post-procedure abolished a rise in cortisol concentrations from 0 to 5 hours, calves experienced a significant increase that was greater than calves dehorned without anesthesia at 6 and 7 hours. Calves receiving local anesthesia plus cautery in addition to scoop dehorning had almost no change in cortisol concentrations throughout the 24 hour period measured. A similar study by Sylvester (1998a) also evaluated differences in the integrated cortisol response of dehorning by scoop dehorning with or without cautery in calves untreated or treated with a cornual nerve block with 6 mL of 2% lidocaine 30 minutes prior to procedures. This study found no significant difference in the integrated cortisol response between calves dehorned by scoop alone (283.5 ± 48.6 nmol/L · hr) or scoop with cautery (210.6 ± 56.7nmol/L · hr). Cortisol concentrations in both of these treatment groups were significantly higher than calves treated with a cornual nerve block and then undergoing dehorning by scoop (140.4 ± 40.5 nmol/L · hr) or scoop plus cautery (70.02 ± 19.0 nmol/L · hr).

In other dehorning research, the effect of analgesic therapy on cortisol response has been measured. For example, Stilwell *et al.* (2008) looked at the effects of treatment with 2.2 mg/kg flunixin meglumine injected 1 hour prior to disbudding versus 5 minutes prior to disbudding versus saline injection in 10 to 40 day old calves. This study found all groups experienced significantly higher cortisol concentrations 1 hour after dehorning procedures, but by 3 hours, calves treated with flunixin were not significantly different from undehorned animals, while cortisol concentrations in animals treated with saline were significantly higher. However, cortisol concentration in saline treated calves and calves treated with flunixin were not significantly different from each other and by 6 to 24 hours, all groups experienced similar cortisol concentrations.

Many studies look at the effects of nerve blocks on cortisol response to dehorning. Doherty *et al.* (2007) found that 10 – 12 week old Holstein calves experienced a significantly lower cortisol response at 30 and 60 minutes post dehorning after a cornual nerve block of either 10 mL of 5% lidocaine or 10 mL of 2% lidocaine administered 30 minutes prior to dehorning as compared compared to untreated, dehorned calves. It was also noted that at 60 minutes post procedure, 10 mL of 5% lidocaine significantly

attenuated cortisol response in comparison to 10 mL of 2% lidocaine administered 30 minutes prior to dehorning. McMeekan *et al.* (1998a) evaluated the effect of timing of cornual nerve block administration using 0.25% bupivicaine on cortisol response in 3 to 4 month old calves. They found calves administered a cornual nerve block at 20 minutes prior to dehorning and then again 4 hours post dehorning experienced a significantly lower cortisol AUC (9,556 ± 1,674 nmol/L · min) than calves dehorned alone (18,111 ± 2,219 nmol/L · min), calves administered the cornual nerve block only at 20 minutes prior (16,257 ± 1,925 nmol/L · min), and calves administered the cornual nerve block immediately prior (11,397 ± 2,270 nmol/L · min). Another study by McMeekan *et al.* (1998b) found between calves undergoing scoop dehorning or scoop dehorning with a cornual nerve block using 6 mL's of 0.25 % bupivicaine administered 20 minutes prior or scoop dehorning with a cornual nerve block immediately prior to dehorning and then calves administered a cornual nerve block 20 minutes prior and then again at 4 hours post, that calves in the latter treatment group had a significantly lower AUC from 0 to 9.33 hours for cortisol as compared to the other treatment groups. However for the first 3.83 hours, all calves receiving a cornual nerve block experienced a significantly lower AUC cortisol response as compared to scoop dehorning without treatment. Additionally, a study by Boandl *et al.* (1989) also found no significant difference between calves treated with a cornual nerve block of 2% lidocaine with a 1:100,000 dilution of epinephrine and hot iron dehorned versus untreated and dehorned calves. Graf and Senn (1999) found a cornual nerve block with 2% lidocaine significantly diminished cortisol response in 4 to 6 week old calves as compared to those injected with saline from 20 to 90 minutes post dehorning.

Some studies have looked at cortisol responses when a multi-modal approach to pain management is utilized and a local anesthetic is combined with other forms of analgesia. For example, Grondahl-Nielson *et al.* (1999) evaluated the effects of treatment with cornual nerve block, 0.2 mg/kg of xylazine and 0.1 mg/kg butorphanol IM, or no treatment on cortisol response in 4 to 6 week old Friesian calves. This study found that cortisol increased significantly for calves in the untreated and dehorned group immediately after dehorning as compared to the other treatment groups. However, the increase was temporary, and from 10 minutes and beyond there was no significant

difference among treatment groups. A study by Sutherland *et al.* (2002b) in 3 to 4 month old Friesian calves evaluated the effects on cortisol response of a cornual nerve block alone or a cornual nerve block combined with 4 to 5.3 mg/kg phenylbutazone IV, or a cornual nerve block with 3 to 3.75 mg ketoprofen IV. The study found the only treatment that significantly tapered the cortisol AUC for the first 24 hours after dehorning procedures were calves administered a cornual nerve block plus ketoprofen. In a study mentioned previously by Milligan *et al* (2004), calves treated with a cornual nerve block of 5 mL of 2% lidocaine mixed with 0.05 mg/mL epinephrine plus 0.03 ml/kg of 10% ketoprofen given IM 10 minutes prior to procedures experienced significantly lower cortisol concentrations from 0 to 3 hours compared to calves given a cornual nerve block alone. A study by Heinrich *et al.* (2009) found in 6 to 12 week old calves treated with a cornual nerve block with 5 mL of 2% lidocaine and 0.05 mg/mL of epinephrine 10 minutes prior to hot iron cauterization dehorning experienced significantly higher serum cortisol concentrations from 0 to 6 hours post dehorning as compared to calves administered the cornual nerve block plus a single IM dose of 0.5 mg/kg meloxicam. However no differences were noted at 24 hour after dehorning. Another recent study by Duffield *et al.* (2010) found there to be no difference in cortisol response at 3 and 6 hours post electro-cautery dehorning in 4 to 8 week old calves treated with 3 mg/kg ketoprofen IM plus a cornual nerve block versus calves given an IM injection of sterile saline plus a cornual nerve block. Local anesthesia was used during disbudding procedures in a study with 3 to 5 week old calves by Stilwell *et al*. (2009). They found that calves administered 2.2 mg/kg flunixin IV plus a cornual nerve block had decreased cortisol concentrations at 3 hours post procedures as compared to those calves given the cornual nerve block alone and untreated calves. However, by 6 hours and beyond, no significant difference in cortisol levels among treatment groups were observed. Another study by Stilwell *et al.* (2010) examined the effects of 0.2 mg/kg xylazine administered IM 10 minutes prior to disbudding alone or in combination with 5 mL 2% lidocaine administered as a cornual nerve block on the cortisol response of calves disbudded by a hot – iron. This study found no treatment effect at mitigating cortisol response to disbudding, as both treatment groups were significantly higher than control calves from 10 to 60 minutes post disbudding. A study by Lepkova *et al.* (2007) evaluated

differences in cortisol response for C_{max}, T_{max}, and time to return to baseline for calves undergoing general anesthesia with IV xylazine (0.1 mg/kg) and ketamine (2 mg/kg), sedation with xylazine (0.2 mg/kg) plus local anesthesia with 2% lidocaine, or local anesthesia alone with 2% lidocaine injected by the zygomatic nerve. This study found C_{max} for cortisol to be lowest for calves undergoing sedation plus local anesthesia (82.53 ± 6.04 nmol/L) which was significantly less than calves receiving local anesthesia alone (113.86 ± 25.65 nmol/L). Cortisol concentrations for calves receiving general anesthesia fell in between these two treatment groups (110.62 ± 45.96 nmol/L) and was not significantly different from either treatment group. Calves receiving sedation and general anesthesia also had the shortest T_{max} and the fastest return to baseline serum cortisol concentrations.

Conclusion

The literature available focusing on pain management in cattle during castration and or dehorning is plentiful. As demonstrated, there has been several studies looking at the effects of castration and/or dehorning on plasma cortisol concentrations. Additionally, several analgesic regimens have been used in effort to relieve pain during these procedures, with varying results. Until a drug obtains a label for analgesia, continued research addressing this matter will need to be performed. The study in chapter 2 will present the use of oral sodium salicylate and an injectable combination of xylazine, ketamine and butorphanol as other analgesic regimens that could be used during castration and dehorning and the effects of each on biomarkers of pain.

Tables

Table 1.1 Cortisol and Castration

Author	Size	Age	Method	Treatment Groups	Sampling Schedule	Cortisol Concentrations	Significance
Fell 1986	19	4 – 11 wks	Rubber ring Surgical	a) Rubber ring b) Surgical c) Control	Immediately prior, 15, 30, 60 min, 2, 3, 4, and 24 h, 6 d	C_{max} (nmol/L) a) 10.2 ± 2.6 b) 3.2 ± 0.6 c) 1.1 ± 0.1 4 h (nmol/L) a) 1.5 ± 0.2 b) 0.9 ± 0.1 c) 1.2 ± 0.2	Between 15 min and 2 h, (b) was significantly higher than (a).
Cohen 1990	17 Holstein	28 – 36 wk	Surgical Chemical	a) Control b) Surgical c) Chemical with α – hydroxyproprionic acid	0, 2, 3, 6, 12, 24, 36, and 48 h, 3, 4, 5, 6, 7, and 8 d	3 h (nmol/L) a) ~ 5.5 b) ~ 57.9 c) 46.4 ± 1.4 6 h (nmol/L) a) ~ 24.8 b) 64.0 ± 1.8 c) ~ 41.4 2 d (nmol/L) a) ~ 19.3 b) ~ 35.9 c) ~ 22.1	AUC for (b) was greater than (c)
King 1991	142 Cross	Early: 11 ± 2 wks	Newberry Burdizzo	Early Castration a) Surgical	Immediately prior and	Early Castration 2 min (nmol/L)	During early castration (c) was

		Late: 23 ± 2 wks		b) Burdizzo c) Control Late castration a) Surgical b) Burdizzo c) Control	after, 3, 6, 12, 24, and 30 h	a) 31.5 ± 8.0 b) 28.7 ± 8.3 c) 61.2 ± 12.4 3 hr (nmol/L) a) ~ 71.7 b) ~ 49.7 c) ~ 53.4 Late Castration 2 min(nmol/L) a) 51.3 ± 11.3 b) 88.3 ± 8.6 c) 63.5 ± 5.2 3 hr (nmol/L) a) 122.5 ± 11.6 b) 106.5 ± 22.9 c) 66.5 ± 23.5 6 hr (nmol/L) a) 75.9 ± 14.9 b) 42.2 ± 11.3 c) 47.5 ± 7.5	significantly higher than (b) at 2 min For late castration (b) was significantly higher than (a) castration at 2 min At 3 h, in late castration calves, (a) and (b) were significantly higher than (c). At 6 h there was a significantly higher cortisol concentration for (a) than (b), but neither were significantly different from (c) No statistical comparison was made across ages
Mellor 1991	11 Friesian	< 1 wk	Rubber ring	a) Handling b) Rubber ring c) 50 µg ACTH IV	Immediately prior, 0, 15, 30, 60, 90, 120, 180, 240 min.	All concentrations at 11 nmol/L or less	No significant difference between (a) and (b) at any of the time points.
Faulkner 1992	268 Cross	24 – 36 wks	Newberry Knife	a) 0.07 mg/kg butorphanol + 0.02 mg/kg xylazine IV, 90 sec prior to castration	0, 3, and 7 d	3 d (nmol/L): a) 56.5 b) 62.8 c) 38.0	There was not a significant difference between treatment groups for calves

				b) Castration c) Butorphanol + xylazine, no castration d) No castration		d) 31.8 7 d (nmol/L): a) 48.9 b) 62.5 c) 51.6 d) 38	castrated but there was between calves castrated and those not on day 3 On day 7 (b) was significantly higher than (a), (c), and (d)
Robertson 1994	36 Ayrshire	1,3, and 6 wks	Burdizzo Surgical Banding	a) Handling only b) Burdizzo c) Surgery d) Rubber ring	-20, -1, 12, 24, 36, 48, 60, 72, 84, 96, 138, and 180 min	C_{max} (nmol/L), T_{max} (min) at 6 d a) ~ 40, 12 b) ~ 80, 24 c) ~ 105, 12 d) ~ 58, 36 C_{max}(nmol/L), T_{max}(min), 3 wk a) ~35, 12 b) ~ 55, 24 c) ~ 63, 24 d) ~ 40, 12 C_{max}(nmol/L), T_{max}(min), 6 wks a) ~ 20, 12 b) ~ 60, 24 c) ~ 105, 24 d) ~ 25, 12	C_{max} for (c) was significantly greater than (a), (b), and (d). (b) was significantly higher than (a). C_{max} for 6 and 42 d old calves was significantly higher than 21 d calves
Chase 1995	12 Angus 6 Hereford 24 Brahman	80 – 88 wks	Newberry knife Banding	a) Uncastrated b) 25 mL lidocaine 2% injected into both spermatic chords, surgical castration 3 min later c) EZE latex rubber	Pre surgical sample, post surgical 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, 26, 28, and 35 d.	Immediately post castration (nmol/L) b) 54.6 c) 42.5 day 0 before tx to day (nmol/L)	(b) was significantly higher than (a) and (c) immediately after. (b) and (c) was significantly higher than (a) 2 days after.

				bands		a) 40.0 b) 51.3 c) 45.2	
Molony 1995	40 Ayrshire	1 wk	Burdizzo Surgical Rubber ring	a) Handling alone b) Burdizzo c) Surgical d) Burdizzo applied for 10s distal to rubber ring e) Rubber ring	20 min prior and at 12, 24, 36, 48, 60, 72, 84, 96, 132, and 180 min after treatment.	C_{max} (nmol/L), T_{max} (min) a) ~ 22, 12 b) ~ 80, 24 c) ~ 96, 24 d) ~ 70, 12 e) ~ 51, 12	C_{max} for (c) was significantly higher than all other groups. By 24 min, both (c) and (b) were significantly different than the other treatments. At 96 min, (c) was significantly different from (b) and (d). There was no significant difference between any groups by 132 min.
Fisher 1996	56 Friesian	22 wks	Burdizzo Surgical	a) Control b) SC 0.1 mg HSA-GnRH c) Burdizzo d) Burdizzo w/ 8 mL 2% lidocaine in each testicle and 3 mL SC on each side of scrotum 15 min prior d) Burdizzo w/ 8 mL 2% lidocaine in each testicle and 6 mL SC in scrotum 15 min prior e) Surgical	Mean cortisol calculated from: -2-0, 0.25-1.5, 2-6,8-24,48, & 72 h	0.25 – 1.5 h (nmol/L) a) 13.8 b) 24.6 c) 62.4 d) 40.6 e) 85.3 f) 54.3 2 – 6 h (nmol/L) a) 14.6 b) 34.5 c) 30.3 d) 30.3 e) 59.8 f) 47.7	From 0 – 2 h: (a) significantly lower than (c), (d),(e), and (f). (c) was significantly higher than (d), but lower than (e). (e) was significantly higher than all treatment groups From 2 – 6 h: (e) was highest followed

				f) Surgical w/ local anesthetic		8 – 24 h (nmol/L) a) 15.7 b) 25.7 c) 24.6 d) 22.9 e) 19.6 f) 29.0 C_{max} (nmol/L) a) 40.3 b) 63.5 c) 86.6 d) 73.1 e) 126.9 f) 97.7 AUC 0-10 h (nmoL· L^{-} h) a) 15.7 b) 134.9 c) 128.0 d) 111.2 e) 291.4 f) 227.3	by (f) but they were not significantly different from each other From 8 – 24 h: All treatment groups were not significantly different from each other C_{max}: (e) was highest which was significantly different from (f), (c), and (d). AUC: (e) and (f) were significantly higher from the other treatment groups.
Fisher 1997	40 Friesian	20 wks	Surgical	a) Untreated control b) Oral metyrapone c) SURG castration d) Oral metyrapone (3 g) every 4 hours from -44 to 4 hours and surgical castration	Mean cortisol calculated from: -2 – 0, 0.25 – 1.5, 2 – 6, and 8 – 12h	0.25 - 1.5 h (nmol/mL) a) 16.6 b) 10.5 c) 82.5 d) 46.4 2 – 6 h (nmol/L) a) 14.1 b) 10.8	0.25 - 1.25 h: (c) was significantly greater than (a),(b), and (d). (d) was significantly higher than (a) and (b) From 2 – 6 and 8 – 12 hr (c) was not

	c) 36.7 d) 37.0 8 – 12 h (nmol/L) a) 13.0 b) 15.5 c) 33.4 d) 31.2 1 d (nmol/L) a) 6.6 b) 9.9 c) 10.2 d) 13.5 3 d (nmol/L) a) 4.4 b) 7.7 c) 15.2 d) 13.5 7 d (nmol/L) a) 3.3 b) 3.3 c) 16.3 d) 13.5 C_{max} (nmol/L) a) 35.6 b) 33.7 c) 118.9 d) 77.8 AUC ($nmol \cdot L^{-1} \cdot h$) a) 170.5 b) 165.0	significantly different from (d) but both were higher than (a) and (b) C_{max} was greatest for (c) which was significantly greater than (a), (b), and (d). (d) was significantly greater than (a) and (b) AUC was greatest for (c) but it was not significantly different from (d), both were significantly higher than (a) and (b)

						c) 480.6 d) 434.3	
Fisher 1997	30 Friesian	22 wks	Surgical	a) Untreated control b) IV cortisol administration of 12 mg (0 hr), 6 mg (30, 60, 70, 100, 130, & 160), 2 mg every 30 min until 430 min, & 1 mg at 460 min c) Surgical castration	-2, -1.5, -1, -0.5, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 24, and 72 h	C_{max} (nmol/L) a) 54.4 b) 114.0 c) 111.7 AUC 0 – 12 h, (nmol·L^{-1}·h) a) 262.1 b) 595.9 c) 582.2	C_{max} was not significantly different between (b) and (c), but both were significantly higher than (a) AUC was also not significantly different between (b) and (c), but both were significantly higher than (a) By 24 h, there were no significant differences between treatment groups
Fisher 2001	52 Angus, Angus cross and Simmental	56 wks	Surgical Banding	a) 6 – 7 mL lidocaine in each testis, 6 mL SC b) Surgical, 6 – 7 mL lidocaine in each testis, 6 mL SC along incision line, 15 min prior to castration c) Banding, 6 – 7 mL lidocaine in each testis, 6 mL SC along banding area, 15 min prior to banding	0, 1, 2, 4, 7, 14 d	Day 0 (nmol/L) a) 54 b) 46 c) 51 Day 1 (nmol/L) a) 49 b) 57 c) 46 Day 2 (nmol/L) a) 40 b) 52 c) 41 Day 4 (nmol/L)	No significant difference between groups (b) and (c) at any of the time points Calves in (b) did have significantly higher serum cortisol concentrations at 7 and 10 days than (a), but was not different at any of the other time points.

						a) 37 b) 52 c) 44 Day 7 (nmol/L) a) 34 b) 60 c) 54 Day 14 (nmol/L) a) 43 b) 73 c) 65	
Early 2002	40 Friesian	22 wks	Surgical	a) Control b) Surgical c) 3 mg/kg ketoprofen IV 20 min prior d) 9 mL of 2% lidocaine to each testis 20 min prior e) b + c	-2, -1.5, -1, -0.5, -0.25, 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 24, and 72 h	AUC (nmol/L·h^{-1}) a) 156.7 ± 14.8 b) 485.9 ± 76.4 c) 215.5 ± 38.3 d) 360.9 ± 41.9 e) 324.5 ± 54.5 C_{max} (nmol/L) a) 52.4 ± 12.8 b) 126.4 ± 17.0 c) 68.2 ± 14.1 d) 60.9 ± 7.42 e) 79.5 ± 1.1 T_{max} (h) b) 0.31 ± 0.04 c) 0.29 ± 0.04 d) 2.63 ± 0.77 e) 4.61 ± 1.75	(d) failed to reduce AUC as compared to (b) ($P > 0.05$). (c) and (e) reduced ($P < 0.05$) the AUC as compared to (b). C_{max}($P < 0.05$) was greater in (b) than (c), (d), and (e) T_{max} was longer for (e) than (c)
Stafford 2002	190 Friesian Cross	8 – 16 wks	Ring Band Surgical	a) Handling alone b) 3 mL lidocaine in	-30 min, immediately prior and	C_{max}, T_{max} (nmol/L, h) a) not sig	Attenuation in cortisol response for

	Emasc-ulator Clamp	each testicle, control c) b + 3 mg/kg IV ketoprofen, control d) 28µg/kg ACTH IV e) 2 rubber rings on the scrotal neck f) e + 3 mL lidocaine 20 min prior g) e + f + 3 mg/kg IV ketoprofen 20 min prior h) Band i) h + 3 mL lidocaine 20 min prior j) h + i + 3 mg/kg IV ketoprofen 20 min prior k) Surgical castration, cord broken by traction l) k + 3 mL lidocaine 20 min prior m) k + l + 3 mg/kg IV ketoprofen 20 min prior n) Surgical castration, emasculator o) n + 3 mL lidocaine 20 min prior p) n + o + 3 mg/kg IV ketoprofen 20 min prior q) Clamp castration r) q + 3 mL lidocaine 20 min prior	post, 30 min, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h	b) not sig c) not sig d) 99 ± 3, 2 e) 76 ± 11, 1.5 f) 24 ± 3, 0 g) 31 ± 4, 0 h) 101 ± 6, 1 i) 28 ± 6, 0 j) 26 ± 5, 0 k) 68 ± 7, 0.5 l) 66 ± 14, 2 m) 30 ± 14, 1.5 n) 56 ± 12, 2.5 o) 84 ± 4, 1 p) 31± 6, 0.5 q) 64 ± 7, 0.5 r) 53 ± 5, 0.5 s) 21 ± 2, 0	(f) and (g) as compared to (e). (f) and (e) were not significantly different from pre-treatment values) There was an attenuation in cortisol response for (i) and (j) as compared to (h). (i) and (j) also did not significantly rise from pre-treatment values. Surgical castration with traction caused significant elevations in cortisol for (k) and (l) for 0.5 to 3 and 0.5 to 4 h respectively from (a). They were both significantly higher than (m) as well from 0.5 to 3.5 h. (q) produced a significant increase in cortisol from 0.5 to 1.5 h. The same occurred for (r). Calves in (s) did not experience a

				s) q + r + 3 mg/kg IV ketoprofen 20 min prior			significant rise in cortisol
Ting 2003a	50 Holstein x Friesian	56 wks	Burdizzo	a) Sham b) Burdizzo alone c) 3 mg/kg ketoprofen IV 20 min prior d) Local anesthesia with 2% lidocaine 20 min prior e) Caudal epidural with 0.05 mg/kg 2% xylazine + 0.4 mg/kg 2% lidocaine 10 min prior	-2, -1.5, -1, -0.5, -0.25, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 24, 72 h after treatment	0.25 – 1 h (nmol/L) a) 12.01 b) 67.11 c) 33.58 d) 35.39 e) 43.70 2 – 6 h (nmol/L) a) 10.38 b)14.64 c) 5.23 d) 21.79 e) 25.23 6.5 – 12 h (nmol/L) a) 8.7 b)15.2s5 c) 6.62 d) 14.25 e) 13.75 1 d (nmol/L) a) 8.99 b) 14.54 c) 22.51 d) 30.01 e) 17.90 3 d (nmol/L) a) 14.6 b) 16.82 c) 24.31	From 0.25 to 1 h, (c), (d), and (e) were significantly lower than (b), but not significantly different from each other From 2 to 6 h, (b) was still significantly higher than (c), (d), and (e). However (c) was also significantly lower than (d) and (e) From 6.5 to 12 h, (b), (d), and (e) were not significantly different from each other however all were significantly higher than (c) By 3 d there was no significant difference among treatment groups The AUC for (b), (d), and (e) were significantly higher

						d) 21.86 e) 26.76 AUC(nmol/L) · h a) 126 b) 263 c) 125 d) 266 e) 200 C_{max}(nmol/L) a) 27.3 b) 101.0 c) 66.9 d) 66.1 e) 76.1	than (c) and (a) For C_{max}, (c), (d), and (e) were significantly lower than (b) but significantly higher than (a)
Ting 2003b	50 Holstein x Friesian	48 wks	Surgical	a) Control b) Surgical c) 3 mg/kg keto-profen IV 20 min prior to b d) 1.5 mg/kg ketoprofen IV 20 min + immediately prior to b e) 1.5 mg/kg ketoprofen IV 20 min + immediately prior to b + 3 mg/kg ketoprofen at 24 h post	-2, -1.5, -1, -0.5, -0.25, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 24, 72 h after treatment	0.25 – 1.5 h (nmol/L) a) 8.33 b) 40. 65 c) 39.16 d) 32.87 e) 34.30 2 – 6 h (nmol/L) a) 7.87 b) 30.39 c) 9.1 d) 7.29 e) 8.13 6.5 – 12 h (nmol/L) a) 7.89 b) 14.04 c) 15.16	From 0.25 to 0.5 h, all treatment groups had a significantly higher cortisol response than (a) From 2 – 6 h calves from (b) experienced a significantly higher cortisol response than (a), (c), (d), and (e) From 6.5 to 12 h, all treatment groups were significantly greater than (a). C_{max} for all treatment

						d) 14.76 e) 17.96 C_{max} (nmol/L), T_{max} (h) a) 15.0, n/a b) 67.7, 0.4 c) 75.7, 0.32 d) 65.7, 0.35 e) 67.7, 0.40 AUC(nmol·L^{-1}·h) a) 106 b) 324 c) 189 d) 186 e) 238	groups was significantly greater than (a) AUC was greatest for (b) which was significantly greater than all other treatment groups. (c), (d), and (e) were also significantly greater than (a)
Schwartz-kopf Genswein 2005	17 Holstein	7.5 – 11 wks	Surgical	a) Day 1: control – no castration b) Day 2: sham castration c) Day 3: castration	a) 0, 15, 30, 60, 120 and 240 min b) 15, 30, 60, 120, and 240 min c) 15, 30, 60, 120, 240 min, 24 and 48 h	15 min (nmol/L) a) 12.1 ± 1.7 b) 16.3 ± 1.9 c) 51.3 ± 2.8 30 min(nmol/L) a) 11.6 ± 1.1 b) 14.9 ± 1.9 c) 56.0 ± 5.2 60 min(nmol/L) a) 11.9 ± 1.7 b) 11.9 ± 1.4 c) 43.9 ± 6.9 120 min(nmol/L) a) 13.8 ± 1.9 b) 13.8 ± 2.2 c) 49.7 ± 6.9	During actual castration, 0 – 120 min was significantly higher than 240 and 24 and 48 h thereafter All time points for (c) were significantly higher than (a) and (b) (except for 240 minutes for (a))

						240 min(nmol/L) a) 18.8 ± 3.3 b) 15.2 ± 1.9 c) 25.4 ± 4.1	
Pang 2006	50 Holstein x Friesian	22 wks	Banding Burdizzo	a) Untreated control b) Banding c) 1.4 mg/kg carprofen IV 20 min prior to banding d) Burdizzo e) 1.4 mg/kg carprofen IV 20 min prior to burdizzo	-2, -1.5, -1, -0.5, -0.25, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, 24, and 72 h	AUC 0 – 2 h (nmol · L^{-1}·h) (a) 39.2 ± 11.0 (b) 147.6 ± 11.0 (c) 123.9 ± 11.6 (d) 92.2 ± 11.3 (e) 81.7 ± 10.9 AUC 2 – 6 h (nmol · L^{-1}·h) (a) 60.7 ± 12.9 (b) 102.4 ± 12.8 (c) 85.8 ± 13.5 (d) 92.2 ± 13.1 (e) 64.8 ± 12.8 AUC 6 – 12 h (nmol · L^{-1}·h) (a) 76.7 ± 11.3 (b) 110.6 ± 11.2 (c) 72.6 ± 11.8 (d) 121.1 ± 11.4 (e) 96.0 ± 11.1 AUC 0 – 12 h (nmol · L^{-1}·h) (a) 39.2 ± 11.0 (b) 147.6 ± 11.0 (c) 123.9 ± 11.7	From 0 – 2 h, there was no significant difference between (b) and (c). (b) was significantly higher than (d) and (e). There was also no significant difference between (d) and (e) From 6 to 12 h, (b) was significantly higher than (c), however was not significantly different from (d) or (e) (e) had the lowest C_{max}, however it was only significantly different from (b). There were no other significant differences among treatment groups. T_{max} was the different between (b) and (c) versus (d)

						(d) 92.2 ± 11.2 (e) 81.7 ± 10.9 C_{max} (nmol/L), T_{max} (h) (a) 48.6 ± 8.5, n/a (b) 117.8 ± 8.5, 1.2 ± 0.1 (c) 95.7 ± 9.0, 1.2 ± 0.11 (d) 95.0 ± 8.5, 0.5 ± 0.1 (e) 91.1 ± 8.5, 0.4 ± 0.10	and (e), however not different within method of castration. Banding T_{max} was longer than Burdizzo Administration of carprofen failed to prevent rise in cortisol levels for either method.
Coetzee 2007	20 Angus cross	16 – 26 wks	Newberry knife Henderson castration tool	a) Uncastrated b) Castration c) Sodium salicylate at 50 mg/kg IV immediately prior + b d) Oral acetylsalicylic acid at 50 mg/kg immediately prior + b	Immediately prior and after, 10, 20, 30, 40, 50 min, 1, 1.5, 2, 4, 6, 8, 10, and 12 h	Baseline: 137.60±15.3 nmol/L to 145.64 ±25.74.nmol/L C_{max} (nmol/L), T_{max} (min) a) 192.00 ± 8.69, 20 b) 190.60 ± 24.88, 20 c) 168.80 ± 22.61, 40 d) 235.00 ± 18.01, 30 AUC (µmol · min/L) a) 42.75 ± 4.14 b) 54.34 ± 8.22 c) 50.90 ± 6.58 d) 63.97 ± 4.86	(c) significantly lower than (d) at 20, 40 , and 90 min post castration Elevations in serum cortisol decreased to below baseline values for (b), (c), and (d) by 60 min. The AUC for (d) was significantly higher than (a)
Thuer 2007	70 Simmental or Simmental x Holstein	3 – 4 wks	Rubber ring Burdizzo	a) Rubber ring + 10 mL of NaCl injected into the spermatic cord and SC 5 min prior b) a + 10 mL 2% lidocaine block of the spermatic cord and SC 5 min prior	Prior to anesthesia, 0, 20, 40 min, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 24, 48, and 72 h	20 min (nmol/L) a) ~ 65 b) ~ 42 c) ~ 40 d) ~ 30 e) ~29 f) ~ 25	At 20 min post, (c) was significantly higher than (d). By 90 min both returned to baseline concentrations and remained there throughout the rest

				c) Burdizzo + 10 mL of NaCl in the spermatic cord and SC 5 min prior d) c + 10 mL of 2% lidocaine block of the spermatic chord and SC 5 min prior e) Control (handling) + 10 mL of NaCl injected into the spermatic cord and SC 5 min prior f) e + 10 mL 2% lidocaine block of the spermatic cord and SC 5 min prior		40 min (nmol/L) a) ~ 62 b) ~ 40 c) ~ 29 d) ~ 22 e) ~ 15 f) ~ 20 1 h (nmol/L) a) ~ 40 b) ~ 30 c) ~ 32 d) ~ 17 e) ~ 10 f) ~ 18 1.5 h (nmol/L) a) ~ 24 b) ~ 19 c) ~ 40 d) ~ 18 e) ~ 8 f) ~ 15	of sampling. AUC for the first hour after castration and C_{max} was also significantly higher for (c) than (d) (a) caused a significant increase at 1.5 h and 4 h. By 6 h, serum levels returned to baseline. (b) only caused a slight increase which returned to baseline after 1 h. AUC from 0 – 2.5 h and C_{max} were not significantly higher between (a) and (b)
Boesch 2008	30 Cross	< 1wk	Burdizzo	a) 10 mL 2% lidocaine (2 mL/ spermatic cord, 3 mL in spermatic neck) 20 min prior b) 10 mL 0.5 % bupivicaine 20 min prior c) 10 mL saline 20 min prior	-1.25, -25, immediately before and after, 20, 35, 50, 1:05, 1:20, 1:35, 1:50, 2:05, 2:20, 2:35, 2:50. . 14.0, 15, 16, 17 h	Immediately post (nmol/L) a) ~ 120 b) ~ 75 c) ~ 90 35 min (nmol/L) a) ~ 105 b) ~ 70 c) ~ 80	Trend toward higher peak concentrations in (c) > (b) > (a) (P = 0.061) Total AUC from 0 to 11 h was significantly higher in (c) > (b) > (a).

						125 min (nmol/L) a) ~ 50 b) ~ 45 c) ~ 57	
Coetzee 2008	10 Angus cross	16 – 26 wks	Newberry Henderson Castration tool	a) Castration b) Simulated castration	-24, -12, immediately prior and after, 10, 20, 30, 45 min, 1, 1.5, 2, 2.5, 3, and 4 h	Baseline concentrations: -24 h: 76.06 ± 11.97 - 12 h: 50.84 ± 10.99 C_{max} (nmol/L): a) 128.80 ± 9.06 b) 36.58 ± 31.94 Both decreased to less than baseline by 2.5 hours T_{max} (h): a) 0.68 ± 0.25 b) 0.53 ± 0.16 Total AUC (h · nmol/L) a) 137.87 ± 6.11 b) 44.5 ± 39.98	No significant differences among treatment groups for any parameters
Stillwell 2008	40 Friesian	24 ± 2 wks	Burdizzo	a) Control (treated with 5 mL of SC 0.9 % saline) b) Caudal epidural with 4 mL of 2 % Lidocaine 5 minutes prior to castration + a c) b + 8 mL(2.2 mg/kg) flunixin meglumine injected SC in the neck d) b + 5 mL (1.4 mg/kg) carprofen	-5, 6, 24, and 48 h	6 h a) 36.78 ± 5.24 b) 21.56 ± 5.9 c) 17.69 ± 4.28 d) 15.12 ± 4.47 24 h a) 46.99 ± 7.15 b) 36.46 ± 7.15 c) 32.57 ± 5.82 d)24.66 ± 6.07 48 h	(c) and (d) had significantly lower cortisol at 6 h than (a). By 24 h only (d) had significantly lower cortisol than (a), and (c) was not significantly different than (d) or (a). By 48 h, cortisol was significantly lower for (d) than (a), (b), and (c).

				injected SC in the neck		a) 24.89 ± 4.97 b) 36.28 ± 4.07 c) 32.45 ± 4.06 d) 15.81 ± 4.25	(a) was significantly lower by 48 h than at 6 and 24 h. (c) had significantly higher cortisol at 24 and 48 h than at 6 h. (d) had significantly increased cortisol at 24 h compared to -5 min but not different from 6 and 48 h.
Marti 2010	47 Holstein	12 wks	Rubber ring	a) Intact b) 3 mL in each testis, and 2 mL around scrotum of 2% Lidocaine and 3 mg/kg of flunixin meglumine IM 20 min prior to castration	-120, 0, 30, 60, 90, and 180 min	30 – 180 min (nmol/L) a) 13.2 ± 1.56 b) 5.6 ± 1.56 AUC 0 – 180 (nmol/L/h) a) 32 ± 4.6 b) 19 ± 4.6	(a) was significantly higher than (b) for mean cortisol concentration from 30 to 180 min. As well the AUC was higher (P = 0.06) for (a) as compared to (b)
Gonzalez 2010	43 steers 46 bulls	steers 30 wks ± 3 wks	Banding	a) Sham b) 0.07 mg/kg xylazine epidural, then 1.1 mg/kg flunixin meglumine IV 30 min prior to sham c) Banding d) 0.07 mg/kg xylazine epidural, then 1.1 mg/kg flunixin meglumine	-0.5, 0.5, 1, 2, 4, 24, and 48 h, 7 and 14 d	Salivary cortisol 4 h (nmol/L) a) 4.6 b) 3.4 c) 10.0 d) 4.1 24 h (nmol/L) a) 3.1 b) 2.5 c) 4.7 d) 3.5	(c) had a significantly higher salivery cortisol at 1 (P <0.05) and 2 h (P< 0.03) after castration band castrated calves had greater salivary cortisol than control calves at 2 h (P < 0.02)

				IV 30 min prior to banding		14 d (nmol/L) a) 3.1 b) 2.7 c) 2.7 d) 2.7	non medicated calves had greater salivary cortisol at 0.5, 1, and 2 h than medicated calves (P < 0.05)
Stewart 2010	30 Friesian	16 wks	Surgical	a) Sham castration b) Surgical castration c) 5 mL of lidocaine in each testicle with SQ infiltration 10 min prior + a d) 5 mL of lidocaine in each testicle with SQ infiltration 10 min prior + b	-20, -10, 15, and 20 min	15 min (nmol/L) a) ~ 19.3 b) ~ 80.0 c) ~ 22.1 d) ~ 52.4 20 min (nmol/L) a) ~ 16.6 b) ~ 74.5 c) ~ 22.0 d) ~ 55.18	Castration increased cortisol in (b) significantly greater than (d)

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796 **Table 1.2 Dehorning and Cortisol**

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Author	Sample size	Age	Method	Treatment Groups	Sampling Schedule	Cortisol Concentrations	Significance
Laden 1985	18 Holstein	8 wks 12 wks	Electric	a) Dehorned at 8 wks b) Control	-15, 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 72 h	5 min: (nmol/L) a) 0.56 b) 0.15 15 min: (nmol/L) a) 0.75 b) 0.24	Plasma cortisol elevated (P < 0.01) above baseline at 15, 30 minutes for (b) and at 5, 15, 30, and 60 min for

						30 min: (nmol/L) a) 0.84 b) 0.22 60 min: (nmol/L) a) 0.64 b) 0.15	(a). Significant difference between (b) and (a) at 5, 15, 30, and 60 min.
Boandl 1989	24 Holstein	7 – 16 wks	Electric	a) Handling b) Untreated Dehorned c) Cornual nerve block + a d) Cornual nerve block: 5 mL lidocaine HCl 2% Epi 1:100,000 + (b)	Baseline sample, prior, 30 min post	30 min (nmol/L) a) 26.5 b) 78.1 c) 46.4 d) 82.5	(b) and (d) not significantly different from each other
Wohlt 1994	13 Holstein	3 – 4 wks	Cauterized on day Buddexx the next day	a) Sham b) Electric cautery c) Buddex	1-2, 5 min post restraint, 5,15, 30, and 45 min, 1, 2, 3, 4, 8, and 12h	C_{max}(nmol/L): a) 30.3 b) 60.4 c) 57.1	Plasma cortisol peaked 5 min post sham and 15 min post dehorning (b) and (c)
Morrisse 1995	164 Mont-beliard	4 – 8 wks	Caustic Paste at 4 wks Cauterized at 8 wks	a) Control, 4 wks b) a+ cornual nerve block: 4 mL 2% lidocaine 15 min prior c) Caustic paste, 4 wks d) c + cornual nerve block e) Control, 8 wks f) e + cornual nerve block	Prior, 1, 4, and 24 h post treatment	1 h (nmol/L) a) 10.8 ± 19.3 b) 10.5 ± 8.3 c) 49.7 ± 21.2 d) 40.3 ± 26.4 e) 10.2 ± 8.0 f) 14.6 ± 11.8 g) 33.6 ± 13.8 h) 27.6 ± 45.3	Calves in (c) and (d) were significantly higher than (a) and (b) at 1 h Calves in (g) and (h) were significantly higher than (e) and (f) at 24 h

				g) Cauterization, 8 wks h) g + cornual nerve block		4 h (nmol/L) a) 22.1 ± 17.6 b) 6.9 ± 6.4 c) 32.3 ± 30.6 d) 13.8 ± 8.3 e) 13.8 ± 14.4 f) 22.4 ± 17.4 g) 8.0 ± 7.5 h) 26.8 ± 19.6 24 h (nmol/L) a) 6.9 ± 5.8 b) 9.9 ± 13.5 c) 8.6 ± 10.2 d) 8.0 ± 6.6 e) 6.4 ± 5.2 f) 16.8 ± 11.9 g) 17.7 ± 11.9 h) 35.6 ± 17.4	
Petrie 1995	55 Friesian	6 – 8 wks	Scoop Cautery	a) Sham b) 3 mL 2% lidocaine 20 min prior to sham c) Scoop d) 3 mL 2% lidocaine 20 min prior to scoop e) Cautery f) 3 mL 2 % lidocaine 20 min prior to cautery g) 0.31 mg ACTH IV	-70, -10, 15, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480 min post treatment	Total AUC (nmol/L·min) a) 4,386.8 ± 1,426.4 b) 5,024.1 ± 1,296.7 c) 15,210.4 ± 3,327.4 d) 16,871.3 ± 3,975.7 e) 8,467 ± 1,591.9 f) 8,660.5 ± 2,041.7 g) 18,639.8 ± 2,276.2 AUC -70 min – 2 h (nmol/L) a) 786.3 ± 281.4	(c): mean cortisol concentration returned to control values by 6.5 h AUC (-70 min – 2 h) of (c) was significantly greater than (a), (b), (d), (e), and (f)

						b) 1,616.8 ± 427.7 c) 6,100.2 ± 1,161.5 d) 2,827 ± 102.1 e) 3,732.9 ± 769.8 f) 2,858.3 ± 789.1 g) 9,731.0 ± 1,409.9 AUC 2 – 9.5 h (nmol/L) a) 3,332.9 ± 1,247.1 b) 3,404.6 ± 935.3 c) 9,110.2 ± 2,466.6 d) 1,601.5 ± 3,327.4 e) 4,723 ± 935.3 f) 5,799 ± 1,528.5 g) 8,908.8 ± 929.8	AUC (2 – 9.5 h) of (c) and (d) were significantly greater than (a), (b), (e), and (f) but not significantly different from each other.
McMeekan 1997	30 Friesian	14 – 16 wks	Scoop	a) Control – not dehorned b) Shallow scoop dehorned c) Deep scoop dehorned	-0.25, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, and 9 h	AUC -0.25 – 9 h (nmol/L · min) a) 3724.7 ± 973.9 b) 24,762.0 ± 3,225.2 c) 23,059.8 ± 2,728.7 C_{max} (nmol/L) a) ~ 16 b) ~ 80 c) ~ 77	(b) and (c) had significant rises after dehorning until 4.5 h. Cortisol returned to baseline by 8 h (b) and 6 h (c). There was no significant difference at any time point between (b) and (c)
McMeekan 1998a	70 Friesian	12 – 16 wks	Barnes Scoop	a) Handling b) Cornual nerve block: 6 mL, 0.25% bupivicaine 20 min prior to (a) c) b + cornual nerve	-0.33, 0, 0.33, 0.66, 1, 1.33, 1.66, 1.83, 2.33, 2.66, 2.83, 3.33, 3.83, 4.33,	AUC 0 – 3.83 h (nmol/L · min) a) 2,195 ± 853 b) 2,562 ± 824 c) 1,358 ± 375 d) 12,725 ± 1,374	Total AUC: (d), (e), and (f) were significantly higher than (g), (a), (b), and (c). Also (g) was significantly

				block 4 hours after (a) d) scoop dehorning e) b + d f) cornual nerve block w/ 6 mL of 0.25% bupivicaine immediately prior g) c + d	4.83, 5.33, 6.33, 7.33, 8.33, 9.33 h	e) 4,963 ± 1,112 f) 3,459 ± 834 g) 2,457 ± 1,120 4.33 – 9.33 h (nmol/L) a) 721 ± 324 b) 1,871 ± 694 c) 1,180 ± 300 d) 5,387 ± 1,091 e) 11,297 ± 1,647 f) 7,938 ± 1,809 g) 7,109 ± 1,229 0 – 9.33 h (nmol/L) a) 2,916 ± 100 b) 4,433 ± 137 c) 2,538 ± 497 d) 18,111 ± 2,219 e) 16,257 ± 1,925 f) 11,397 ± 2,270 g) 9,566 ± 1,674	higher than (a), (b), and (c). For AUC from 0 to 3.83 hours, (d) was significantly higher than (e), (f), and (g). For AUC from 4.33 to 9.33 h, (d), (e), and (f) were significantly greater than (g)
McMeekan 1998b	100 Friesian	12-16 wks	Scoop	a) Handling b) Cornual nerve block: 6 mL 0.25 bupivicaine 20 prior to (a) c) 3 mL 10% ketoprofen IV 20 min prior to (a) d) cornual nerve block: 6 mL lidocaine 20 min prior + c + a	-0.33, 0, 0.33, 0.66, 1, 1.33, 1.66, 1.83, 2.33, 2.66, 2.83, 3.33, 3.83, 4.33, 4.83, 5.33, 6.33, 7.33, 8.33, 9.33 h	C_{max} (nmol/L) (a,b,c,d,e) all between 5 - 10 f) 77.25 h) 60.7 i) 13.8	

				e) b + c + a f) Scoop dehorning g) b + f h) c + f i) c + d + f			
Sylvester 1998	60 Friesian	20 – 26 wks	Scoop Cautery	a) Control b) Cornual nerve block: 6 mL 2% lidocaine 30 min prior + c c) Cornual nerve block + scoop + cautery d) Cornual nerve block + scoop e) Scoop + cautery f) Scoop	-0.66, -0.25, 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, and 36 h.	AUC (nmol/L · hr) a) 51.3 ± 10.8 b) 59.4 ± 14.5 c) 70.02 ± 19.0 d) 140.4 ± 40.5 e) 210.6 ± 56.7 f) 283.5 ± 48.6 36 h post (nmol/L) a) 10.3 ± 3.2 b) 9.2 ± 2.4 c) 21.3 ± 4.3 d) 29.2 ± 9.2 e) 26.0 ± 6.0 f) 28.6 ± 6.2	The AUC for (f) was significantly higher than (d), (c), (b), and (a) but from (e). (e), (d), and (c) were not significantly different from each other but were higher than (a) and (b) At 36 h post, there was no significant difference between (c), (d), (e), and (f), but all were significantly different from (a) and (b)
Graf 1999	53 calves	4 – 6 wks	Cautery	a) Cornual nerve block with 2% lidocaine 20 min prior b) Saline injection 20 min prior c) none	-40, -30, -20, -10, 0, 5, 10, 20, 40, 60, 90, 120, 150, 180, 210, and 240 min	5 min (nmol/L) a) ~21 b) ~42 c) ~ 22 10 min(nmol/L) a) ~ 30 b) ~ 64 c) ~ 40 20 min(nmol/L)	Plasma cortisol concentrations were significantly elevated for (b) and (c) as compared to (a) at 20 through 90 min.

						a) ~23 b) ~78 c) ~49 60 min (nmol/L) a) ~ 10 b) ~36 c) ~ 33 90 min (nmol/L) a) ~ 10 b) ~ 26 c) ~ 25	
Grondahl-Nielson 1999	48 Friesian	4 – 6 wks	Electric	a) Cornual nerve block: 2% lidocaine, 15 min prior to sham w/ cold iron b) a + hot iron c) 0.2 mg/kg xylazine, 0.1 mg/kg butorph-anol IM 20 min prior d) a + c, 15 min prior e) Hot iron dehorned f) control	-25 to sedation, 1, 1.5, 2, and 4 h	(increase from baseline) - 25 – 5 min (nmol/L): a) ~ 0.2 b) ~ 0.4 c) ~ 0.5 d) ~ 1 e) ~ 2.5 10 – 30 min (nmol/L): a) ~ -0.25 b) ~ -0.3 c) ~ 0.3 d) ~ -0.1 e) ~ 1.25 40 – 1.5 hr (nmol/L) a) ~ -0.3 b) ~ 0.02	Plasma cortisol in group (e) increased significantly more immediately after dehorning than all other groups. No other statistical differences

						c) ~ 1.5 d) ~ 0.5 e) ~ 1
Sutherland 2002a	28 Friesian	12 – 16 wks	Scoop	a) Handling b) Dehorning c) Cornual nerve block: 6 mL 2% lidocaine 15 min prior to dehorning. 2 h after lidocaine, 6 mL of bupivicaine injected d) c + cautery	-0.5, 0, 0.5, 1 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6.5, 7, 7.5, 8, 9, 9.5, 10, 10 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 17, 18, 19, 20, 21, 22, 23, 24 h	C_{max} (nmol/L), T_{max} (h) a) ~ 45, 0.5 b) 157, 0.5 c) 150, 7 d) ~ 84, 4
Sutherland 2002b	93 Friesian	12 – 16 wks	Scoop	a) Handling b) Dehorning c) Cornual nerve block: 6 mL 2% lidocaine 15 min prior + 6 mL of 0.25% bupivicaine 2 h after initial treatment + b d) c + 4-5.3 mg/kg phenylbutazone IV + a e) c + d + b f) c + 3-3.75 mg/kg IV ketoprofen + a g) c + f + b h) c = IV ACTH 0.28 µg/kg + a i) c + h + b	-0.5, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 17, 18, 19, 20, 21, 22, 23, 24 h	AUC 0 – 24 h (nmol /L h^{-1}) a) 24,160 (2,629) b) 44,907 (4,171) c) 39,006 (6,130) d) 29,824 (6,383) e) 40,916 (5,268) f) 30,748 (5,261) g) 35,293 (6,057) h) 70,844 (7,357) i) 61,150 (6,901) j) 63,738 (6,784) k) 54,638 (2,438)

				j) ACTH, then ACTH again 6 h later k) ACTH + b k) ACTH 6 h prior + b		
Stafford 2003	100 Friesian	12 wks	Scoop	a) Sham b) Scoop c) Cornual nerve block: 5 mL 2% lidocaine 15 min prior to sham, 3 mg/kg ketoprofen IV d) c + dehorn e) 0.1 mg/kg xylazine IV 20 min before sham f) e + dehorn g) c + e + sham h) d + f + dehorn i) c + e + 2 mg/kg tolazoline IV 5 min prior + a j) d + f + 2 mg/kg tolazoline IV 5 min before dehorn	-0.5 h before treatment, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 h after treatment.	C_{max} (nmol/L), T_{max} (h) a) ~ 5, 0 b) 76, 0.5 c) ~ 20, 5.5 d) 20, 4 e) ~ 40, 0 f) ~ 60, 5.5 g) ~ 57, 0 h) ~ 65, 5.5 i) ~ 90, 0.5 j) ~100, 0.5
Milligan 2004	40 Holstein	0.3 – 2 wks	Electric	a) Cornual nerve block: 5 mL of 2% lidocaine w/ 0.05 mg/mL Epi b) Cornual nerve block + 0.03 mL/kg of 10% ketoprofen IM 10 min prior	prior, 3 and 6 h	Time 0 (nmol/L): a) 68.4 ± 14.3 b) 87 ± 12.7 3 h (nmol/L): a) 86.3 ± 18.2 b) 64.8 ± 12.1 6 h (nmol/L):

						a) 96.9 ± 19.6 b) 111.1 ± 10.7	
Schwartz-kopf Genswein 2005	29 Holstein	3.5 – 8.5 wks	Electric	a) Day 1: control – no dehorning a) Day 2: sham dehorning b) Day 3: dehorning	a) 0, 15, 30, 60, 120, and 240 min b) 15, 30, 60, 120, and 240 min c) 15, 30, 60, 120, 240 min, 24 and 48 h	15 min (nmol/L) a) 17.7 ± 2.2 b) 14.6 ± 1.37 c) 46.9 ± 1.93 30 min (nmol/L) a) 14.6 ± 1.37 b) 14.9 ± 1.37 c) 51.3 ± 3.6 60 min (nmol/L) a) 11.9 ± 1.1 b) 12.1 ± 1.37 c) 30.9 ± 2.8 120 min(nmol/L) a) 10.7 ± 1.37 b) 12.4 ± 1.7 c) 18 .8 ± 1.9 240 min (nmol/L) a) 16.3 ± 1.9 b) 17.7 ± 1.7 c) 11.0 ± 1.1	
Doherty 2007	32 Holstein	10 – 12 wks	Electric	a) 10 mL 5% lidocaine 30 min prior to sham b) 10 ml 5% lidocaine 30 min prior to dehorn	-0.5, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24, 48 and 72 h	30 min (nmol/L) a) ~30 b) ~ 35 c) ~ 60 d) ~ 80	Cortisol significantly higher in (d) at 30 and 60 min. Cortisol significantly higher for (d) than

				c) 10 ml 2% lidocaine 30 min prior to dehorn d) 10 ml saline 30 min prior to dehorn		60 min (nmol/L) a) ~ 10 b) ~ 15 c) ~ 20 d) ~ 45	(a), (c), and (b). (c) was also significantly higher than (a) and (b) at 60 min
Lepkova 2007	18 Czech Red Pied	Adult cows	Foetot-omy wire	a) General anesthesia: IV xylazine (0.1 mg/kg) and ketamine (2 mg/kg) b) IM xylazine (0.2 mg/kg), then 20 min later zygomatic nerve block (2% lidocaine) c) Zygomatic nerve block with 20 mL 2% lidocaine	immediately before and post, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h.	C_{max} (nmol/L) a) 110.62 ± 45.86 b) 82.53 ± 6.04 c) 113.86 ± 25.65 T_{max} (h) a) 0.42 ± 0.19 b) 0.00 ± 0.00 c) 0.25 ± 0.27 Time to return to baseline (h) a) 2.5 ± 1.23 b) 1.92 ± 1.11 c) 3.83 ± 2.18	C_{max} was highest for (c) which was significantly higher than (b) but not significantly different from (a).
Sylvester 2008a	60 Friesian	20 – 26 wks	Scoop Cautery	a) Control b) 6 mL of local anesthetic via cornual nerve block	-0.66, -0.25, 0.25, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 36	36 h (nmol/L) a) 10.3 ± 3.2 b) 9.2 ± 2.4 c) 21.3 ± 4.3 d) 29.2 ± 9.2 e) 26.0 ± 6.0 f) 28.6 ± 6.2 AUC (nmol/L·h^{-1}) a) 51.3 ± 10.8 b) 59.4 ± 13.5	At 36 h (c), (d), (e), and (f) were significantly higher than (a) and (b) but not significantly different from each other The AUC was greatest for (f) which was significantly greater than (a), (b), (c), and (d). (e) and

						c) 70.2 ± 19.0 d) 140.4 ± 40.5 e) 210.6 ± 56.7 f) 283.5 ± 48.6	(f) were not significantly different from each other
Sylvester 2002b	57 Friesian	20 – 26 wks	Scoop Guillotine Shears Saw Embryo-tomy wire	a) Control, handling b) Barnes scoop c) guillotine shears d) Butcher's saw e) Embryotomy wire f) 40 mg IV ACTH	-0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, and 36 h	36 h (nmol/L) a) 14.0 ± 2.7 b) 30.8 ± 5.1 c) 42.9 ± 7.8 d)32.9 ± 6.7 e) 31.1 ± 9.5 f) 13.0 ± 1.9 C_{max} (nmol/L) a) 62.1 ± 10.8 b) 118.8 ± 8.1 c) 102.6 ± 10.8 d) 105.3 ± 8.1 e) 89.1 ± 8.1 f) 124.2 ± 13.5 AUC (nmol/L ·hr^{-1}) a) 94.5 ± 13.5 b) 391.5 ± 29.7 c) 286. 2 ± 48.6 d) 340.2 ± 37.8 e)367.2 ± 54.0 f)353.7 ± 43.2	No significant differences in C_{max} or duration of the cortisol response between calves dehorned (b, c, d, and e) Calves in (c) had a significantly lower cortisol concentration at 2 and 2.5 h as compared to (b), (d), and (e)
Stillwell 2008	20 Holstein x		Chemical	a) Sham, injected with saline	-5, 1, 3, 6, and 24 h	1 h (nmol/L) a) 10.18 ± 4.14	(b), (c), and (d) were significantly

	Friesian			b) 2 mg/kg flunixin IV 5 min prior c) 2 mg/kg flunixin IV 1 h prior d) Chemical disbudding, no treatment		b) 67.07 ± 29.27 c) 61.42 ± 25.40 d) 66.82 ± 11.26 3 h (nmol/L) a) 6.50 ±7.55 b) 10.27 ± 7.67 c) 24.19 ± 39.01 d) 24.73 ± 14.18 6 h (nmol/L) a) 15.68 ± 13.06 b) 10.58 ± 12.08 c) 26.90 ± 31.21 d) 22.08 ± 22.18 24 h (nmol/L) a) 4.38 ± 2.98 b) 14.10 ±7.74 c) 20.12 ± 28.53 d) 10.24 ± 12.28	higher than (a) at 1 h post disbudding At 3 h, (d) was significantly higher than (a), but not significantly higher than (b) or (c). (b) and (c) were not significantly different from (a) By 6 and 24 h, there was no significant difference among treatment groups.
Heinrich 2009	60 Holstein	6 – 12 wks	Electric	a) Placebo Injection + corneal nerve block: 5 mL 2% lidocaine + 0.05 mg/mL epinephrine 10 min prior b) Single IM 0.5 mg/kg dose of meloxicam + cornual nerve block	0.5., 1, 1.5, 2, 4, 6, and 24 h	average increase from baseline: (nmol/L) a: 13.6 ± 7.38 b: 2.6 ± 7.0 24 hrs after (nmol/L): a: 34.8 ± 3.64 b: 35.1 ± 2.74	Cortisol significantly increased for (a) and (b) after dehorning from baseline (sham). Elevation in cortisol was significantly less for (b) from dehorning for up to 6 h after. No difference at 24 h
Stillwell 2009	32 Holstein	3.5 ± 1 wk	Dis-budding	a) Saline injection 5 min prior	-5, 1, 3, 6, and 24 h	1 h (nmol/L) a) 62.64 ± 10.32	At 1 h post, calves in (a) were significantly higher than all other

				b) 2% lidocaine c) 2.2 mg/kg flunixin IV + b d) saline injection 5 min prior to sham		b) 32.88 ± 26.59 c) 13.98 ± 11.49 d) 14.54 ± 9.25 3 h (nmol/L) a) 19.44 ± 14.14 b) 18.37 ± 8.07 c) 6.25 ± 5.74 d) 12.32 ± 12.32 6 h (nmol/L) a) 16.60 ± 18.41 b) 17.91 ± 12.61 c) 12.51 ± 9.63 d) 20.15 ± 13.88 24 h (nmol/L a) 12.34 ± 12.05 b) 16.62 ± 13.88 c) 9.18 ± 8.56 d) 13.26 ± 14.09	treatment groups At 3 h, (c) was significantly lower than (a), (b), and (d) At 6 and 24 h, there was no significant difference among treatment groups
Stillwell 2009	35 Holstein	3 ± 0.5 wks	Caustic Paste	a) saline injection 5 min prior to caustic paste b) 2% lidocaine prior to caustic paste c) 2.2 mg/kg flunixin IV + b d) Saline injection 5 min prior to sham	-5, 10, 30, and 50 min	10 min (nmol/L) a) 25.54 ± 15.15 b) 19.11 ± 11.40 c) 23.14 ± 16.67 d) 16.84 ± 7.06 30 min (nmol/L) a) 41.39 ± 14.85 b) 16.71 ± 10.69 c) 20.67 ± 12.98 d) 20.20 ± 11.19	Calves in (a) were significantly higher than (b), (c), and (d) at 30 and 60 min.

						50 min (nmol/L) a) 42.32 ± 14.47 b) 14.73 ± 8.80 c) 19.80 ± 9.67 d) 14.34 ± 8.57	
Stillwell 2009	16	4 ± 1 wk	Caustic paste	a) Saline injection 5 min prior to caustic paste b) 2% lidocaine prior to caustic paste c) Saline injection 5 min prior to sham	-5, 90, 120, 150, 180 min	90 min (nmol/L) a) 40.5 ± 17.7 b) 23.3 ± 18.6 c) 15.7 ± 9.9 120 min (nmol/L) a) 11.9 ± 16.4 b) 5.8 ± 7.6 c)12.8 ± 12.9 150 min (nmol/L) a) 20.1 ± 8.5 b) 28.1 ± 15.7 c) 22.4 ± 6.2 180 min (nmol/l) a) 27.2 ± 5.3 b) 43.3 ± 9.8 c) 16.5 ± 14.3	(a) was significantly higher than (b) and (c) at 90 min (b) was significantly higher than (a) and (b) at 180 min
Duffield 2010	40 Holstein	4 – 8 wks	Cautery	a) 3 mg/kg ketoprofen IM + cornual nerve block: 5 mL 2% lidocaine 10 min prior b) equal volume saline IM + cornual nerve block 10 min prior	-10 min, 3, and 6 h	3 h (nmol/L) a) ~ 34 b) ~ 32.5 6 h (nmol/L) a) ~37 b) ~ 33.5	No differences in serum cortisol concentrations at any time

Stillwell 2010	41 Holstein	5.3 ± 0.5 wks	Electric Dis-budding	a) 0.2 mg/kg IM xylazine 10 min prior and saline 8 min prior b) 0.2 mg/kg IM xylazine 10 min prior and cornual nerve block with 2% lidocaine 8 min prior c) Sham disbudded after xylazine and lidocaine d) Sham disbudded after IM saline and lidocaine	5 min after treatment, 10, 25, 40, 60 min	10 min (nmol/L) a) 94.82 ± 9.54 b) 86.34 ± 9.10 c) 77.78 ± 9.54 d) 18.54 ± 9.54 25 min(nmol/L) a) 76.89 ± 9.54 b) 80.79 ± 9.10 c) 68.43 ± 9.84 d) 16.17 ± 9.54 40 min(nmol/L) a) 54.22 ± 9.54 b) 63.66 ± 9.10 c) 51.45 ± 9.54 d) 10.84 ± 9.54 60 min(nmol/L) a) 37.17 ± 9.54 b) 57.11 ± 9.10 c) 33.20 ± 9.54 d) 10.19 ± 9.54	(a), (b), and (c) were significantly higher than (d) at 10, 25, and 40 min after disbudding. (a) and (b) were also significantly higher than (d) at 60 min.

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References

Abrahamsen, E.J. Chemical Restraint in Ruminants. *Current Veterinary Therapy Food Animal Practice.* 5th ed. St Louis, Mo: Saunders Elsevier, 2009; 546 – 549.

Appleby, M. C. 2005. The relationship between food prices and animal welfare. *J. Anim. Sci.* 83: E 9 – 12.

American Veterinary Medical Association. 2009. Welfare Implications of Castration in Cattle. Available at: http://www.avma.org/reference/bakcgrounders/castration_cattle_bgnd.pdf (accessed June 2, 2010).

American Veterinary Medical Association. 2010. Welfare Implications of the Dehorning and Disbudding of Cattle. Available at: http://www.avma.org/reference/bakcgrounders/dehorning_cattle_bgnd.pdf (accessed June 2, 2010).

Anderson, D. E., and W. W. Muir. 2005. Pain management in cattle. *Veterinary Clinics of North America. Food Animal Practice.* 21: 623 – 635.

Bengtsson, B., A. Menzel, P. Holtenius, and O. Jacobson. 1996. Cryosurgical dehorning in calves: a preliminary study. *The Veterinary Record*. 138. 234 – 237.

Bertoni, G., E. Trevisi, F. Piccioli-Cappelli. 2004. Effects of acetyl-salicylate used in post-calving of dairy cows. *Veterinary Research Communications.* 28: 217 – 219.

Boandl, K. E., J. E. Wohlt, and R. V. Carsia. 1989. Effects of handling, administration of a local anesthetic, and electrical dehorning on plasma cortisol in holstein calves. *J. Dairy Sci.* 72: 2193 – 2197.

Boesch, D., A. Steiner, L. Gygax, and M. Stauffacher. 2008. Burdizzo castration of calves less than 1-week old with and without local anaesthesia: short-term behavioural responses and plasma cortisol levels. *App. Anim. Beh. Sci.* 114: 330 – 345.

Chamberlain, P.L. and S. D. Brynes. 1998. The regulatory status of xylazine for use in food-producing animals in the United States. *J. vet. Pharmacol. Therap*. 21: 322 – 329.

Chase, Jr. C. C., R. E. Larsen, R. D. Randel, A. C. Hammond, and E. L. Adams. 1995. Plasma cortisol and white blood cell responses in different breeds of bulls: a comparison of two methods of castration. *J. of An. Sci*. 73: 975 – 980.

Coetzee, J. F., R. Gehring, A. C. Bettenhausen, B. V. Lubbers, S. E. Toerber, D. U. Thomson, B. Kukanich, M. D. Apley. 2007. Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration. *J. vet. Pharmacol. Therap*. 30(4): 305 – 313.

Coetzee, J. F., B. V. Lubbers, S. E. Toerber, R. Gehring, D. U. Thomson., B. J. White, M. D. Apley. 2008. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am. J. Vet. Res.* 69(6): 751 – 761.

Coetzee, J. F., A. L. Nutsch, L. A. Barbur, R. M. Bradburn. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. *BMC Veterinary Research.* 1 – 19.

Cohen, R. D. H, B. D. King, L. R. Thomas, and E. D. Janzen. 1990. Efficacy and stress of chemical versus surgical castration of cattle. *Can. J. Anim. Sci.* 70: 1063 – 1072.

Craigmill, A. L., M. Rangel-Lugo, P. Damian, and J. E. Riviere. 1997. Extralabel use of tranquilizers and general anesthetics. *J. Amer. Vet. Med. Assoc.* 211(3): 302 - 304.

DEA. Drug Scheduling. U.S. Drug Enforcement Administration. Available at http://www.justice.gov/dea/pubs/scheduling.html. (Accessed 3 October 2010).

Dodman, N. H., H. Levine, and M. H. Court. 1992. Clinical assessment of analgesic effects of butorphanol in cattle. *Animal Pain.* 396 – 401.

Doherty, T. J., H. G. Kattesh, R. J. Adcock, M. G. Welborn, A. M. Saxton, J. L. Morrow, and J. W. Dalley. 2007. Effects of a concentrated lidocaine solution on the acute phase stress response to dehorning in dairy calves. *J. Dairy Sci.* 90: 4232 – 4239.

Duffield, T.F. 2008. Current data on dehorning calves. Proceedings of the American Association of Bovine Practitioners Conference. 25 – 28.

Duffield, T. F., A. Heinrich, S. T. Millman, A. DeHaan, S. James, and K. Lissemore. 2010. Reduction in pain response by combined use of local anesthesia and systemic ketoprofen in dairy claves dehorned by heat cauterization. *Can. Vet. J.* 51: 283 – 288.

Earley, B. and M. A. Crowe. 2002. Effects and ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *J. Anim. Sci.* 80: 1044 – 1052.

Edmondson, M.A. 2007. Local and regional anesthesia. *Vet Clin Food Anim*. 24: 211 – 226.

Farm Animal Welfare Council. April 16, 2009. "Five Freedoms" http://www.fawc.org.uk/freedoms.htm. (Accessed October 3, 2010).

Faulkner, P. M. and D. M. Weary. 2000. Reducing pain after dehorning in dairy calves. *J. Dairy Sci.* 83: 2037 – 2041.

Faulkner, D. B., T. Eurell, W. J. Tranquilli, R. S. Ott, M. W. Ohl, G. F. Cmarik, and G. Zinn. 1992. Performance and health of weanling bulls after butorphanol and xylazine administration at castration. *J. Anim. Sci.* 70: 2970 – 2974.

Federation of State Medical Boards of the United States. 1998. Model guidelines for the use of controlled substances for the treatment of pain. Euless, Tx: The Federation.

Fell, L. R., R. Wells, D. A. Shutt. 1986. Stress in calves castrated surgically or by the application of rubber rings. *Aus. Vet. J.* 63: 16 – 18.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *J. Anim. Sci.* 74: 2336 – 2343.

Fisher, A. D., M. A. Crowe, E. M. O Naullain, M. L. Monoghan, D. J. Prendiville, P. O'Kiley, and W. J. Enright. 1997. Effects of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, in vitro interferon-γ production, leukocytes, acute-phase proteins, growth, and feed Intake. *J. Anim. Sci.* 75: 1899 – 1908.

Fisher, A. D., M. A. Crowe, E. M. O Naullain, M. L. Monoghan, J. A. Larkin, P. O'Kiley, and W. J. Enright. 1997. Effects of cortisol on in vitro interferon- γ production, acute phase proteins, growth, and feed intake in a calf castration Model. *J. Anim. Sci.*75: 1041 – 1047.

Food and Drug Administration Center for Veterinary Medicine. January 5, 2006. Guidance For Industry: Development of target animal safety and effectiveness data to support approval of non-steroidal anti-inflammatory durgs (NSAIDS) in animals. www.fda.gov/downloads/AnimalVeterinary/GuidancecomplianceEnforcement/GuidanceforIndustry/UCM052663.pdf. (Accessed September 11, 2010).

Fisher, A. D., T. W. Knight, G. P. Cosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effects of surgical or banding castration on stress responses and behavior of bulls. *Aust. Vet. J.* 4: 279 – 284.

Fulwider, W.K., T. Grandin, B.E. Rollin, T.E. Engle, N.L. Dalsted, and W.D. Lamm. 2008. Survey of dairy management practices of one hundred thirteen north central and northeastern united states dairies. *J. Dairy. Sci.* 91: 1686 – 1692.

Garcia-Villar, R., P.L. Toutain, M. Alvinerie, and Y. Ruckebusch. 1981. The pharmacokinetics of xylazine hydrochloride: an interscpecific study. *J. vet. Pharmacol. Therap.* 4: 87 – 92.

Gaynor, J. S., and W. W. Muir. 2002. Handbook of Veterinary Pain Management. 2nd ed. Mosby Elsevier. St. Louis, MO.

Gehring, R., J. F. Coetzee, J. Tarus-Sang, and M.D. Apley. 2008. Pharmacokinetics of ketamine and its metabolite norketamine administered at sub-anesthetic dose together with xylazine to calves prior to castration. *J. Vet. Pharmacol. Therap*. 32: 124 – 128.

Gonzalez, L. A., K. S. Schwartzkopf-Genswein, N. A. Caulkett, E. Janzen, T. A. McAllister, E. Fierheller, A. L. Schaefer, D. B. Haley, J. M. Stookey, and S. Hendrick. 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, Escherichia coli, and salivary cortisol. *J. Anim. Sci.* 88(2): 802 – 810.

Gottschalk, A., D.S. Smith. 2001. New concepts in acute pain therapy: pre-emptive analgesia. *American Family Physician.* 63(10): 1979 – 1984.

Graf, B. and M. Senn. 1999. Behavioral and physiological responses of calves to dehorning by heat cauterization with or without local aneasthesia. *Applied Anim. Behav. Sci.* 62: 153 – 171.

Graham, Martin. August 1997. GSR in cows in response to electrical stimulation. Presented at ASAE annual international meeting. St. Joseph, MI

Grondahl-Nielsen, C., H. B. simonsen, J. Damkjer Lund, M. Hesselholt. 1999. Behavioral, endocrine, and cardiac responses in young calves undergoing dehorning without and with the use of sedtion and analgesia. *The Veterinary J.* 158: 14 – 20.

Haskell, S.R.R, R. Gehring, M. A. Payne, A. L. Craigmill, A. I. Webb, R. E. Baynes, J. E. Riviere. 2003. Update on FARAD food animal drug withholding recommendations. *J. Am. Vet. Med. Assoc*. 223: 1277 – 1278.

Heinrich, A., T. F. Duffield, K. D. Lissemore, E. J. Squires, and S. T. Millman. 2009. The impact of meloxicam on postsurgical stress associated with cautery dehorning. *J. Dairy Sci.* 92: 540 – 547.

Heleski, C. R., A. G. Mertig, and A. J. Zanella. 2004. Assessing attitudes toward animal welfare: a national survey of animal science faculty members. *J. Anim. Sci.* 82: 2806 – 2814.

Heleski, C. R., A. G. Mertig, and A. J. Zanella. 2005. Results of a national survey of US veterinary college faculty regarding attitudes toward farm animal welfare. *J. Am. Vet. Med. Assoc.* 226: 1538 – 1546.

Hewson, C. J., I. R. Dohoo, K. A. Lemke, H. W. Barkema. 2007. Canadian veterinarians' use of analgesics in cattle, pigs, and horses in 2004 and 2005. *Can. Vet. J.* 48: 155 – 164.

Hjemdahl, P. 1993. Plasma catecholamines – analytical challenges and physiological limitations. *Baillieres Clin. Endocrinol. Metab*. 7: 307 – 353.

Irwin, J. 2004. AgFacts: Castrating Calves. Available at: http://www.dpi.nsw.gov. au/_data/assets/pdf_file/0008/174167/castrating-calves.pdf (accessed 2 June 2010).

Kehlet. H., J. B. Dahl. 1993. The value of "multimodal" or "balanced analgesia" in postoperative pain treatment. *Anesth Analg.* 77: 1048 – 1056.

Kent, J. E., M. V. Thrusfield, I. S. Robertson, and V. Molony. 1996. Calstration of calves: a study of methods used by farmers in the United Kindgom. *The Veterinary Record.* 138: 384 – 387.

Kent, J. E. 1999. Mutilations: a necessary evil. *The Veterinary Journal*. 158: 1 – 3.

King, B. D., R. D. H. Cohen, C. L. Guenther, and E. D. Janzen. 1991. The effect of age and method of castration on plasma cortisol in beef calves. *Can. J. Anim. Sci.* 71: 257 – 263.

Knight, T. W., G. P. Cosgrove, M. G. Lambert, A. F. Death. 1999. Effects of method and age at castration on growth rate and meat quality of bulls. *New Zealand Journal of Agriculture Research*. 42: 255 – 268.

Knight, T. W., G. P. Cosgrove, A. F. Death, and C. B. Anderson. 2000. Effect of method of castrating bulls on their growth rate and liveweight. *New Zealand Journal of Agriculture Research.* 43: 187 – 192.

Laden, S.A., J. E. Wohlt, P. K. Zajac, and R. V. Carsia. Effects of stress from electrical dehroning on feed intake, growth, and blood concentration of Holstein heifer calves. 1985. *J Dairy Sci.* 68: 3062 – 3066.

Lamont, L. A. 2008a. Adjunctive analgesic therapy in veterinary medicine. *Vet Clin Small Anim*. 38: 1187 – 1203.

Lamont, L. A. 2008b. Multimodal pain management in veterinary medicine: the physiologic basis of pharmacologic therapies. *Vet Clin Small Anim.* 38: 1173 – 1186.

Landoni, M. F., F. M. Cunningham, and P. Lees. 1995. Pharmacokinetics and pharmacodynamics of ketoprofen in calves applying PK/PD modeling. *J. vet. Pharmacol Therap*. 18: 315 – 324.

Lepkova, R., J. Sterc, V. Vecerek, J. Doubek, K. Kruzikova, and I. Bedanova. 2007. Stress responses in adult cattle due to surgical dehorning using three different types of anaesthesia. *Berliner und Munchener tierarztliche Wochenschrift.* 120 (11-12): 465 – 469.

Lin, H. C., and M. G. Riddell. Preliminary study of the effects of xylazine or detomidine with or without butorphanol for standing sedation in dairy cattle. 2003. *Veterinary Therapeutics.* 4(3): 285 – 291.

Livingston, A. Comparative and Veterinary Pharmacology. pg 159 – 183. 2010 Springer-Verlag. Pain and analgesia in domestic animals. Berlin.

Lusk, J. L., and F. B. Norwood. 2008. A survey to determine public opinion about the ethics and governance of farm animal welfare. *J. Am. Vet. Med. Assoc*. 233: 1121 – 1126.

Marti., S., A. Velarde, J.L. de la Torre, A. Bach, A. Aris, A. Serrano, X. Manteca, and M. Devant. 2010. Effects of ring castration with local anesthesia and analgesia in Holstein calves at three months of age on welfare indicators. *J. Anim. Sci.* 2010

McCartney, C. J. L., A. Sinha, and J. Katz. 2004. A qualitative systematic review of the role of N-methyl-D-aspartate receptor antagonists in preventive analgesia. *Anesth. Analg.* 98: 1385 – 1400

McMeekan, C. M., D. J. Mellor, K. J. Stafford, R. A. Bruce, R. N. Ward, and N. G. Gregory. 1997. Effects of shallow scoop and deep scoop dehorning on plasma cortisol concentrations in calves. *New Zealand Veterinary Journal*. 45: 72 – 74.

McMeekan, C. M., D. J. Mellor, K. J. Stafford, R.A. Bruce, R. N. Ward, and N. G. Gregory. 1998a. Effects of local anesthesia of 4 or 8 hours duration on the acute cortisol response to scoop dehorning in calves. *Aust. Vet. J.* 76: 281 – 285.

McMeekan, C. M., K.J. Stafford, D. J. Mellor, R. A. Bruce, R. N. Ward, and N. G. Gregory. 1998b. Effects of regional anesthesia and/or a non steroidal anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. *Research in Veterinary Science.* 64: 147 – 150.

McQuay, H. J. 1992. Pre-emptive analgesia [editorial]. *Br. J. Anaesth.* 69: 1 – 3.

Mellor, D.J. 1991. Effects of castration on behavior and plasma cortisol concentrations in young lambs, kids and calves. *Research in Veterinary Science.* 51. 149 – 154.

Milligan, B. N., T. Duffield, and K. Lissemore. 2004. The utility of ketoprofen for alleviating pain following dehorning in young diary calves. *Can Vet J.* 45: 140 – 143.

Misch, L. J., T. F. Duffield, S. T. Millman, K. D. Lissemore. 2007. An investigation into the practices of dairy producers and veterinarians in dehorning dairy calves in Ontario. *Can. Vet. J.* 48: 1249 – 1254.

Molony, V., J. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after different methods of castration of calves. *App. Anim. Behav. Sci*. 46: 33 – 48.

Molony, V., and J. E. Kent. 1997. Assesment of acute pain in farm animals using behavioral and physiological measurements. *J. Anim. Sci.* 75: 266 – 272.

Morisse, J. P., J. P. Cotte, and D. Huonnic. 1995. Effect of dehorning on behavior and plasma cortisol responses in young calves. *App. Anim. Behav. Sci.* 43: 239 – 247.

Muir, W. M., and C. J. Woolfe. 2001. Mechanisms of pain and their therapeutic implications. *J. Am. Vet. Med. Assoc.* 219: 1346 – 1356.

Myers, M. J., M. I. Scott, C. M. Deaver, D. E. Farrell, and H. F. Yancy. 2009. Biomarkers of inflammation in cattle determining the effectiveness of anti-inflammatory drugs. J. Vet. Pharacol. Therap. 33: 1 – 8.

Morrow-Tesch, J. L. 2001. Evaluating management practices for their impact on welfare. *J. Am. Vet. Med. Ass*. 219:1374 – 1376.

Nolan, A. 2001. Patterns and management of pain in animals. International congress and symposium number 246. 93 – 99.

Norwood, F. B, J. L. Lusk, R. W. Prickett. 1997. Consumer Preferences for Farm Animal Welfare: Results of a nationwide telephone survey. Department of Agricultural Economics. Oklahoma State University.

Pang, W. Y., B. Early, T. Sweeney, M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-γ production, acute-phase proteins, feed intake, and growth. 84: 351 – 359.

Paul-Murphy, J., J. W. Ludders, S. A. Robertson, J. S. Gaynor, P. W. Hellyer, and P. L. Wong. 2004. The need for a cross-species approach to the study of pain in animals. *J. Am. Vet. Med. Ass*. 224:692 – 697.

Petrie, N.J, D.J. Mellor, K.J. Stafford, R.A. Bruce, and R.N. Ward. 1995. Cortisol Response to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal.* 44: 9 – 14.

Pozzi, A., W.W. Muir, F. Traverso. Prevention of central sensitization and pain by N-methyl-D-aspartate receptor antagonists. 2006. *J. Amer. Vet. Assoc.* 228(1): 53 – 60.

Roberstosn, I. S., J. E. Kent, and V. Molony. 1994. Effect of different methods of castration on behavior and plasma cortisol in calves of three ages. *Research in Veterinary Science*. 56: 8 – 17.

Rollin, B.E. 2004. Annual meeting keynote address: Animal agriculture and emerging social ethics for animals. *J. Anim. Sci. 82*: 955 – 964.

Schwartzkopf-Genswein, K. S., M. E. Booth-McLean, T. A. McAllister, and G. J. Mears. 2005 Physiological and behavioral changes in Holstein calves during and after dehorning and castration. *Can. J. Anim. Sci*. 85: 131 – 138.

Sellers, G. H. C. Lin, M.G. Riddell, W. R. Ravis, S. H. Duran, and M. D. Givens. 2009. Pharmacokinetics of licocaine in serum and milk of mature Holstein cows. *J. vet. Pharmacol. Therap*. 32: 446 – 450.

Smith, G. W., J. L. Davis, L. A. Tell, A. I. Webb, and J. E. Riviere. 2008. Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. *J. Am. Vet. Med. Assoc*. 232: 697 – 701.

Stafford, K. J., D. J. Mellor, and C. M., McMeekan. 2000. A survey of the methods used by farmers to castrate calves in New Zealand. *New Zealand Veterinary Journal.* 48: 16 – 19.

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. *Research in Veterinary Science.* 73: 61 – 70.

Stafford, K.J., D.J. Mellor, S.E. Todd, R.N. Ward, and C.M. McMeekan. The effect of different combinations of lignocaine, ketoprofen, xylazine, and tolazoline on the acute cortisol response to dehorning in claves. 2003. New Zealand Veterinary Journal. 51(5): 219 – 226.

Stafford, K. J., and D. J. Mellor. 2005a. The welfare significance of the castration of cattle: a review. New Zealand Vet. J. 53(5): 271 – 278.

1141

1142 Stafford, K. J., and D. J. Mellor. 2005b. Dehorning and disbudding distress and its

1143 alleviation in calves. *The Veterinary Journal*. 169: 337 – 349.

1144

1145

1146 Stewart, M. G. A. Verkerk, K. J. Stafford, A. L. Schaefer, and J. R. Webster. 2010.

1147 Noninvasive assessment of autonomic activity for evaluation of pain in calves, using

1148 surgical castration as a model. *J. Dairy. Sci.* 93: 3602 – 3609.

1149

1150 Stillwell, G., M. S. Lima, and D. M. Broom. 2008. Effects of nonsteroidal anti

1151 inflammatory drugs on long-term pain in calves castrated by use of an external clamping

1152 technique following epidural anesthesia. *Am. J. Vet. Res*. 6: 744 – 750.

1153

1154 Stillwell, G., M. S. Lima, D. M. Broom. 2008. Comparing plasma cortisol and behavior

1155 of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia.

1156 *Livestock Science.* 119: 63 – 69.

1157

1158 Stillwell, G., R. Campos de Carvalho, M. S. Lima, and D. M. Broom. 2009. Effect of

1159 caustic paste disbudding, using local anesthesia with and without analgesia, on behavior

1160 and cortisol in calves. *Applied animal behavior science.* 116: 35 – 44.

1161

Stillwell, G., R. C. Carvalho, N. Carolino, M. S. Lima, and D. M. Broom. 2010. Effect of hot-iron disbudding on behavior and plasma cortisol of calves sedated with xylazine. *Research in Veterinary Science*. 88: 188 – 193.

Stroud, L.R., B. A. Solomon, and E. Shenassa, E. 2007. Long-term stability of maternal prenatal steroid hormones from the National Collaborative Perinatal Project: Still valid after all these years. *Psychoneurolendocrinology*. 32(2):140 - 150.

Sutherland, M. A., D. J. Mellor, K. J. Stafford, N. G. Gregory, R. A. Bruce, and R. N. Ward. 2002a. Effect of local anesthetic combined with wound cauterization on the cortisol response to dehorning in calves. *Aust. Vet. J.* 80: 165 – 168.

Sutherland, M.A., D. J. Mellor, K. J. Stafford, N. G. Gregory, R. A. Bruce, and R. N. Ward. 2002b. Cortisol responses to dehorning of calves given 5-h local anesthetic regimens plus phenylbutazone, ketoprofen, or adrenocorticotropic hormone prior to dehorning. *Research in Veterinary Science*. 73: 115 – 123.

Sylvester 1998a

Sylvester, S. P., K. J. Stafford, D. J. Mellor, R. A. Bruce, and R. N. Ward. 1998b. Acute cortisol responses of calves to four methods of dehorning by amputation. *Aust. Vet. J.* 86(2): 123 – 126.

Thuer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods in calves. *The Veterinary Journal*. 173: 333 -342.

Ting, S. T. L., B. Earley, and M. A. Crowe. 2003a. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. *J Anim. Sci*. 81: 1253 – 1264.

Ting, S. T. L., B. Earley, J. M. L. Hughes, and M. A. Crowe. 2003b. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. *J. Anim. Sci.* 81: 1281 – 1293.

Underwood, W. J. 2002. Pain and distress in agricultural animals. *J. Am. Vet. Med. Ass*. 221:208 – 211.

Van Reenan, C. G., N. E. O'Connell, J. T. N. Van der Werf, S. M. Korte, H. Hopster, R. B. Jones, and H. J. Blokhuis. 2005. Responses of calves to acute stress: individual consistency and relations between behavioral and physiological measures. *Physiology and Behavior*. 85: 557 – 570.

Vinuela-Fernandez, I., E. Jones, E. M. Welsh, and S. M. Fleetwood-Walker. 2007. Pain mechanisms and their implications for the management of pain in farm and companion animals. *The Vet. J.* 174: 227 – 239.

Whittem, T., D. A. Freeman, K. Parton, and D. W. Hanlon. 1996. The pharmacokinetics of salicylate in dairy cattle are not altered by simultaneous invtravenous ceftiofur sodium and DL-lysine-acetyl salicylate. *J. vet. Pharmacol. Therap*. 19: 104 – 108.

Wohlt, J.E., M.E. Allyn, P.K. Zajac, and S. Katz. 1994. Cortisol increases in plasma of Holstein heifer claves from handling and method of electrical dehorning. *J Dairy Sci*. 77: 3725 – 3729.

CHAPTER 2: Pharmacokinetics and physiologic effects of xylazine-ketamine-butorphanol administered intramuscularly alone or in combination with orally administered sodium salicylate on biomarkers of pain in Holstein calves following concurrent castration and dehorning

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Introduction

Societal concerns for the moral and ethical treatment of animals and livestock have increased, especially since the early 1990s (Rollin, 2004). In particular, the negative public perception of procedures involved with castration and dehorning is mounting with calls for the development of practices minimizing pain and suffering associated with common animal husbandry practices in cattle. The use of analgesic therapy during painful procedures such as castration and dehorning has been suggested by organizations such as the American Veterinary Medical Association; however, FDA-approved drug labels for the treatment of pain in cattle do not currently exist (AVMA, 2009). In order to enable the cattle industry to effectively respond to these challenges, research is necessary for evaluating the welfare implications of routine animal husbandry practices and identifying practical and cost-effective strategies for relieving pain in cattle.

The development of robust biomarkers for the objective measurement of pain is necessary for evaluating the efficacy of analgesic treatment regimens during routine

1252 animal husbandry procedures such as castration and dehorning. This process is
1253 especially complex in a prey species, such as cattle, that inherently conceal pain
1254 (Underwood, 2002). In previous research (Fisher *et al.*, 2002; Knight *et al*., 2000;
1255 Mellor *et al.*, 2000; Ting *et al.*, 2003, Pang *et al*., 2006; Gonzalez *et al.*, 2008; Faulkner *et*
1256 *al*., 2002) biomarkers for the evaluation of pain and distress associated with castration
1257 and dehorning have included serum cortisol concentration, heart rate, measurement of the
1258 presence of acute phase proteins and in vitro interferon-γ production, behavior scoring,
1259 average daily gain, feed intake, chute activity, and vocalization. The magnitude of the
1260 increase in serum cortisol concentration (as indicated by the change in peak concentration
1261 height [C_{max}]) and duration; the integrated response (as indicated by the AUEC), or both
1262 has been reported to correspond with the predicted noxious stimulus of these during the
1263 procedure (Mellor *et al.*, 2000). The results of studies using ADG as a pain parameter
1264 have been equivocal. For example, one study revealed calves undergoing castration have
1265 a decrease in ADG when compared to calves not undergoing castration; however, a
1266 treatment affect was not observed (Faulkner *et al.*, 1992). Additionally, information is
1267 deficient on the use of chute exit speed and EDA for the objective measurement of pain
1268 associated with castration and dehorning. Chute exit speed has been used in temperament
1269 and reactivity studies in cattle (Mellor *et al.*, 2008; Curley *et al.*, 2006). We therefore
1270 hypothesized that evaluation of exit speed could be used to determine the effect of a
1271 painful procedure and sedative drug on calf behavior. EDA is a measurement of electrical
1272 resistance of a tissue path between two electrodes applied to the skin and can be
1273 influenced by changes in sympathetic outflow during times of pain, anxiety, and stress
1274 (Benford *et al.*, 2004). We hypothesized that sympathetic outflow may increase after

castration and dehorning although the findings of one study reporting EDA assessment in rats undergoing surgery were equivocal (Richardson *et al.,* 2007).

Furthermore, there are many published studies describing the effects of either castration or dehorning, but there are currently no studies that describe the pain response following both castration and dehorning procedures performed in series. In a recent survey of veterinarians (Coetzee *et al.,* 2010), 90% of respondents indicated these procedures are commonly performed at the same time in many production systems. Castration by surgery (pulled and cut respectively) alone caused a peak in cortisol concentrations of 68 nmol/L in 2 to 4 month calves and 129 nmol/L in 5.5 month calves at 30 minutes after the procedure (Stafford and Mellor, 2005). Another study (Doherty *et al.*, 2007) looking at dehorning alone found hot iron dehorning to cause a increase of plasma cortisol to approximately 80 nmol/L after 30 mintues and 45 nmol/L after 60 minutes in untreated 10 to 12 week Holstein calves.

To mitigate pain in livestock, pre-emptive analgesia could be administered prior to painful procedures through the use of various drug regimens. The goal of pre-emptive analgesia is to prevent central sensitization or wind-up pain (Kissin, 2005). Agents that could be used during administration of preemptive analgesia include non-steroidal anti-inflammatory drugs, opioids, α2-agonists, and *N*-methyl *D*-aspartate receptor antagonists (Thurman *et al.*, 2006). Salicylic acid derivatives, including aspirin (acetylsalicylic acid) and sodium salicylate (salicylate), were the first NSAIDs to be used in modern medicine and are still widely used for their analgesic, antipyretic, and anti-inflammatory properties (Langston 2003). In previous bovine castration studies, plasma concentrations of sodium salicylate above 25 µg/mL have coincided with decreased peak cortisol concentrations as

compared to castration with no analgesia (Coetzee *et al.*, 2007). Although the veterinary forms of aspirin are marketed with label indications for the treatment of fever, inflammation, and pain relief, these have never been approved by the FDA Center for Veterinary Medicine for these indications (USP Veterinary Pharmaceutical Information Monographs, 2004). Salicylate is more soluble in water than aspirin and may offer a convenient and cost-effective means of providing an NSAID in the drinking water. However, the use of sodium salicylate is only permitted under the Animal Medicinal Drug Use Clarification Act (AMDUCA) under the supervision of a veterinarian to alleviate suffering provided use does not result in a violative tissue residue (AMDUCA 1994).

The pain response associated with castration and dehorning performed concurrently on calves and the mitigation of this response has not been described. Furthermore, there is some data describing the pharmacokinetic parameters and the associated effects of IM administration of xylazine, ketamine, and butorphanol (Gehring *et al.,* 2008; Sellers *et al.,* 2010). However, studies using salicylate administered PO through free-choice water consumption alone or in combination of xylazine, ketamine, and butorphanol prior to castration and dehorning are deficient in the published literature. If sodium salicylate provided in the drinking water alone or in combination with parenteral sedative-analgesia attenuates signs of distress without causing recumbency, this would offer veterinarians and producers a practical and cost-effective way to reduce pain and distress associated with castration and dehorning. The purpose of the study reported here was to evaluate the individual and combined effects of xylazine, ketamine, and butorphanol administered IM alone or in combination with continuous exposure to

sodium salicylate administered PO through free-choice water consumption on ADG, chute exit speed, EDA, and cortisol response of calves following castration and dehorning in series.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (KSU) (No. 2694). Because this study involved cattle that would experience unmitigated pain as a result of inclusion of a placebo treatment group following castration and dehorning, all calves were assessed 3 times daily for behavioral signs of excessive pain for a 72-hour period after castration and dehorning. Parameters including attitude, gait, appetite, lying, scrotal swelling and horn bud assessment were assigned a score from 0 (pre-study levels) to 5 (significantly altered) with a score of 3 or greater requiring notification of the University Veterinarian. A rescue analgesic protocol for flunixin meglumine at 2.2 mg/kg IV twice daily was scheduled if calves were noted to have scores of 3 or greater in one or more categories after castration and dehorning.

Animal Husbandry

In June of 2008, 40 horned, sexually intact male Holstein calves between 2 to 4 months of age and weighing between 108 to 235 kg were acquired from 3 farms located in Kansas. On arrival, scrotal circumference, horn-base diameter, and horn length was measured. Additionally, all calves received an SC injection of tulathromycin[a] (2.5 mg/kg) as metaphylactic treatment against bovine respiratory disease, an 8-way clostridial vaccine,[b] a 4-way modified-live viral respiratory disease vaccine,[c] and pour-on

for the treatment and removal of external parasites[d]. For sustained fly control, application of the pour-on was repeated every 7 to 10 days for the duration of the study. Five pens (8 calves/pen) were used to house calves in a dry lot confinement facility at Kansas State University (KSU). Ad libitum access to brome hay was provided to each calf. A ration (3.6 kg/calf/day) from a typical beef feedlot receiving diet was provided for the duration of the study. With the exception of the use of buckets for calves in the SAL treatment group, water was provided ad libitum with self-filling water troughs throughout the study.

Three days prior the start of each phase, the 8 calves (n = 2/treatment) assigned to that specific phase were transferred from the dry lot facility to the Animal Resource Facility at KSU and individually allocated to indoor pens (area, 13.40 m^2). Over a 2-day period, calves were adapted to housing in individual pens during which time each calf was restrained with a rope halter within their respective pen for at least 10 to 15 minutes. Each calf was conditioned to walking through an alleyway and restraint in a cattle chute one time prior to the start of the phase. Calves were housed in the Animal Resource Facility for 10 days for completion of Periods 1 and 2 of the study.

Study Design

A 2-period, parallel design study **(Figure 1)** was conducted with treatments arranged in a 2 x 2 x 2 factorial arrangement. The factors were Period (sham castration and sham dehorning (Period 1) or castration and dehorning (Period 2)), sodium salicylate administration (Yes or No), and XKB administration (Yes or No). Prior to study

commencement, calves (n=40) were blocked by bodyweight and randomly assigned treatment groups using random number generating software package[e] so that average weight, scrotal circumference, horn diameter, and horn length were balanced across the treatment groups. The treatment groups (n = 10 calves per group) were (i) 0.9% sterile sodium chloride administered IM (PLACEBO); (ii) 2.5 to 5 mg/mL of sodium salicylate[f] administered PO through free-choice water consumption initiated 24 hours (day -3) prior to Period 1 until 48 hours (day 2) after Period 2 (SAL); (iii) 0.05 mg/kg xylazine[g] + 0.1 mg/kg ketamine[h] + 0.025 mg/kg butorphanol[i] administered IM immediately prior to castration and dehorning in Period 1 and Period 2(XKB); and (iv) a combination of treatments ii and iii (SAL + XKB). Scrotal circumference was measured at the point of maximum scrotal diameter by use of a scrotal circumference tape.[j] Horn diameter (millimeters) was measured with calipers at the base of the horn near the head as it enters the frontal sinus. Horn length was measured from the base of the horn to the tip on the lateral aspect.

The study was completed in five 10-day duration phases from June 30, 2008 to August 11, 2008. Eight calves were assigned to 1 of the 5 phases (2 calves per treatment group per phase as described). The group with the heaviest calves was assigned to the first phase while the lightest calves were assigned to the last phase to minimize variations in body weight, scrotal circumference, and horn diameter by the time the procedures were performed. Each phase was divided into 2 periods with the procedures occurring exactly 48 hours from the other: sham castration and sham dehorning on day -2 (Period 1) and castration and dehorning on day 0 (Period 2). All castration and dehorning procedures were performed by the same veterinarian (JBR).

Determination of Mean Change in Body Weight

Body weights of calves were determined by use of a squeeze chute with a scale that was used for the entire study.[k] All calves were weighed approximately 1 week before the start of the study (June 20th, 2008). The 8 calves assigned to that respective phase were weighed in the morning of days -3, -2, 0, 1, and 2 to determine the mean change in body weight. The calves were then weighed at 4, 6, and 13 days after actual castration and dehorning (Period 2).

Jugular Vein Catheterization

To facilitate the intensive blood sampling schedule and minimize stress invoked on the animal that could potentially confound cortisol concentration measurements, catheters were placed in the left jugular vein of each calf on the morning of day -3 (approximately 24 hours before Period 1). On that morning, calves were individually restrained by a squeeze chute. The area over the jugular vein was clipped and aseptically prepared by use of povidone iodine soap and 70% isopropyl alcohol solution. The catheter insertion site was infiltrated with approximately 0.5 mL of 2% lidocaine hydrochloride SC.[l] A 10 to15 mm stab incision was made through the skin with a No. 21 surgical blade to facilitate placement of a 14 gauge X 13 cm catheter in the jugular vein.[m.] The indwelling catheter was sutured to the skin to ensure catheter placement and an injection port was secured. In order to maintain catheter patency during the study period, 3 mL of flush solution (3 USP units of heparin sodium/mL in saline solution [0.9% NaCl][n]) was

instilled into the indwelling catheter. A blood sample was collected from calves in the SAL and SAL + XKB groups prior to release from the squeeze chute to determine baseline salicylate concentrations.

Sham Castration, Sham Dehorning, Castration, and Dehorning

Approximately 30 minutes prior to commencement of Period 1 (Figure 1) on day -2, calves were fitted with a rope halter and relocated as a group into a holding pen with an adjacent alleyway leading to the squeeze chute. Approximately 2 minutes prior to sham castration, calves were individually led into a squeeze chute with a rope halter and a blood sample was collected for measurement of the baseline serum cortisol concentration (all treatment groups) and pre-study plasma SAL concentrations (SAL and SAL + XKB). The order of castration and dehorning was predetermined before the start of each phase to maintain consistency between study days with order of the treatment groups starting first with PLACEBO, followed by SAL , then XKB, and ending with SAL + XKB. The order was repeated a second time for a total of 8 calves. At time point 0 of day -2 (Period 1), a volume of saline solution equivalent to the volume of XKB administered to calves in the XKB groups was administered IM to the PLACEBO and SAL groups. For the XKB and SAL + XKB groups, 0.025 mg/kg butorphanol tartrate, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine were administered concurrently IM at time point 0. Immediately after drug/ placebo administration, the scrotum was cleaned with a 0.1% chlorhexidine solution, the apex of the scrotum was manually extended and elongated ventrally and each testicle was then repeatedly manipulated (4 to 5 times for the left and right testicle) dorsally and ventrally within the scrotum for approximately 20 seconds (sham castration). The head

was then restrained with a halter by extending and flexing the neck laterally to the right and the hair trimmed around the base of the left horn (sham dehorning); this process was similarly repeated for the right horn (sham dehorning). The 5-minute blood sample was collected in the chute prior to release of the calf. The calf was then released from the chute through another alleyway (set up for measurement of chute exit speed) and restrained prior to each successive sampling of blood at the intervals described. The process was repeated on each calf in Period 1.

During Period 2, calves were similarly restrained and blood sampled as in Period 1. The scrotum was cleaned as described. Castration was performed by use of a closed surgical castration technique without the provision of local anesthesia. The apex of the scrotum was secured manually, extended distally, and the distal third of the scrotum was removed with a No. 10 scalpel blade. The right testicle and spermatic cord was exteriorized by blunt dissection of the scrotal fascia. The cremaster muscle was stripped ventrally via digital manipulation and traction. Then, the testicular artery and vein, epididymis, and vas deferens were stripped ventrally by digital manipulation and traction. The remaining connective tissue was incised with the scalpel blade. The same procedure was used to remove the left testicle. After castration the head was restrained similar to Period 1. The left horn was removed by use of a Barnes dehorning instrument.[o] Hemostasis was achieved through thermocautery by use of a hot iron.[p] The head was released and restrained as described in Period 1. The right horn was removed with the same procedure for the left horn. The head was released from restraint, the 5-minute blood sample was collected, and the calf was released from the squeeze chute as described in Period 1. This process was repeated on each calf during Period 2.

Determination of Chute Exit Speed

Upon release from the squeeze chute into the alleyway as described in Period 1, the calf passed through a series of 2 wireless photo sensors[q] positioned 1.5 m and 3 m, respectively, from the exit of the chute. The time elapsed for each calf to travel 1.5 m between these 2 sensors (chute exit speed) was recorded by an electronic timer[q] equipped with a printer.[q]

Blood Sample Collection

Blood samples were collected immediately prior to sham castration and sham dehorning and castration and dehorning in Periods 1 and 2 (i.e. 0 minutes) and at 5, 10, 20, 30, 40, and 50 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours. At approximately 30 seconds prior to each sample collection, 5 mL of blood was drawn from the indwelling catheter of the left jugular vein and directly returned; this process was repeated 3 times so that the third repetition was completed immediately prior to the scheduled sample collection. At the designated time, blood was drawn from the indwelling catheter into 20-mL luer lock syringes[r] and transferred to evacuated tubes containing lithium heparin (sample total volume, 6 mL) and evacuated tubes with no additive (sample total volume, 8 mL); additionally, 5 mL of flush solution was injected into the indwelling catheter after sample collection to maintain patency of the catheter. The evacuated tubes were immediately stored on ice until centrifugation for 10 minutes at 3,000 X g to separate blood components. Plasma or serum was then transferred into cryovials and frozen at –80°C prior to sample analysis.

Electrodermal Activity

EDA was measured by use of a commercially available pain assessment device.[s] The device consisted of 2 electrodes that transmit an electric current when touched on a hairless area of an animal's skin. These electrodes were placed across the nasal planum of each calf when determining a reading. A numerical score between 0 and 9.9 was digitally displayed on the device with 0 corresponding to calm or no pain and 9.9 corresponding to tense or severe pain. Readings were taken immediately prior to procedures in both Periods 1 and 2 and then at 5, 10, 20, 30, 40, and 50 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours after the initial reading. Readings were also taken at castration and at dehorning. The EDA was measured only during phases 3, 4, and 5 of the study (n = 6 animals/ treatment).

Sodium Salicylate Administration

Four 19-L plastic buckets were weighed and the results recorded. Sodium salicylate powder[t] was added to 10 L of tap water in plastic buckets to achieve a final concentration of 2.5 to 5 mg sodium salicylate/mL of water. Fifteen to 45 mL of molasses was mixed to increase palatability depending on the level of water consumption by the calf. The weight of each bucket containing the medicated solution was recorded. Sodium salicylate powder was provided in the drinking water 24 hours prior to Period 1 by hanging the bucket containing the medicated water from a chain in each pen of the calves in the SAL and SAL + XKB treatment groups. Calves in the SAL and SAL + XKB groups were provided the medicated solution ad libitum.

Water buckets were checked three times a day. After near completion of the medicated solution in the bucket, the remaining contents were weighed, dumped out and the bucket refilled with a freshly prepared volume of medicated solution as described. On days -3 and -1, 12 hours prior to sham castration and castration, respectively, 2 buckets with differing concentrations of the medicated solution (1.5 mg/mL and 2.5 mg/mL or 2.5 mg/mL and 5 mg/mL) were offered to calves to improve the consumption of salicylate and to achieve maximum plasma salicylate concentrations. Calves in the SAL and SAL + XKB groups were offered the medicated solution from 24 hours prior to period 1 to 48 hours after period 2. Forty-eight hours after period 2, calves were offered a final bucket of the medicated solution. Calves were allowed to finish the bucket of medicated solution, and then a bucket of fresh tap water was offered. Calves in the PLACEBO and XKB groups were offered tap water ad libitum via self-filling water units.

Determination of Serum Cortisol Concentration

Serum cortisol concentrations were determined by use of a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analysis system[u] as described (Coetzee *et al.*, 2007). A minimum sample volume of 100 µL of serum were used for analysis by the assay. The calibration range for the assay was 28 to 1,380 nmol of cortisol/L. The analytical sensitivity was 5.5 nmol of cortisol/L. Cortisol samples were analyzed within 3 months of collection. Cortisol stability has been verified previously[26] in human serum after 42 years of storage at -20ºC. The laboratory technician performing the analysis was masked to the assignment of samples to the treatment groups.

Determination of Plasma Drug Concentration of Xylazine, Ketamine, and Butorphanol

Plasma concentrations of xylazine ($H^{+;}$ m/z, 221.2 → 90.1), butorphanol ($H^{+;}$ m/z, 328.3 → 157.1), and ketamine ($H^{+;}$ m/z, 238.1 → 125.0) were determined with a high-pressure liquid chromatography,[v] and mass spectrometry-mass spectrometry[w] method. Fifty microliters of an internal standard (Ketamine-D_4 [100 ng/mL] in 50:50 acetonitrile:water; m/z 242.2 → 129.0) was used for ketamine and xylazine determination. Norketamine- D_4 [100 ng/mL] in 50:50 acetonitrile:water; (m/z 228.1 → 129.0) was used as an internal standard for butorphanol. The internal standards combined with 400 µL of acetonitrile were added to each 100 µL aliquot of study plasma and blank plasma to create standards and quality controls. Each sample was vortexed for approximately 20 seconds to precipitate the proteins and centrifuged for 10 minutes at 6,500 X g. Approximately 400 µL of supernatant was filtered by use of a 0.45µm filter.[x] The fluid volume of the filtrate was evaporated under nitrogen at 40°C by use of a dry-down unit. Dried extracts were reconstituted in 100 µL of starting mobile phase (5:95 0.2% acetic acid in H_2O:0.2% acetic acid in acetonitrile), vortexed, and transferred to autosampler vials for injection. The mobile phase consisted of 0.2% acetic acid in H_2O (A; starting mobile phase) and 0.2% acetic acid in acetonitrile at a flow rate of 0.4 mL/min (B; transitioning mobile phase). The mobile phase gradient consisted of 5% of B from 0 to 1.0 minutes, a linear gradient to 80% of B at 4.5 minutes, and then return to the starting mobile phase. The total runtime of analysis was 7 minutes. Analyte separation was achieved by use of a C18 column[y] maintained at 40°C. The method was accurate and

precise across a linear dynamic range of 0.5 ng/mL to 100.0 ng/mL. Quality controls of known concentrations were analyzed during sample analysis for monitoring of method performance. The precision and accuracy of 45 quality control samples instrumented over 5 analytical runs was found to be ≤ 2.1% and ≤ 4.5% (xylazine), ≤ 9.9% and ≤ 10.7% (butorphanol), and ≤ 8.3% and ≤ 5.8% (ketamine), respectively. All samples were analyzed within 6 months of collection. Xylazine and ketamine stability have been verified after 2 months of storage at -20ºC as compared with fresh plasma samples.[26] However, the stability of butorphanol has not been reported. The laboratory technician (JH) performing the analysis was masked to the assignment of samples to the treatment groups (XKB and SAL + XKB).

Determination of Plasma Drug Concentration of Salicylate

Plasma salicylate concentrations were determined by use of a fluorescence polarization immunoassay kit[z] as described (Coetzee *et al.*, 2007). The limit of quantification range was 5 μg to 800 μg of salicylate/mL. Quality control samples (10 to 400 μg of salicylate/mL in typical untreated bovine serum) were analyzed and compared to the calibration curve prior to analysis of study samples. Deviation of quality control concentrations greater than 10% warranted recalibration. A calibration curve was constructed with 6 calibration points (duplicate samples in typical untreated bovine serum; 0, 50, 100, 200, 400, and 800 μg of salicylate/mL). All samples were analyzed within 5 months of collection (SLB).

Pharmacokinetic and Pharmacodynamic Analysis

The pharmacokinetic and pharmacodynamic parameters (T_{max}, C_{max}, and mean concentration) of salicylate and cortisol were analyzed descriptively by inspection of the time-concentration curve. The area under the curve (AUC) for salicylate and the area under the effect curve (AUEC) for cortisol was calculated by use of the trapezoidal rule.

Noncompartmental pharmacokinetic analysis of xylazine, ketamine, and butorphanol time concentration data was performed (RG) by use of a commercially available software program.[aa] Pharmacokinetic parameters determined were AUC (first to last measured concentration) determined by the trapezoidal rule, slope of the terminal portion of the time-concentration curve (λ_z), terminal elimination half-life ($T_{½\lambda z}$), time to maximum drug concentration (T_{max}), maximum drug concentration (C_{max}), total body clearance per fraction of drug absorbed (Cl_F), volume of distribution per fraction of drug absorbed (Vz_F), and mean residence time (MRT). The parameters are represented in the following equations:

$$T\frac{1}{2}el = 0.693/\lambda_z \quad [1]$$

$$Cl/F = D/AUC \quad [2]$$

$$Vz/F = \frac{Dose}{AUC\ x\ \lambda z} \quad [3]$$

$$MRT = \frac{AUMC}{AUC} \quad [4]$$

$AUC_{0-\infty} = AUC + Clast/\lambda_z$ [5]

where C_{last} is the last measured concentration and AUMC represents the area under the moment curve.

Statistical Analysis

Individual and combined effects of xylazine, ketamine, butorphanol and salicylate were analyzed statistically. All calves receiving XKB (treatment groups XKB and SAL + XKB) were compared to those calves not receiving XKB (treatment groups PLACEBO and SAL). The same was performed for calves receiving salicylate. The effect of study day was determined by evaluating the interaction between phase and treatment. Additionally, individual treatment groups were compared to each other for statistical analysis. The cortisol data within each period were evaluated (SSD) for evidence of departure from normality by use of a univariate procedure of SAS.[bb] There was significant evidence of departure from normality for several of the cortisol parameters; therefore, data were ranked by use of the rank procedure of SAS. An ANOVA was conducted on unranked and ranked data by use of the mixed procedure of SAS with fixed effects of period, salicylate treatment, combined xylazine, ketamine, and butorphanol treatment; and the interactions of these 3 effects. Means and standard errors reported are LS means and pooled SEM. The least LSM and SEM results reported are for the unranked data. The *P* values reported to assess significance among the LSM are those derived from the analysis of the ranked data. Data for ADG, chute exit speed, and EDA

were analyzed (JFC) by use of JMP, a commercial software program.[cc] Statistical significance was designated *a priori* at $P < 0.05$.

Results

Rescue analgesia was not administered during this study as the result of an absence of overt signs of pain after castration and dehorning. Scrotal circumference ranged from 12.5 to 23.5 cm; horn-base diameter ranged from 22.3 to 50.9 mm, and horn length ranged from 23.4 to 73.4 mm. There was no evidence of a treatment day (phase)*treatment interaction for cortisol response (p= 0.16) weight gain (*P*= 0.24), chute exit speed (*P*=0.13) or EDA (*P*= 0.67). Therefore data were pooled across study days for the analysis.

Mean Change in Body Weight—A comparison of the mean ADG ± SEM body weight change results between treatment groups are summarized **(Figure 2)**. Two calves from the PLACEBO group and 2 calves in the SAL + XKB group developed thrombophlebitis during different phases of the study and therefore were not included in the statistical analysis. Castration and dehorning significantly impacted ADG among all treatment groups (*P* = 0.043). Calves in the SAL and SAL + XKB treatment groups had a significantly (*P* = 0.0286) higher ADG for the first 13 days after castration and dehorning than those calves in the PLACEBO and XKB treatment groups. The LSM of the ADG for the SAL and SAL + XKB groups were 1.187 ± 0.275 kg/day and 1.172 ± 0.305 kg/day, respectively, as compared with 0.098 ± 0.336 kg/day for the PLACEBO

group. A large scrotal circumference was associated with a decrease in ADG following castration and dehorning ($P = 0.004$).

Chute Exit Speed—A comparison of the mean ± SEM chute exit speed results are summarized **(Figure 3)**. Administration of XKB significantly prolonged the time taken to exit the chute during Period 1, compared with the PLACEBO and SAL groups during Periods 1 and 2. The chute exit speed for one calf in the PLACEBO group and one calf in the SAL + XKB groups during Period 1 was missed because of a failure to reset the timer and was not included in the statistical analysis. Another calf in the SAL + XKB group became sternally recumbent in between the sensors and therefore an accurate time was not determined. One calf in the PLACEBO group and 1 calf in the SAL group became sternally recumbent in the squeeze chute during the dehorning procedure in Period 2, however this did not influence the chute exit speed.

Electrodermal Activity—A comparison of the EDA of the 4 treatment groups over time are summarized **(Figure 4)**. A treatment effect ($P = 0.017$) was observed, and specifically the EDA of calves in the XKB (from 10 to 50 minutes and 1.5 hours) and SAL + XKB (10 minutes to 1.5 hours) were significantly ($P < 0.050$) lower when compared to the other treatment groups. There was also a significant ($P < 0.001$) difference in EDA depending on the time point measured after treatment. A significant difference ($P = 0.001$) was observed between the phase of the study and time the EDA was recorded. There was also a significant ($P < 0.001$) difference between the treatment

group and the time EDA was recorded. It should be noted that there was no period effect ($P = 0.300$) on EDA (sham castration and dehorning versus castration and dehorning).

Serum Cortisol Concentrations- A comparison of the mean ± SEM serum cortisol concentration results measured during Periods 1 and 2 are summarized **(Figures 5 and 6)**. All parameters (C_{max}, T_{max}, $AUEC_{0\ to\ 1\ h}$, $AUEC_{1\ to\ 6\ h}$, and $AUEC_{6\ to\ 24\ h}$) for serum cortisol concentration results were significantly ($P < 0.001$) different in Period 2 versus Period 1. Cortisol T_{max} was significantly ($P < 0.001$) shorter in Period 2, while cortisol C_{max}, $AUEC_{0\ to\ 1\ h}$, $AUEC_{1\ to\ 6\ h}$, and $AUEC_{6\ to\ 24\ h}$ were significantly ($P < 0.001$) greater in Period 2 compared with Period 1.

A comparison of T_{max} and C_{max} for serum cortisol concentration are summarized **(Figures 7 and 8)**. Because of the large variability in individual serum cortisol concentrations among calves receiving XKB in Period 1 compared with the serum cortisol concentration of calves not receiving XKB, a significant difference was not detected between the mean serum cortisol concentration ($P = 0.384$). The cortisol T_{max} for calves in the SAL + XKB group was significantly less than the PLACEBO ($P = 0.015$) and XKB ($P = 0.006$) groups during Period 2. A significant ($P = 0.254$) difference was not detected for cortisol C_{max} among calves treated with XKB and those not treated with XKB during Period 2; additionally, a significant ($P = 0.345$) difference was not detected for cortisol C_{max} between calves treated with salicylate and those that did not receive salicylate treatment during Period 2.

The AUEC estimates for serum cortisol concentration for calves receiving XKB group and calves receiving SAL are summarized **(Figure 9)** and compared among 3 distinct time intervals(ie, $AUEC_{0 \text{ to } 1 \text{ h}}$, $AUEC_{1 \text{ to } 6 \text{ h}}$, and $AUEC_{6 \text{ to } 24 \text{ h}}$). The AUEC estimates for serum cortisol concentrations among the 4 treatment groups are summarized **(Table 1)**. A period effect was detected between Period 1 and Period 2 for all 3 time intervals. For $AUEC_{0 \text{ to } 1 \text{ h}}$, the AUEC was a significantly ($P = 0.007$) less during Period 2 for calves receiving XKB, compared with those not receiving XKB. Furthermore, the $AUEC_{0 \text{ to } 1 \text{ h}}$ of the XKB group was significantly lower than the PLACEBO groups ($P = 0.016$) and SAL groups ($P = 0.042$) during Period 2. A significant difference was not detected for $AUEC_{1 \text{ to } 6 \text{ h}}$ ($P = 0.389$) and $AUEC_{6 \text{ to } 24 \text{ h}}$ ($P = 0.208$) between the calves that received XKB and those that did not. A significant difference ($P = 0.872$) for $AUEC_{0 \text{ to } 1 \text{ h}}$ in calves receiving salicylate was not detected during Period 2, compared with those not receiving salicylate; however, $AUEC_{1 \text{ to } 6 \text{ h}}$ was significantly ($P = 0.024$) less during Period 2 for those calves receiving salicylate. Additionally, $AUEC_{1 \text{ to } 6 \text{ h}}$ was significantly less in the SAL group when compared to the PLACEBO ($P = 0.030$) and XKB groups ($P = 0.028$) during Period 2. There was a lower $AUEC_{6 \text{ to } 24 \text{ h}}$ for the SAL group as compared with XKB group in Period 2; however, this was not statistically significant($P = 0.064$).

Xylazine, Ketamine, and Butorphanol Pharmacokinetic Parameter Estimates—Pharmacokinetic parameter estimates (T_{max}, C_{max}, AUC, V_z_F, Cl_F, MRT, and $T_{1/2\lambda z}$) for xylazine, ketamine, and butorphanol were determined by noncompartmental analysis and summarized **(Table 2)**. Additionally, the plasma profiles

were summarized **(Figures 10 and 11)**. The V_z_F per fraction of the dose absorbed was significantly ($P = 0.045$) greater in the SAL+ XKB group, compared with that in the XKB group.

Salicylate Pharmacokinetic Parameter Estimates—The T_{max}, C_{max}, AUC, and mean plasma drug concentration were determined for SAL and SAL + XKB and summarized **(Table 3)**. Dot plots representing the mean dose of sodium salicylate consumed by the SAL and SAL + XKB groups and the corresponding plasma salicylate concentration was constructed **(Figures 12 and 13)**. Calves in the SAL and SAL + XKB group received doses of sodium salicylate that ranged from 13.62 to 151.99 mg of salicylate/kg from 24 hours prior to period 1 to 48 hours after period 2.

Discussion

As concern for improving the welfare of livestock increases, the need for pain management research in cattle becomes more necessary. The objective of the study reported here was to determine the pharmacokinetic parameters of xylazine, ketamine, and butorphanol administered IM and sodium salicylate administered PO and to compare their effect on biomarkers of pain and distress following sham (Period 1) and actual castration and dehorning (Period 2). Our results revealed that the treatment of cattle prior to castration and dehorning with either salicylate alone or in combination with xylazine, ketamine, and butorphanol increased ADG and decreased cortisol concentrations. Currently, protocols for the provision of analgesic therapy are not routinely employed during the majority of routine animal husbandry practices. In a survey (Coetzee *et al.*,

2010) of bovine practitioners, 21% of U.S veterinarians reported using analgesia at the time of castration. In a similar Canadian survey (Hewson *et al.,* 2007), 6.9% of beef calves and 18.7% of dairy calves (both < 6 months old) reportedly received treatments to provide pain relief during castration. In a survey (Fulwider *et al*., 2008) of dairy practices in the Northeastern and Central United States, 12.4% of dairy personnel administered an anesthetic at the time of dehorning and 1.8% provided analgesic treatment. This may be due to the absence of FDA-approved, long-acting, and cost-effective analgesic drugs that have established withdrawal times.

It is noteworthy that studies examining the combined effect of castration and dehorning are deficient in the published literature even though 90% of veterinarians responding to a survey (Coetzee *et al*., 2010) report castrating and dehorning calves at the same time. Several studies (Fisher *et al.,* 1996, 1997, 2001; Mellor *et al.,* 2000; Ting *et al*., 2003; Pang *et al.,* 2006; Gonzalez *et al.,* 2008; Coetzee *et al.,* 2007, 2008; Earley *et al.,* 2002; Stafford *et al.,* 2002, 2003; Wohlt *et al.,* 1994; Grondahl-Nielsen *et al.,* 1999; Stillwell *et al.,* 2008) have evaluated acute changes in serum cortisol concentration as a method to determine the extent and duration of distress associated with either castration or dehorning in cattle. Given that many veterinarians and producers dehorn calves at the time of castration (Coetzee *et al.,* 2010), evaluation of castration and dehorning in series and concurrent treatment regimens may be more relevant to current practices in the cattle industry in the United States. In a previous study using 2 to 4 month old untreated bull calves, a peak serum cortisol concentration of 68 nmol/L was reported within 30 minutes of surgical castration, and the duration of the elevation in serum cortisol concentration

above pretreatment serum cortisol concentration was greater than 4 hours (Stafford *et al.*, 2002). During a study in 3-month-old calves dehorned with a Barnes dehorner, serum cortisol concentration increased to 76 nmol/L within a 0.5 hours after dehorning, declined to 45 nmol/L between 1.5 to 2.5 hours after dehorning, and decreased further to pretreatment concentrations within 4.5 to 8 hours after dehorning (Stafford *et al.*, 2003). In the present study, the mean serum cortisol concentration of calves in the PLACEBO group ranged from 141.46 to 34.94 nmol/L at 20 and 360 minutes after castration and dehorning, respectively. These values are higher than some studies reported in which castration or dehorning were performed alone. This increase may reflect the cumulative effect of performing both castration and dehorning procedures in series, differences in study design, or could be random variability.

The development of a drug regimen to reduce weight loss after painful management procedures would make such practices practical and desirable for cattle producers. Furthermore, demonstrating a performance benefit would likely make the addition of analgesic treatments to castration and dehorning protocols more cost effective. The mandated use of analgesia during routine painful procedures would be better received by producers if a performance advantage was observed. Research (Fisher *et al.*, 1996; Faulkner and Weary, 2000) has indicated the use of analgesics and anesthetics influence feed intake and weight gain after painful procedures. For example, investigators (Fisher *et al.*, 1996) found calves treated with local anesthesia during surgical castration, but not burdizzo castration, had a greater ADG than in cattle castrated without a local anesthetic. Another study (Faulkner and Weary, 2000) revealed that

calves treated with ketoprofen prior to and 2 to 7 hours after dehorning, in addition to treatment with xylazine and lidocaine (administered as a local anesthetic at the time of the procedure), gained more weight (1.2 ± 0.4 kg) than control calves only receiving a local anesthetic or xylazine and lidocaine during the first 24 hours after dehorning.

The period effect on serum cortisol concentration could be attributed to pain associated with castration and dehorning which caused a greater physiological rise in serum cortisol concentrations during Period 2 than Period 1. It should be noted elevations in serum cortisol are not necessarily associated with painful stimuli, but also may become elevated in times of stress. This was demonstrated in Period 1 as a rise in cortisol at the time of sham castration and dehorning; however, this was not as great as the rise in Period 2. Several studies (Coetzee *et al*., 2010) report castrating and dehorning calves at the same time. Several studies (Fisher *et al.*, 1996, 1997, 2001; Mellor *et al.*, 2000; Ting *et al*., 2003; Pang *et al.*, 2006; Gonzalez *et al.*, 2008; Coetzee *et al.*, 2007, 2008; Earley *et al.*, 2002; Stafford *et al.*, 2002, 2003; Wohlt *et al.*, 1994; Grondahl-Nielsen *et al.*, 1999; Stillwell *et al.*, 2008) have correlated painful procedures (ie, castration and dehorning) with increased cortisol concentrations; furthermore, the results reported in the present study add additional support to this knowledge base. In a previous dehorning study (Wohlt *et al.*, 1994), serum cortisol concentrations were reported to increase 2-fold in response to stress caused by handling, while peaking 4 to 5-fold in response to dehorning with Buddex or conventional electric dehorners. In the present study, cortisol concentrations experienced a 3-fold increased from time 0 to reach the C_{max} in Period 1 in response to sham castration and dehorning across all treatment

groups and approximately a 4-fold increase in Period 2 in response to castration and dehorning.

Studies investigating the effect of extended dosing of an analgesic and anti-inflammatory compound on ADG in livestock undergoing painful procedures are deficient in the literature. The results of the study reported here support our hypothesis that extended exposure to an NSAID in these situations may be beneficial because ADG was significantly greater over 13 days after castration and dehorning in calves receiving free-choice sodium salicylate in the drinking water. This effect may in part be due to prolonged analgesic effects by the drug, but may also be due to anti-inflammatory effects. This finding has positive implications for the practical utility of providing prolonged analgesia with salicylate in the drinking water before and after castration and dehorning. Additional research on the effectiveness of analgesics on feed intake and ADG over a prolonged period of time after castration and dehorning would be beneficial. This research could determine if analgesia impacts final market weight or cost in feed to compensate for loss in ADG after painful procedures.

Chute exit speed assessment has typically been employed in studies evaluating temperament in cattle. A study (Muller *et al.*, 2008) investigating the effect of injection administration and handler visibility on chute exit speed determined no correlation between the 2 events. The hypothesis that painful procedures, such as castration and dehorning, are associated with faster chute exit speeds has not been tested. There has been a study (Gonzalez *et al.*, 2008) examining chute activity during castration and found

that chute activity was slower with the administration of butorphanol and xylazine. The results of the present study indicated that chute exit speed was slower in calves receiving XKB, especially during Period 1. This can most likely be attributed to the sedative effects of xylazine, ketamine, and butorphanol resulting in a slower reaction time exiting the chute as compared the SAL and PLACEBO groups. However, there was no significant difference between periods in any treatment group. This suggests that chute exit speed may not be a specific indicator of pain and distress, especially in acclimated Holstein calves.

EDA is the measurement of the electrical resistance between 2 electrodes applied to the skin (Benford *et al.*, 2004). EDA can be influenced by changes in resistance as a result of changes in sympathetic outflow (Benford *et al.*, 2004). The Pain Gauge® is purported to be a device capable of measuring EDA although there is a paucity of data to support this use in livestock species. A study that used the Pain Gauge® in rats found it ineffective for accurately assessing postoperative pain because pain scores did not decrease with increasing dosages of analgesic regimens (Richardson *et al.*, 2007). In the present study, a significant decrease in EDA measurement coinciding with the presence of quantifiable plasma drug concentrations was observed in calves receiving XKB. After 90 minutes, EDA increased and was not significantly different from other treatment groups. It is noteworthy that a difference in EDA between the sham and castration and dehorning period was not observed. Therefore, EDA measurement was not a reliable indicator of pain associated with dehorning and castration in calves.

The observed differences in EDA in the XKB treated calves is more likely due to α-2 adrenergic agonist effect of xylazine on eccrine sweat gland output and the effect of sedation. The nasal planum of calves where the EDA measurements were taken contains a dense population of serous nasolabial glands or eccrine glands (Dyce *et al.*, 2002). Unmeyelinated postganglionic sympathetic axons surround eccrine sweat glands secreting water, electrolytes, and mucin when stimulated (Sato, 1997). Therefore these alterations in electrolyte secretion likely changed the conductivity of the skin in XKB treated calves and therefore the EDA measurements. Similarly, differences between phases during recording times were likely due to fluctuations in temperature or humidity between days of the study or individual variation. However, this was not investigated as a part of the present study.

In the present study, xylazine, ketamine, and butorphanol; salicylate; or both were used. Butorphanol is an opioid drug that has partial receptor agonist-antagonist effects. Butorphanol provides analgesia by binding to κ (partial agonist) and μ(antagonist) receptors. When combined with xylazine, butorphanol lowers the dose required to provide analgesia and enhances the sedative effect (Thurmon *et al*., 1996). A dehorning study (Grondahl-Nielsen *et al.*, 1999) investigated the combined effect of xylazine and butorphanol and revealed the co-administration of the drugs alone or in combination with a cornual nerve block significantly decreased the change in cortisol concentration immediately after dehorning, compared with the change in cortisol concentration in untreated calves. Xylazine is an α-2 adrenergic agonist with sedative and analgesic effects when administered to cattle at doses ranging from 0.05 to 0.3 mg of xylazine/kg

(Garcia-Villar *et al*., 1981). Antinociceptive effects have been reported in lambs following IM administration of xylazine (0.05 mg/kg) (Grant and Upton, 2001). Ketamine is an *N*-methyl *D*-aspartate receptor antagonist causing analgesic and dissociative effects when administered IV to calves at doses ranging from 2 to 4 mg/kg (Postner and Burns, 2009). A combination of low-dose of xylazine (0.02 to 0.05 mg/kg), ketamine (0.04 to 0.1 mg/kg), and butorphanol (0.02 to 0.05 mg/kg) administered IV or IM in cattle is reported to provide mild sedation without the side effect of recumbency (Court *et al.,* 2002).

Studies (Sutherland *et al.,* 2002; Sylvester *et al.,* 1998) have shown that plasma cortisol concentrations reach a peak within 30 minutes of dehorning after which levels decrease to a plateau concentration that persists for 5 – 6 hours. Therefore we chose to examine cortisol concentrations over 0 to1 hour because this coincided with peak cortisol concentrations and peak XKB concentrations. In present study, XKB was rapidly absorbed following IM administration and achieved a peak concentration approximately 10 minutes after administration. The administration of xylazine, ketamine, and butorphanol together provided attenuation of serum cortisol during castration and dehorning from 0 minutes to 1 hour after treatment. Therefore, treatment with xylazine, ketamine, and butorphanol is likely to be more effective for controlling acute distress associated with castration and dehorning. The effects of xylazine, ketamine, and butorphanol are relatively short (Thurman *et al.,* 1996); therefore, it was not surprising that the effects of the co-administration of xylazine, ketamine, and butorphanol on serum cortisol concentration did not last > 1 hour. In previous studies (Garcia-Villar *et al.,*

1981), an IV dose of 0.2 mg/kg xylazine was associated with a peak plasma xylazine concentration of 1.050 $\mu g \cdot mL^{-1}$, a $t_{1/2\alpha}$ of 36.48 minutes, and a total body clearance of 42 ml/min/kg. Ketamine administered IV in calves had a $t_{1/2}$ of 60.5 ± 5.4 minutes and a total body clearance of 40.39 ± 6.6 ml/min/kg in another study (Waterman *et al.*, 1981). In another study (Sellers *et al.*, 2010), IV administration of ketamine at a dose of 5 mg/kg demonstrated the following pharmacokinetic parameters; C_{max} of 18.135 ± 22.720 ng/mL, T_{max} of 0.083 hr, an AUC of 4,484 ± 1,398 ng · h/mL, and a $t_{1/2\beta}$ of 1.80 ±0.0 hr. Previous studies (Court *et al*., 1992) in dairy cows administered 0.25 mg/kg IV of butorphanol showed a $t_{1/2}$ to be 82 minutes, total body clearance to be 34.6 ± 77ml/kg/min, and the mean AUC was 7,567 ± 54 ng · min/mL. In the present study the $t_{1/2}$ was 109.43 ± 22.62, 81.45 ± 10.44, and 71.28 ± 7.64 minutes respectively for xylazine, ketamine, and butorphanol. The dosages used in this study were less than doses used in previously mentioned references. The drugs in the present study a longer $t_{1/2}$ than previously mentioned studies with the exception of butorphanol which had a shorter $t_{1/2}$. Total body clearance for all three drugs was also found to be greater than previous studies. The T_{max} for ketamine in the present study was also longer than what was previously reported.

Analysis of the results indicated that there was more variability between Period 1 and Period 2 for the T_{max} of serum cortisol concentration. These differences in T_{max} were most likely the result of individual calf variability in response to treatment with xylazine, ketamine, and butorphanol. A previous study (Coetzee *et al.*, 2008) with 4 to 6 month old bull calves found no significant difference in T_{max} of serum cortisol between calves

surgically castrated versus those undergoing simulated castration. Another study (Ting *et al.*, 2003) found a significantly longer T_{max} in calves blocked with 11 mL of lidocaine or following a caudal epidural with 0.05 mg/kg of xylazine and 0.4 mg/kg of lidocaine HCL when compared to burdizzo castration without analgesia and burdizzo castration following 3 mg/kg of ketoprofen IV. It could be thought that T_{max} would be shorter during painful procedures as a painful stimuli would quickly elevate cortisol concentrations, and this was seen in period 2 versus period 1 for calves receiving salicylate, however was not observed with any of the other treatment groups.

Research investigating the effects of salicylic acid derivatives (ie, salicylate) on the change in biomarkers of pain after castration and dehorning is deficient in the literature. The only study to date using salicylate during castration found administration of a 50 mg/kg IV bolus salicylate to calves prior to castration attenuated cortisol C_{max} as compared to calves receiving oral aspirin (acetyl salicylic acid) immediately prior or those calves left untreated before castration (Coetzee *et al.*, 2007). Studies (Stillwell *et al.*, 2008) incorporating the use of other NSAIDs (eg, carprofen) has provided equivocal results in efficacy of abolishing changes in serum cortisol concentration that are caused by castration and dehorning. Investigators have reported[7] the administration of different concentrations of ketoprofen IV to cattle prior to castration failed to reduce the initial peak in serum cortisol concentration that is correlated with castration; however, serum cortisol concentrations from 2 to 6 hours after castration were significantly reduced. Treatment with salicylate in this study decreased serum cortisol concentrations from 6 to 12 hours after castration and dehorning. AUEC for serum cortisol was examined from 1

to 6 hours because this coincides with a previously described plateau phase where the effect of salicylate should predominate. This decrease in concentration supports the analgesic and anti-inflammatory properties of salicylate. It can be concluded that while sodium salicylate may not provide immediate analgesia at the time of a painful procedure, at the dosing regimen described in this study, it may provide analgesia and reduce inflammation for several hours after painful procedures. Furthermore, this effect could have future implications for the use of sodium salicylate in chronic pain management. Research will be necessary to determine the duration of treatment in order to minimize the cost and maximize the efficiency of treatment with sodium salicylate in the drinking water.

There is limited research revealing estimates of the pharmacokinetic parameters of salicylate administered PO in cattle. Studies have suggested that the bioavailability of salicylate when administered PO in cattle is 61.05% (Barron *et al.*, 2008). A study (Coetzee *et al*., 2007) found that sodium salicylate administered IV at 50 mg/kg at the time of castration attenuated peak cortisol response when plasma drug concentrations where above 25 µg/kg. In the present study, mean plasma salicylate concentrations at the time of castration and dehorning were greater than 25 µg/kg (SAL, 40.36 µg of cortisol/mL; SAL + XKB, 55.11 µg of cortisol/mL). Therefore, the observed attenuation of cortisol response in the present study was in agreement with previous studies.[21] The consumption of salicylate-treated water by calves in the SAL and SAL +XKB groups after castration and dehorning on day 0 (Period 2) at 72 hours past initiation of sodium salicylate treatment decreased markedly. However, the mean plasma drug concentration

of salicylate remained > 25 µg/mL in most calves until treatment with salicylate ceased on day 2. This was likely due to constant access to medicated water as well as dose accumulation attributed to the plasma elimination half-life of 4.31 ± 0.42 hours as previously reported (Barron *et al.*, 2008) for sodium salicylate administered PO.

There is a paucity of research that combines salicylic acid derivatives and ketamine. Therefore, the reason for the increased V_z_F of ketamine when combined with the administration of oral sodium salicylate is unknown. It is unclear if this is associated with variability in the animals, experimental conditions, or if there was a true pharmacokinetic interaction between these compounds.

It is suggested that compounded drugs used in studies must have documented tissue residue information including withdrawal times as well as concentration, carrier, and stability data (AAVPT, 2010). Under the Animal Medicinal Drug Use Clarification Act (AMDUCA), ELDU is permitted for relief of suffering in cattle provided specific conditions are met. These conditions include that (1) ELDU is permitted 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 production purposes; (4) ELDU in feed is prohibited and (5) ELDU is not permitted if it results in a violative food residue (AMDUCA, 1994). The use of salicylate in the manner conducted in this study would be considered extra-label, and therefore use in a production scheme would need to comply with the mentioned guidelines. Aspirin has a FARAD recommended 24 hour meat and milk withdrawal time

(Smith *et al.*, 2008). Further studies are needed to evaluate tissue residues with the use of sodium salicylate as described in this study. Xylazine given at a dose of 0.05 to 0.30 mg/kg IM has a FARAD recommended withdrawal time of 4 days in meat and 24 hours in milk (Haskell *et al.*, 2003). FARAD has suggested that withdrawal times for ketamine at dosages up to 10 mg/kg IM be 3 days for meat and 48 hours for milk (Craigmill *et al.*, 1997). Butorphanol has a suggested withdrawal time of 48 hours (Papich, 1996).

In conclusion, castration and dehorning in series was associated with an increase in plasma cortisol in excess of concentrations previously[33,34] reported for either castration or dehorning in Holstein calves. Co-administration of xylazine, ketamine, and butorphanol alone or in combination with salicylate in the drinking water attenuated serum cortisol concentration after castration and dehorning. Furthermore, the ADG in calves that received free-choice salicylate was significantly greater than calves in the PLACEBO and XKB groups and suggesting NSAID treatment over several days may mitigate negative performance effects associated with castration and dehorning in calves. Chute exit speed was not a specific indicator of pain and distress associated with castration and dehorning; however, administration of XKB significantly increased chute exit speed. EDA measurement was not a specific indicator of pain associated with dehorning and castration but EDA measurement may be influenced by pharmacological effects that were unrelated to analgesic activity in calves. These findings suggest that administration of free-choice salicylate in the drinking water may provide long term performance benefits that were likely associated with persistent NSAID plasma concentrations.

Tables

Table 2.1. A comparison of the AUEC for serum cortisol concentration in calves treated with saline solution administered IM (PLACEBO; [n = 10]); 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; [n = 10]); 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol /kg administered IM (XKB; [n = 10]; and combination of sodium salicylate administered PO and xylazine, ketamine, and butorphanol administered IM (SAL + XKB; [n=10]) during sham castration and sham dehorning (Period 1) and castration and dehorning (Period 2).

Treatment	Period	$AUEC_{0 \text{ to } 1 \text{ h}}$ (h*nmol/L)	$AUEC_{1 \text{ to } 6 \text{ h}}$ (h*nmol/L)	$AUEC_{6 \text{ to } 24 \text{ h}}$ (h*nmol/L)
PLACEBO	1	92.560^{c}	152.06^{cd}	597.36^{bcd}
	2	132.19^{a}	342.9^{a}	756.28^{ab}
SAL	1	84.293^{cd}	119.06^{d}	434.29^{cd}
	2	119.94^{a}	216.36^{bc}	583.64^{ad}
XKB	1	42.102^{e}	123.81^{d}	574.37^{ac}
	2	93.993^{bcd}	322.96^{a}	756.21^{a}
SAL + XKB	1	48.927^{e}	131.36^{d}	455.51^{cd}
	2	104.57^{abc}	259.94^{ab}	637.6^{ab}

Within columns, means with different superscripts differ significantly (P < 0.05)

Table 2.2. A comparison of the mean ± SEM of pharmacokinetic parameter estimates derived from noncompartmental pharmacokinetic analysis of results from calves treated with 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB; (n = 10) or 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption and XKB (SAL + XKB; (n = 10) prior to sham castration and sham dehorning (Period 1) and castration and dehorning (Period 2).

Parameter	***Xylazine***		***Ketamine***		***Butorphanol***	
	XKB	**SAL + XKB**	**XKB**	**SAL + XKB**	**XKB**	**SAL + XKB**
$T_{1/2\lambda z}$ (min)	96.40 ± 20.33 [a]	122.47 ± 24.90 [a]	67.43 ± 11.13 [a]	95.56 ± 9.75 [a]	68.23 ± 7.13 [a]	74.32 ± 8.14 [a]
T_{max} (min)	9.5 ± 0.50 [a]	11 ± 1.00 [a]	10 ± 1.29 [a]	9 ± 1.45 [a]	9.5 ± 0.50 [a]	12 ± 1.33 [a]
C_{max} (ng/mL)	20.95 ± 1.68 [a]	19.50 ± 2.07 [a]	14.97 ± 1.91 [a]	12.32 ± 1.91 [a]	7.07 ± 0.55 [a]	6.21 ± 0.68 [a]
$AUC_{0\ to\infty}$(hr·ng/mL)	16.68 ± 1.44 [a]	17.48 ± 1.19 [a]	12.90 ± 2.4 [a]	12.4 ± 2.06 [a]	6.82 ± 0.47 [a]	6.57 ± 0.49 [a]
Vz_F (L/kg)	6.7 ± 1.09 [a]	8.27 ± 1.54 [a]	12.11 ± 2.15 [a]	18.67 ± 2.15 [b]	6.11 ± 0.59 [a]	6.98 ± 7.10 [a]
CL_F (mL/min/kg)	53.69 ± 4.89 [a]	49.30 ± 2.72 [a]	184.28 ± 33.73 [a]	167.51 ± 33.73 [a]	64.025 ± 4.92 [a]	68.51 ± 8.03 [a]
MRT (min)	96.31 ± 18.15 [a]	120.03 ± 22.48 [a]	67.43 ± 10.46 [a]	95.56 ± 10.46 [a]	85.621 ± 8.14 [a]	94.45 ± 9.57 [a]

[a]All reported parameter estimates within a row for each drug with different superscripts differ significantly ($P < 0.05$)

T_{max} = time to maximum drug concentration. C_{max} = maximum concentration of drug. AUC = area under the curve.

Vz_F = volume of distribution per fraction of dose absorbed (F). CL_F = total body clearance per fraction of dose absorbed.

MRT = mean residence time

Table 2.3. Sodium salicylate plasma drug concentrations in calves treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; [n = 10]) or treated with SAL and 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (SAL + XKB**;**[n = 10]) from 24 hours prior to sham castration and sham dehorning (Period 1) to 48 hours after castration and dehorning, Period 2.

Parameter estimate	SAL		SAL + XKB	
	Mean	SEM	Mean	SEM
AUC (min·μg/mL)	4,923.26	856.33	5,054.18	695.21
Sodium salicylate concentration throughout Period 1 (μg/mL)	32.41	12.86	27.31	6.79
Sodium salicylate concentration throughout Period 2 (μg/mL)	40.36	12.19	55.107	10.795
T_{max} (h)	41.7		68.93	
C_{max} (μg/mL)	61.134	10.312	63.223	10.837
Mean sodium salicylate concentration (μg/mL)	32.20	1.59	30.07	1.23

T_{max} = time to maximum drug concentration; C_{max} = maximum concentration of drug; AUC = Area under the curve.

Figure Legend

Figure 2.1. Flow chart depicting the parallel study design.

Figure 2.2. A comparison of ADG ± SEM for calves treated with saline solution administered IM [PLACEBO; (n = 8)]; 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption [SAL; (n = 10)]; 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM [XKB; (n = 10)]; and both xylazine, ketamine, and butorphanol and sodium salicylate as previously described [SAL + XKB;(n=8)]. A significant ($P < 0.05$) difference between ADGs is indicated by different symbols (◊, ■).

Figure 2.3. A comparison of mean ± SEM chute exit speed for calves treated with saline solution administered IM [PLACEBO, (Period 1, n = 9; Period 2, n=10)]; 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption [SAL, (Period 1 and 2, n = 10)] ; 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg [XKB, (Period 1 and 2, n = 10)] administered IM; and both xylazine, ketamine, and butorphanol and sodium salicylate [SAL + XKB (Period 1, n=8; Period 2, n = 10)] during Period 1 and 2. A significant ($P < 0.05$) difference between chute exit speeds is indicated by different symbols (▲,◊, ■).

Figure 2.4. A comparison of the mean EDA scores between calves treated with saline solution administered IM (PLACEBO; (n = 6); 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; (n = 6) ; 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB; (n = 6) ; and SAL + XKB (n = 6) for both period 1 and period 2.

Figure 2.5. A comparison of mean serum cortisol concentration results in calves treated with saline solution administered IM (PLACEBO [— ◊ —]; (n = 10) ; 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL [— ■ — –]; (n = 10) ; 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB [– ▲ –]; (n = 10) ; and SAL + XKB (— ● —**;** (n = 10) after sham castration and sham dehorning (Period 1). Refer to text for further discussion.

Figure 2.6. A comparison of mean serum cortisol concentration results in calves (n = 10) treated with saline solution administered IM (PLACEBO [—◊ —]; (n = 10) ; 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL [— ■ — –]; (n = 10); 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB [– ▲ –]; (n = 10) ; and SAL + XKB (— ● —**;** (n = 10) castration and dehorning (Period 2). Refer to text for further discussion.

Figure 2.7. A comparison of the mean ± SEM T_{max} for serum cortisol concentration in calves treated with 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB; (n = 20), not treated with XKB (n = 20) , treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; (n = 20) , and not treated with SAL (n = 20) after sham castration and sham dehorning (Period 1) and castration and dehorning (Period 2). A significant ($P < 0.05$) difference between the T_{max} of serum cortisol concentrations is indicated by different letters.

Figure 2.8. A comparison of the mean ± SEM C_{max} for serum cortisol concentration in calves treated with 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg

butorphanol/kg administered IM (XKB; (n = 20) , not treated with XKB (N=20), treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; (n = 20) , and not treated with SAL (n = 20) after sham castration and sham dehorning (Period 1) and castration and dehorning (Period 2). A significant ($P < 0.05$) difference between the C_{max} of serum cortisol concentrations is indicated by different letters.

Figure 2.9. A comparison of the area under the effect curve (AUEC) for serum cortisol concentration in calves treated with 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (XKB; (n = 20) , not treated with XKB (n = 20), treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL; (n = 20) , and not treated with SAL (n = 20) during the 1st hour (■, $AUEC_{0 \text{ to } 1 \text{ h}}$), 1st through the 6th hour (■ , $AUEC_{1 \text{ to } 6 \text{ h}}$), and 6th through 24th hour (□ $AUEC_{6 \text{ to } 24 \text{ h}}$) after sham castration and sham dehorning (Period 1) and castration and dehorning (Period 2). A significant ($P < 0.05$) difference between the AUEC of serum cortisol concentrations is indicated by different letters within the same time period.

Figure 2.10. A comparison of mean ± SEM plasma drug concentration in calves treated with 0.05 mg xylazine/kg (—☐—) + 0.1 mg ketamine/kg (—♦—) + 0.025 mg butorphanol/kg (— X—) administered IM (XKB; (n = 10) immediately prior to castration and dehorning (Period 2).

Figure 2.11. A comparison of the mean ± SEM plasma drug concentration in calves treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (concentration data not shown) and 0.05 mg xylazine/kg (—☐—) +

0.1 mg ketamine/kg (—♦—) + 0.025 mg butorphanol/kg (— X—) administered IM (SAL + XKB; (n = 10)) immediately prior to castration and dehorning (Period 2).

Figure 2.12. A dot plot representing the mean dose of sodium salicylate administered to calves PO through free-choice water consumption in the group treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL [□];(n = 10) and SAL and 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (SAL + XKB [♦];(n = 10) from 24 hours prior to sham castration and sham dehorning (Period 1) to 48 hours after castration and dehorning (Period 2). Dose was calculated from water intake and concentration of salicylate added and then divided by animal weight (kg).

Figure 2.13. A comparison of plasma sodium salicylate concentration results in calves treated with 2.5 to 5 mg sodium salicylate/mL of administered PO through free-choice water consumption (SAL[**—♦—]**; (n = 10) or treated with SAL and 0.05 mg xylazine/kg + 0.1 mg ketamine/kg + 0.025 mg butorphanol/kg administered IM (SAL + XKB[— ■ — –]**;**(n = 10) from 24 hours prior to sham castration and sham dehorning (Period 1) to 48 hours after castration and dehorning (Period 2).

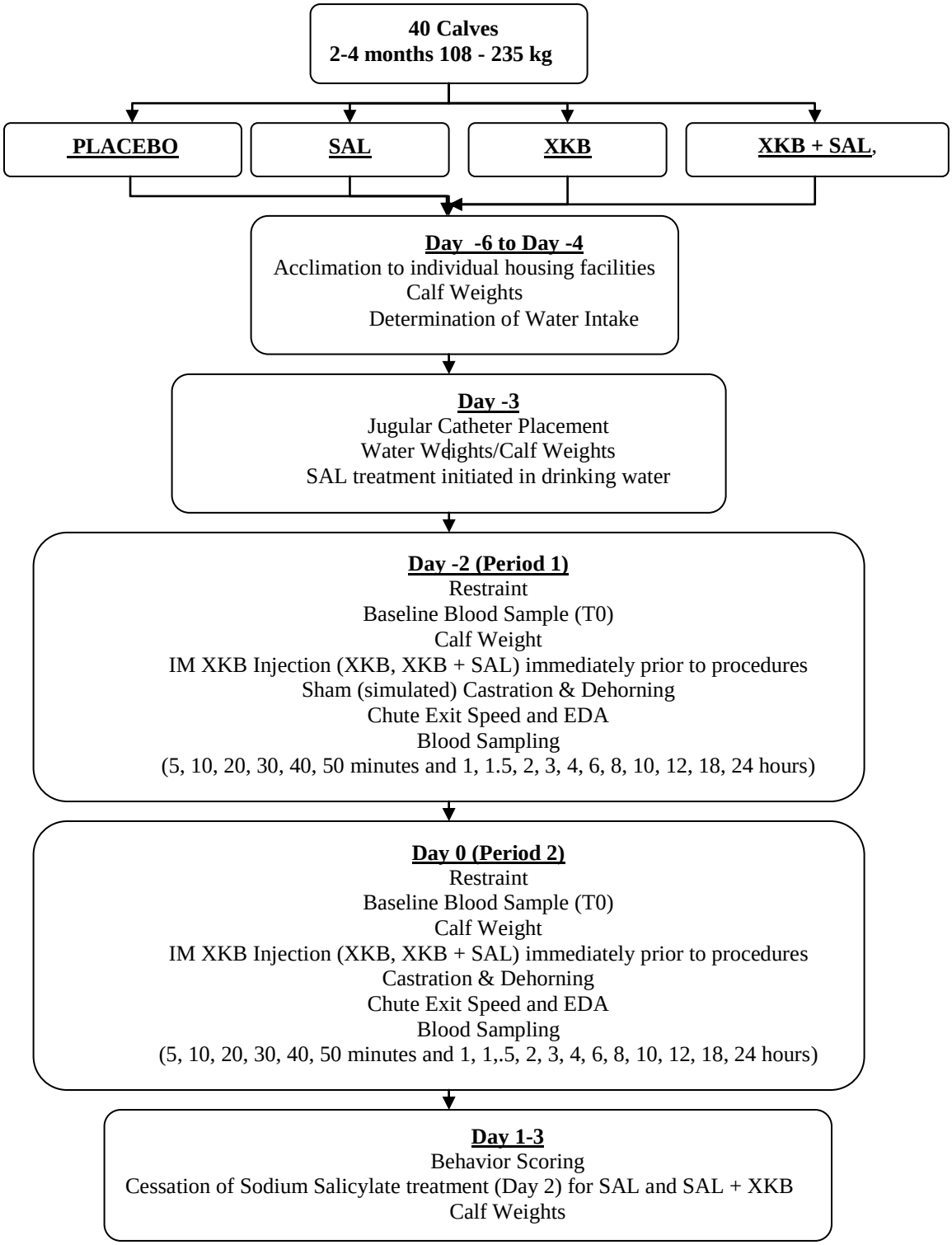
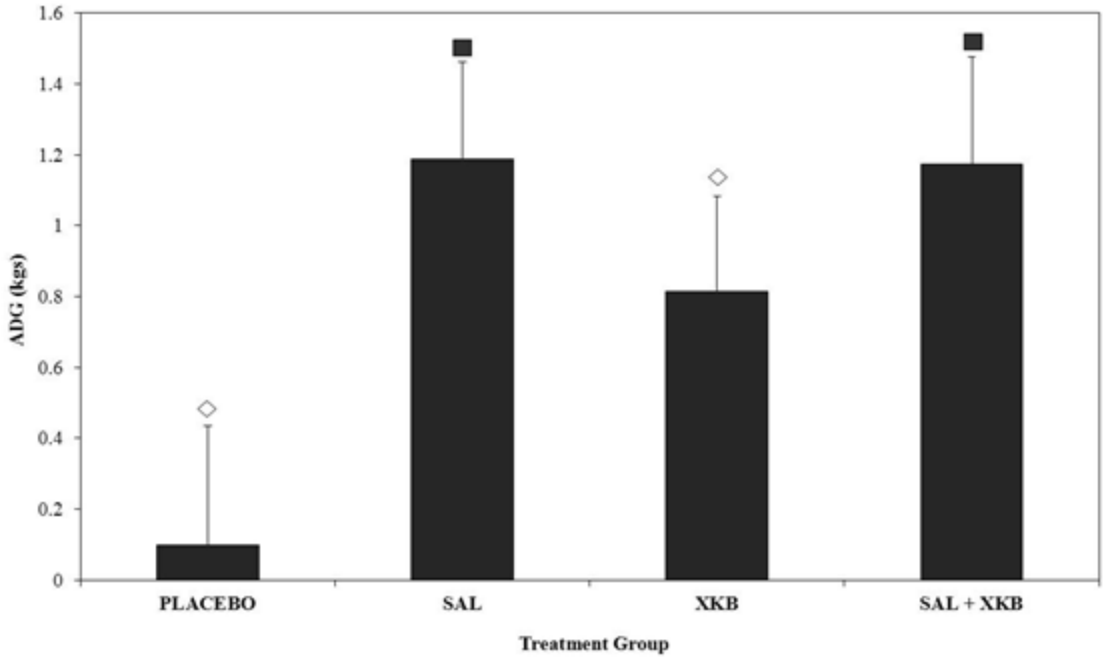
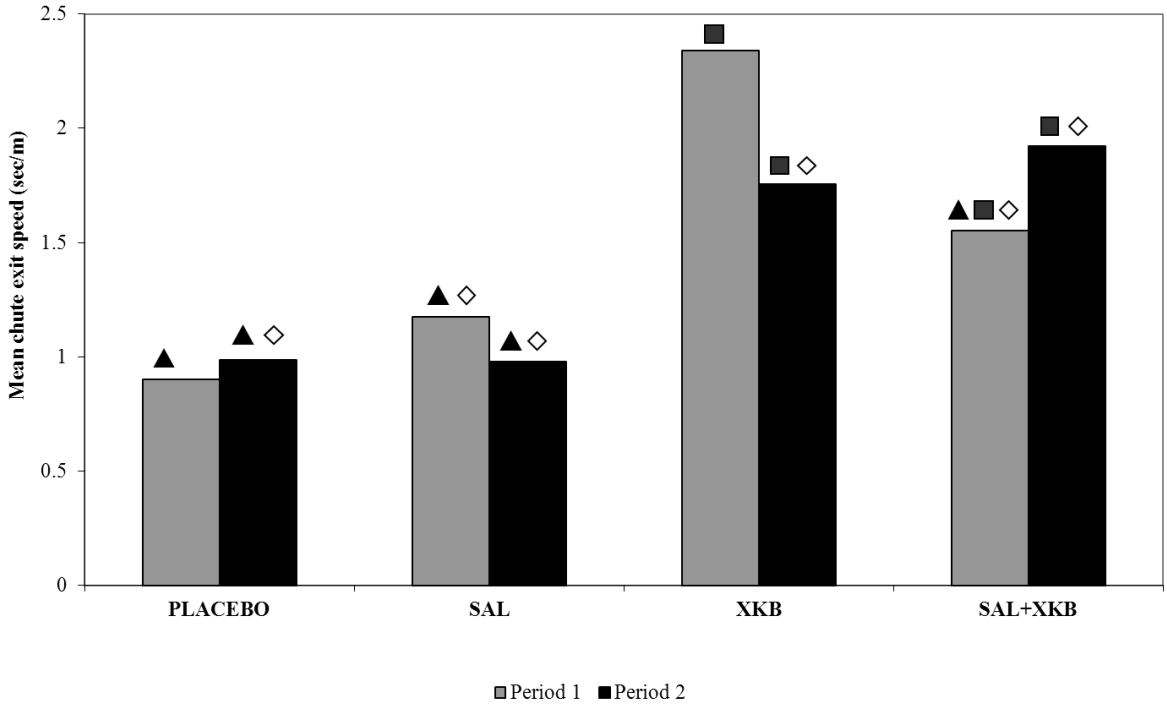

40 Calves
2-4 months 108 - 235 kg
PLACEBO
SAL
XKB
XKB + SAL,
Day -6 to Day -4
Acclimation to individual housing facilities
Calf Weights
Determination of Water Intake
Day -3
Jugular Catheter Placement
Water Weights/Calf Weights
SAL treatment initiated in drinking water
Day -2 (Period 1)
Restraint
Baseline Blood Sample (T0)
Calf Weight
IM XKB Injection (XKB, XKB + SAL) immediately prior to procedures
Sham (simulated) Castration & Dehorning
Chute Exit Speed and EDA
Blood Sampling
(5, 10, 20, 30, 40, 50 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24 hours)
Day 0 (Period 2)
Restraint
Baseline Blood Sample (T0)
Calf Weight
IM XKB Injection (XKB, XKB + SAL) immediately prior to procedures
Castration & Dehorning
Chute Exit Speed and EDA
Blood Sampling
(5, 10, 20, 30, 40, 50 minutes and 1, 1,.5, 2, 3, 4, 6, 8, 10, 12, 18, 24 hours)
Day 1-3
Behavior Scoring
Cessation of Sodium Salicylate treatment (Day 2) for SAL and SAL + XKB
Calf Weights

Figure 2.2


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ADG (kgs)
PLACEBO
SAL
XKB
SAL + XKB
Treatment Group

2135

Figure 2.3



2136

2137

2138

2139

2140

2141

2142

2143

Figure 2.4

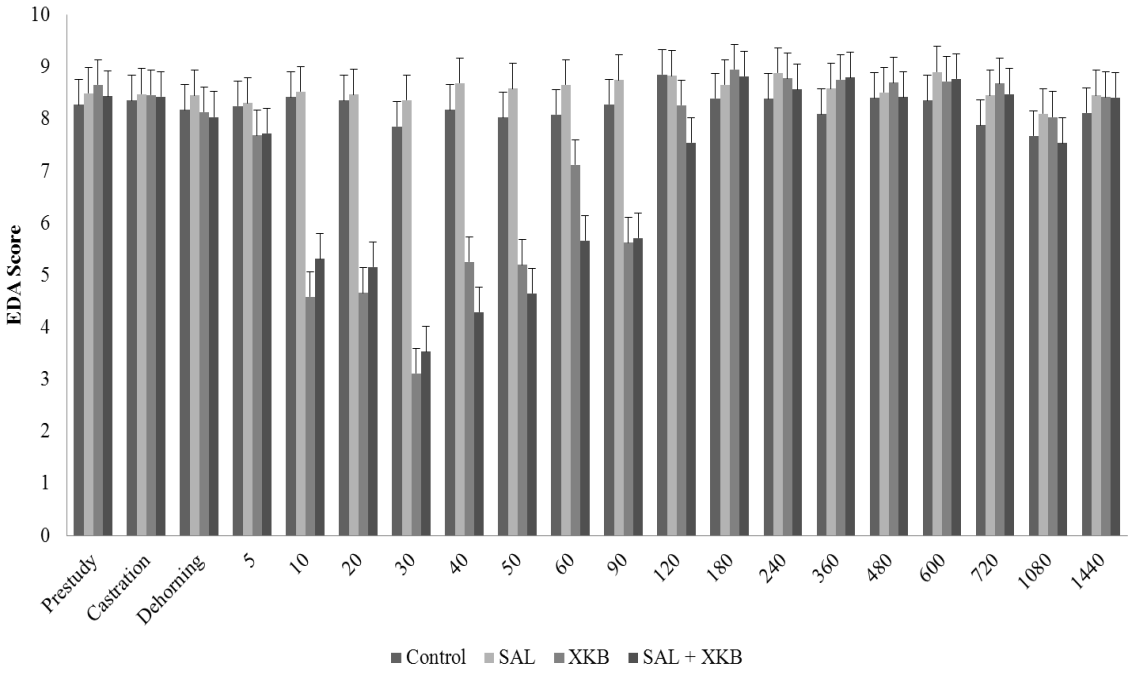

EDA Score
10
9
8
7
6
5
4
3
2
1
0
Prestudy
Castration
Dehorning
5
10
20
30
40
50
60
90
120
180
240
360
480
600
720
1080
1440
Control
SAL
XKB
SAL + XKB

Figure 2.5

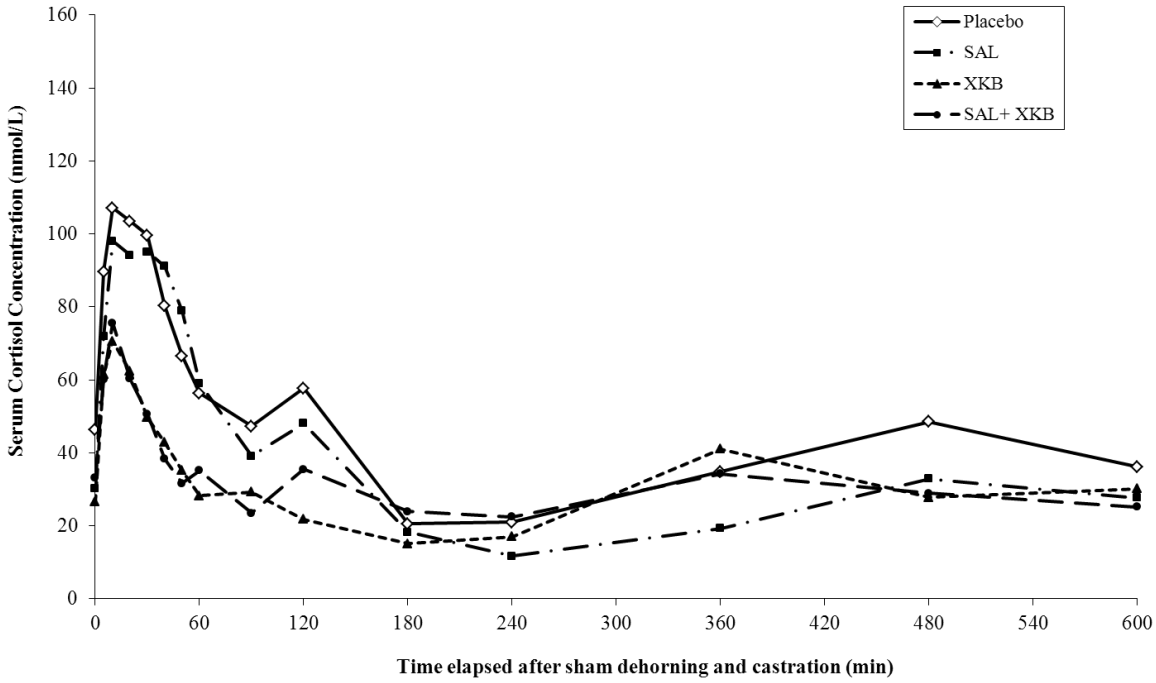

Placebo
SAL
XKB
SAL+ XKB
Serum Cortisol Concentration (nmol/L)
Time elapsed after sham dehorning and castration (min)
0
20
40
60
80
100
120
140
160
0
60
120
180
240
300
360
420
480
540
600

Figure 2.6

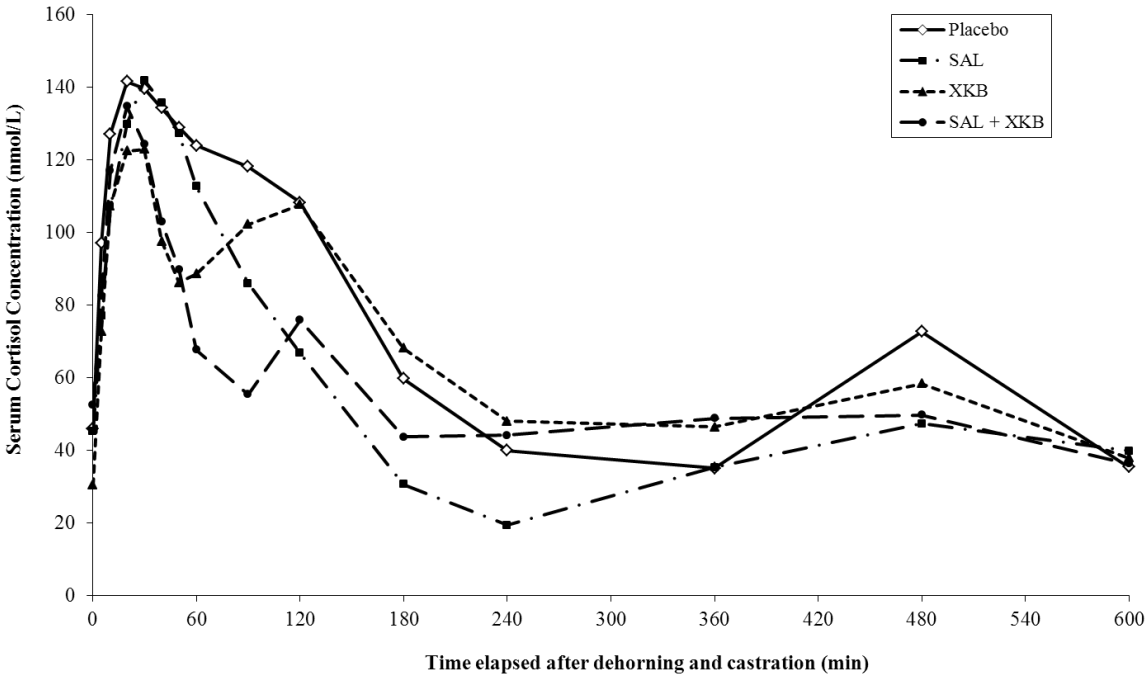

Placebo
SAL
XKB
SAL + XKB
Serum Cortisol Concentration (nmol/L)
Time elapsed after dehorning and castration (min)
0
20
40
60
80
100
120
140
160
0
60
120
180
240
300
360
420
480
540
600

Figure 2.7

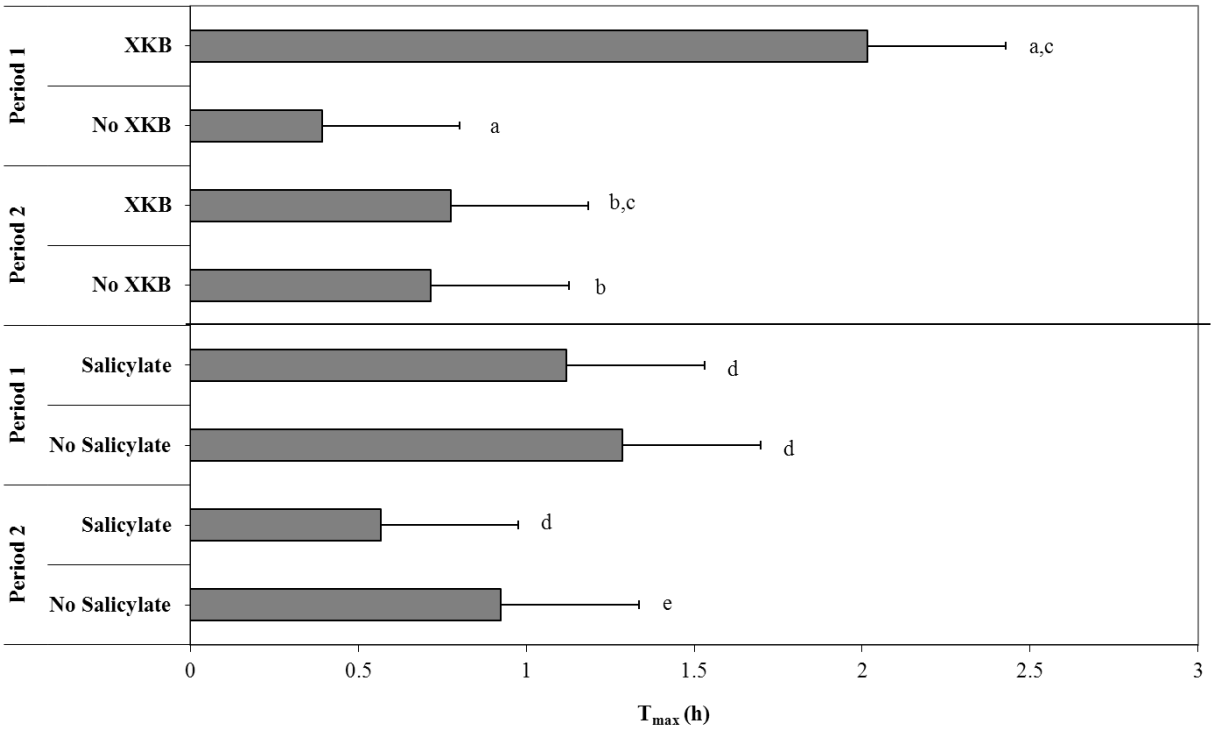

Period 1
XKB
No XKB
Period 2
XKB
No XKB
Period 1
Salicylate
No Salicylate
Period 2
Salicylate
No Salicylate
a,c
a
b,c
b
d
d
d
e
0
0.5
1
1.5
2
2.5
3
T_{max} (h)

Figure 2.8

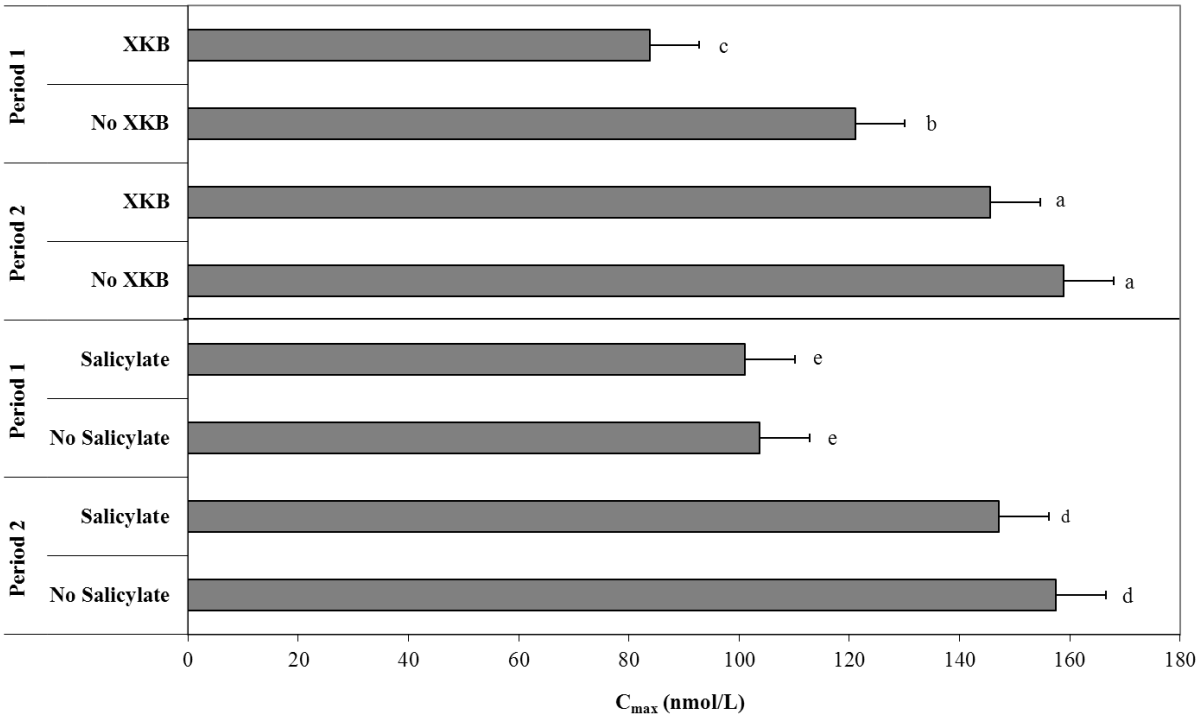

Period 1
XKB
No XKB
Period 2
XKB
No XKB
Period 1
Salicylate
No Salicylate
Period 2
Salicylate
No Salicylate
c
b
a
a
e
e
d
d
0
20
40
60
80
100
120
140
160
180
Cmax (nmol/L)

Figure 2.9

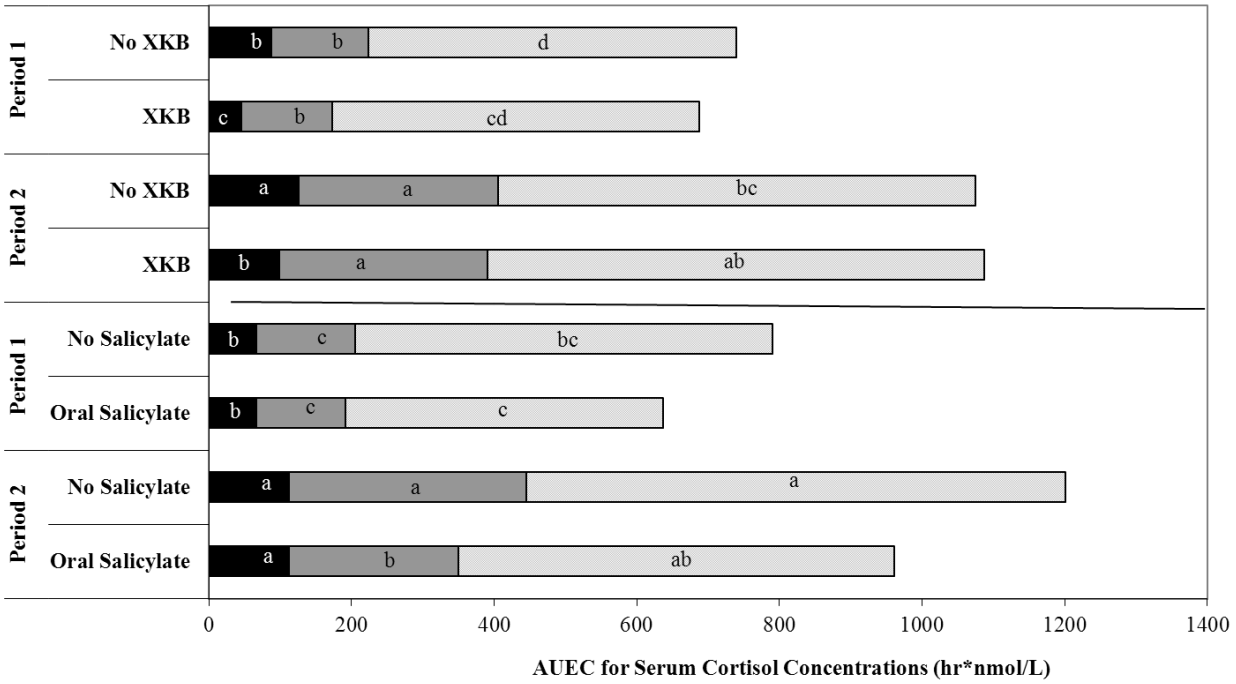

Period 1
No XKB
b
b
d
XKB
c
b
cd
Period 2
No XKB
a
a
bc
XKB
b
a
ab
Period 1
No Salicylate
b
c
bc
Oral Salicylate
b
c
c
Period 2
No Salicylate
a
a
a
Oral Salicylate
a
b
ab
0
200
400
600
800
1000
1200
1400
AUEC for Serum Cortisol Concentrations (hr*nmol/L)

Figure 2.10

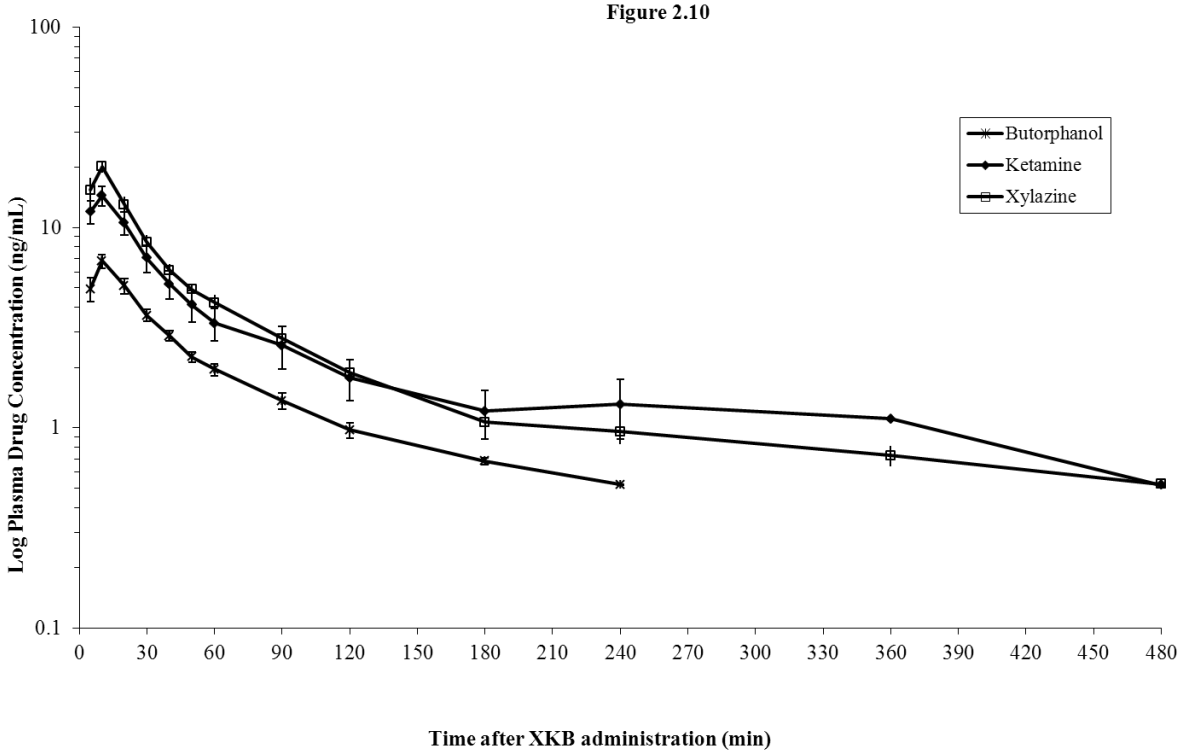

100
10
1
0.1
Log Plasma Drug Concentration (ng/mL)
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
Time after XKB administration (min)
Butorphanol
Ketamine
Xylazine

Figure 2.11

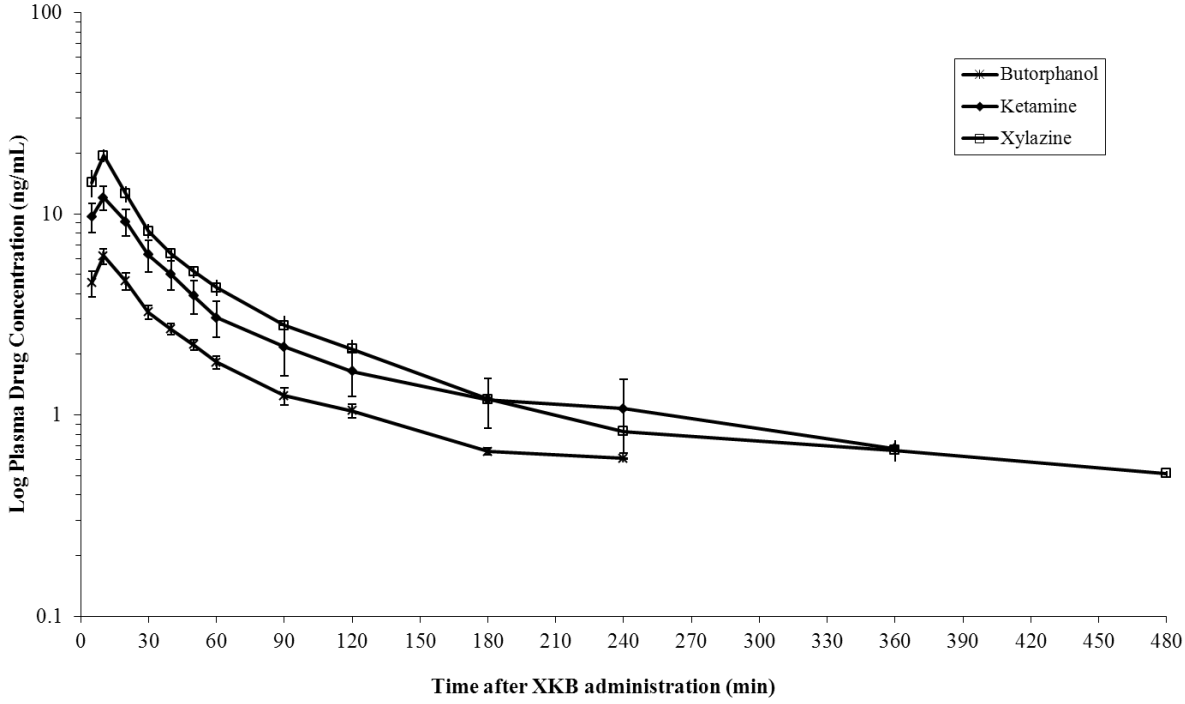

100
10
1
0.1
Log Plasma Drug Concentration (ng/mL)
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
Time after XKB administration (min)
Butorphanol
Ketamine
Xylazine

Figure 2.12

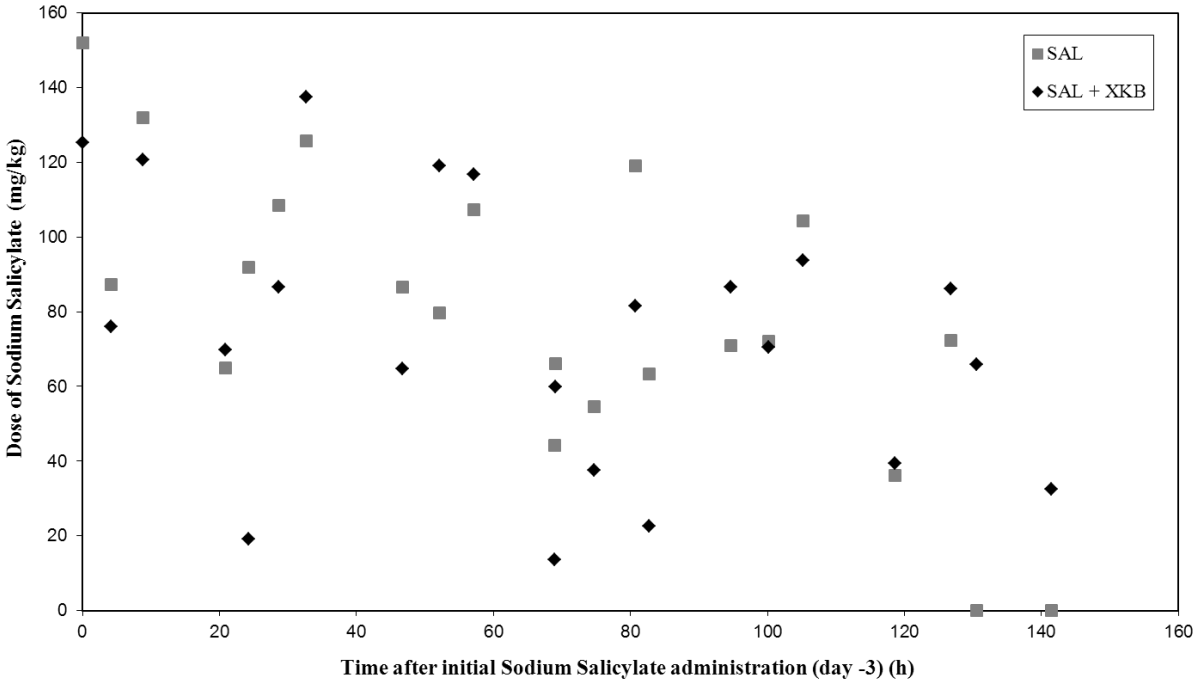
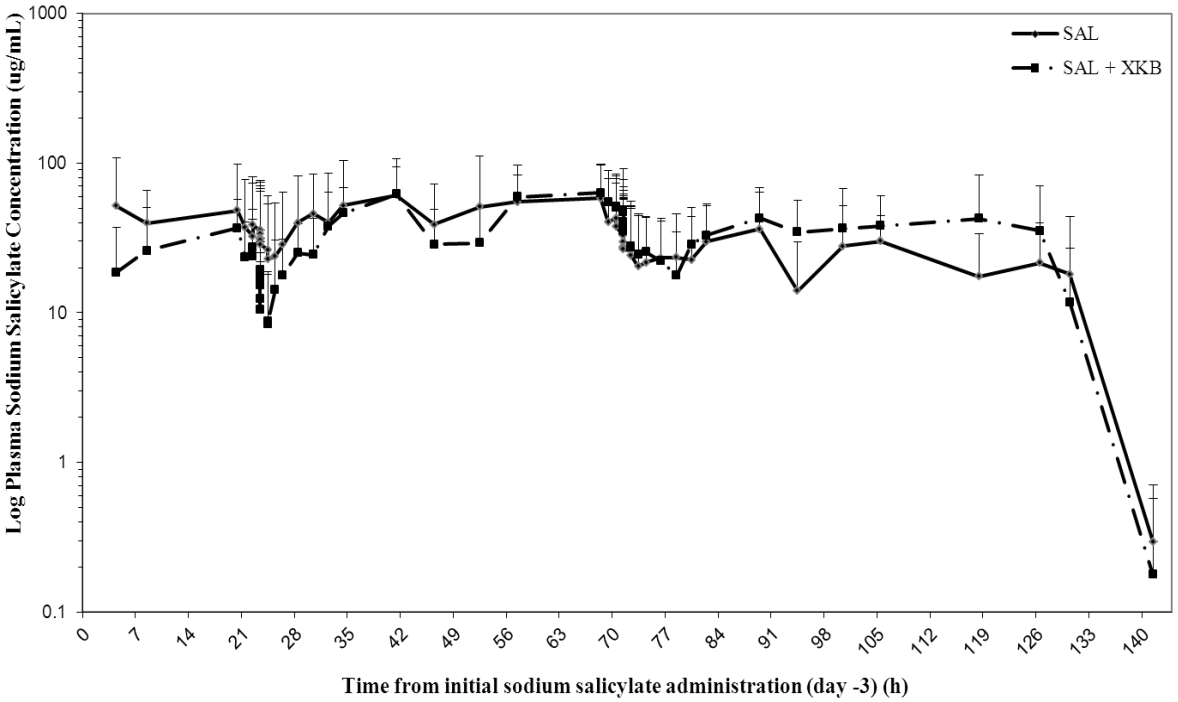

SAL
SAL + XKB
Dose of Sodium Salicylate (mg/kg)
Time after initial Sodium Salicylate administration (day -3) (h)
0
20
40
60
80
100
120
140
160

Figure 2.13


Log Plasma Sodium Salicylate Concentration (ug/mL)
1000
100
10
1
0.1
SAL
SAL + XKB
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
Time from initial sodium salicylate administration (day -3) (h)

References

AAVPT position paper regarding the evaluation of scientific manuscripts that involve the administration of compounded drugs to animals. Available at: *http://www.aavpt.org/ documents/Manustripts_Compound_Drug_Position.pdf.* Accessed June 30, 2010.

Abrahamsen EJ. Chemical Restraint in Ruminants. *Current Veterinary Therapy Food Animal Practice.* 5th ed. St Louis, Mo: Saunders Elsevier, 2009; 546 – 549.

American Veterinary Medical Association (AVMA) Web site. Backgrounder: Welfare implications of the castration of cattle. Available at: www.avma.org/ reference/backgrounders/ castration_cattle_bgnd.asp. Accessed June 5, 2009.

Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA. U.S. Food and Drug Administration website. Available at http://www.fda.gov/RegulatoryInformation/ Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDC Act/AnimalMedicinalDrugUseClarificationActAMDUCAof1994/default.htm. Accessed June 28, 2010.

Barron SL, Gehring R, Reinbold JR, et al. Development of a model for pain during castration and dehorning. Poster Presentation. Merck Merial Summer Scholars Program. Michigan State University, East Lansing, MI. July 2008.

Benford SM and Dannemiller S. Use of electrodermal activity for assessment of pain/stress in laboratory animals. *Animal Laboratory News* 2004; 1: 13 – 23.

Chase Jr. CC, Larsen RE, Randel RD, et al. Plasma cortisol and white blood cell responses in different breeds of bulls: A comparison of two methods of castration. *J Anim Sc* 1995;73: 975 – 980.

Coetzee JF, Gehring R, Bettenhausen AC, et al. Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration. *J vet Pharmacol Therap* 2007; 30(4): 305 – 313.

Coetzee JF, Lubbers BL, Toerber SE, et al. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res* 2008; 69(6): 751 – 762.

Coetzee JF, Nutsch A, Barbur LA, et al. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. *BMC Veterinary Research* 2010; 6.

Court MH, Dodman NH, Levine HD, et al. Pharmacokinetics and milk residues of butorphanol in dairy cows after single intravenous administration. *J vet Pharmacol Ther* 1992; 15(1): 28 - 35.

Craigmill AL, Rangel-Lugo MR, Damian P, et al. Extralabel use of tranquilizers and general anesthetics. *J Am Vet Med Assoc* 1997; 211(3): 302 – 304.

Curley KO, Paschal JC, Welsh TH Jr., et al. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. *J Anim Sci* 2006; 84: 3100 – 3103.

Doherty TJ, Kattesh HG. Adcock, MG, et al. Effects of a concentrated lidocaine solution on the acute phase stress response to dehorning in dairy calves. *J Dairy Sci.* 2007; 90: 4232 – 4239.

Dyce KM, Sack WO, and Wensing CJG. The Head and Ventral Neck of the Ruminants. In: *Textbook of Veterinary Anatomy*. 3rd ed. Philadelphia, PA: Saunders, 2002; 627.

Earley B, and Crowe MA. Effects of ketoprofen alone or in combination with local anesthesia during castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *J Anim Sci* 2002; 80: 1044 – 1052.

Faulkner DB, Eurell T, Tranquilli WJ, et al. Performance and health of weanling bulls after butorphanol and xylazine administration at castratio. *J Anim Sci* 1992; 70:2970 - 2974.

Faulkner PM, and Weary DM. Reducing pain after dehorning in dairy calves. *J Dairy Sci* 2000; 83: 2037 – 2041.

Fisher AD, Crowe MA, Alonso de la Varga ME, et al. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *J Anim Sci* 1996; 74: 2336 – 2343.

Fisher AD, Crowe MA, O'Naullain EM, et al. Effects of cortisol on in vitro interferon-γ production, acute-phase proteins, growth and feed intake in a calf castration model. *J Anim Sci* 1997; 75: 1041 – 1047.

Fisher AD, Knight TW, Cosgrove GP, et al. Effects of surgical or banding castration on stress responses and behavior of bulls. *Aust Vet J* 2001; 79(4): 279 – 284.

Fulwider WK, Grandin T, Rollin BE, et al. Survey of dairy management practices on one hundred thirteen North Central and northeastern United States Dairies. *J. Dairy Sci* 2008; 91: 1686 – 1692.

Garcia-Villar R, Toutain PL, Alvinerie M, et al. The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J Vet Pharmacol Ther* 1981; 4(2): 87 – 92.

Gehring R, Coetzee JF, Tarus-Sang J, et al. Pharmacokinetics of ketamine and its metabolite norketamine administered at a sub-anesthetic dose together with xylazine to calves prior to castration. *J vet Pharmacol Ther* 2008; 32: 124 – 128.

Gonzalez LA, Schwartzkopf-Genswein KS, Caulkett NA, et al. Pain mitigation after band castration of beef calves and its effects on performance, behavior, *Escherichia coli*, and salivary cortisol. *J Anim Sci* 2008; 2: 802 – 810.

Grant C, and Upton RN. The anti-nociceptive efficacy of low dose intramuscular xylazine in lambs. *Res Vet Sci* 2001; 70(1): 47 – 50.

Grondahl-Nielsen C, Simonsen HB, Lund JD, et al. Behavioral, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. *The Veterinary Journal* 1999; 158: 14 – 20.

Haskell SR, Gehring R, Payne M, et al. Update on FARAD food animal drug withholding recommendations. *J Am Vet Med Assoc* 2003; 223(9): 1277 – 1278.

Hewson CJ, Dohoo IR, Lemke KA, et al. Canadian veterinarians' use of analgesics in cattle, pigs and horses in 2004 and 2005. *Can Vet J* 2007; 48:155 – 164.

Kissin I. Preemptive Analgesia at the Crossroad. *Anesth Analg* 2005; 100: 754 –756.

Knight TW, Cosgrove GP, Death AF, et al. Effect of method of castrating bulls on their growth rate and liveweight. *New Zealand Journal of Agriculture Research.* 2000; 43: 187 – 192.

Langston VC. Therapeutic management of inflammation. In: Howard JL, Smith RA eds. In Current *Veterinary Therapy 4: Food Animal Practice*. Philadelphia, PA: WB Saunders CO, 2003; 7 – 12.

Mellor DJ, Cook CJ, and Stafford KJ. Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA eds. In *The biology of animal stress: Basic Principals and implications for animal welfare*. New York, USA: CABI Publishing, 2000; 171 – 198.

Muller R, Schwartzkopf-Genswein KS, Shah MA, et al. Effect of neck injection and handler visibility on behavioral reactivity of beef cattle. *J Anim Sci* 2008; 86: 1215 – 1222.

Niedorf F, Hogr HH, and Kietzmann M. Simultaneous determination of ketamine and xylazine in canine plasma by liquid chromatography with ultraviolet absorbance detection. *J Chromatogr B Analyt technol Biomed Life Sci* 2003; 791 (1-2); 421 – 6.

Pang WY, Early B, Sweeny T, et al. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-γ production, acute-phase proteins, feed intake, and growth. *J Anim Sci* 2006; 84: 351 – 359.

Papich MG. Drug residue considerations for anesthetics and adjunctive drugs in Food Producing animals. *Veterinary Clinics of North: Food Animal Practice* 1996; 12(3): 693 – 705.

Postner LP, and Burns P. Injectable Anaesthetic Agents. Page 283 in Veterinary Pharmacology and Therapeutics. (9th Edn) Eds. J.E. Riviere, M.G. Papich. Wiley-Blackwell. 2009. Ames, Iowa.

Richardson CA, Niel L, Leach MC, et al. Evaluation of the efficacy of a novel electronic pain assessment device, the Pain Gauge®, for measuring postoperative pain in rats. *Laboratory Animals* 2007; 41: 46 – 54.

Rollin BE. Annual meeting keynote address: Animal agriculture and emerging social ethics for animals. *J Anim Sci* 2004; 82: 955 – 964.

Sato K. The physiology, pharmacology, and biochemistry, of the eccrine sweat gland. *Rev Physiol Biochem Pharmacol* 1977; 79: 51 – 131.

Sellers G, HC Lin, MG Riddell, et al. Pharmacokinetics of ketamine in plasma and milk of mature Holstein cows. *J vet Pharmacol Ther* 2010; doi: 10.1111/j.1365-2885.2010.01159.x.

Smith GW, Davis JL, Tell LA, et al. Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. *J Am Vet Med Assoc* 2008; 232(5): 697 – 701.

Stafford KJ, Mellor DJ, Todd SE, et al. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. *Res Vet Sci* 2002; 73(1): 61 – 70.

Stafford KJ, Mellor DJ, Todd SE, et al. The effect of different combinations of lignocaine, ketoprofen, xylazine, and tolazoline on the acute cortisol response to dehorning calves. *New Zealand Veterinary Journal* 2003; 51(5): 219 – 226.

Stafford KJ and DJ Mellor. The welfare significance of cattle: a review. *New Zealand Vet J.* 2005; 53(5): 271 – 278.

Stillwell G, Lima MS, and Broom DM. Effects of nonsteroidal anti-inflammatory drugs on long-term pain in calves castrated by use of an external clamping technique following epidural anesthesia. *Am J Vet Res* 2008; 69(6): 744 – 749.

Stroud LR, Solomon BA, Shenassa E, et al. Long-term stability of maternal prenatal steroid hormones from the National Collaborative Perinatal Project: Still valid after all these years. *Psychoneurolendocrinology* 2007; 32(2):140 - 150.

Sutherland MA, Mellor DJ, Stafford KJ, et al. Cortisol responses to dehorning of calves given a 5-h local anaesthetic regimen plus phenylbutazone, ketoprofen, or adrenocorticotropic hormone prior to dehorning. *Res Vet Sci* 2002; 73: 115 - 123.

Sylvester SP, Mellor DJ, Stafford KJ, et al. Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cautery of the wound. *Aust Vet J* 1998; 76(2): 118 – 122.

Thurmon JC, Tranquilli WJ, Benson GJ. Preanesthetics and anesthetic adjuncts. In: *Lumb and Jones Veterinary Anesthesia*. 3rd ed. Baltimore, MD: Lippincott Williams & Wilkins, 1996; 183 – 209.

Ting STL, Earley B, and Crowe MA. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. *J Anim Sci* 2003; 81: 1253 – 1264.

Ting ST, Earley B, Hughes JM, et al. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. *J Anim Sci* 2003; 81; 1281 – 1293.

Underwood WJ. Pain and Distress in agriculture animals. *J Am Vet Med Assoc* 2002; 221: 208 – 211.

USP Veterinary Pharmaceutical Information Monographs. Anti-inflammatories. *J. Vet. Pharmacol. Ther* 2004; 27(Suppl. 1): 4 – 14.

Waterman AE. The pharmacokinetics of ketamine administered intravenously in calves and the modifying effect of premedication with xylazine hydrochloride. *J vet Pharmacol Ther* 1984; 7(2): 125 – 130.

Wohlt JE, Allyn ME, Zajac PK, et al. Cortisol Increases in Plasma of Holstein Heifer Calves from Handling and Method of Electrical Dehorning. *J. Dairy Sci* 1994; 77: 3725 – 3729.

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To whom it may concern,

My name is Sarah Baldridge and I am a masters degree student at Kansas State University. I was needing to get copyright permission from AJVR so I can submit my manuscript as part of my thesis at K-State. The title of the article is "Pharmacokinetics and physiologic effects of xylazine-ketamine-butorphanol administered intramuscularly alone or in combination with orally administered sodium salicylate on biomarkers of pain in Holstein calves following concurrent castration and dehorning." I need this release by

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I appreciate your help in this matter!
Thanks!

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Chapter 3 - Implications for further research

From the presented research in chapter 2, new insight has been gained on analgesic use in cattle prior to concurrent castration and dehorning. However, still much research is still needed to find a practical and effective method to relieve pain in cattle. Several implications for further research can be made regarding the study design, analgesic regimens used, and methods for measuring pain.

Firstly this study examined the affects of both castration and dehorning performed concurrently on biomarkers for pain. This is a novel approach to study design used as compared to what methods are used in the available literature. Most studies focus only on one of the procedures, while in a normal production system, both are usually performed together. There is a suggestion from the magnitude of cortisol response seen from these calves that performing both of these procedures increased cortisol concentrations as compared to concentrations found from previous studies. This may be a more accurate study design for the measurement of pain and analgesic effects during such studies. This study design is also more applicable to what goes on in a real production setting.

The use of sodium salicylate in cattle during routine procedures is a relatively unexplored option for pain management in the available literature. Therefore a proper dosing schedule has not been established or validated. During this study, calves were not administered salicylate on an mg/kg dosing schedule but based on mg sodium salicylate /mL water depending on the amount of water consumed. A proper dosing regimen is needed to ensure consistency between cattle. During this study, calves were offered as much water as they could drink from which then the dosing schedule administered was calculated based on consumption. Target plasma concentrations were the main influence on the amount of sodium salicylate added to the water each time. In future studies, the

results from this study could be used to find an appropriate dosing schedule to be initiated at the start of the study and followed throughout.

The other method of providing analgesia, the "ket-stun" is becoming a more regularly used technique for providing standing sedation in cattle. During this study, the XKB injection was administered immediately prior to castration and dehorning. While that may be the most practical application for a production scheme, it may not be the ideal method for pain management, as the onset of maximal sedation and analgesia may have taken place after the procedures had already occurred. This fact highlights a dilemma that several of the castration and dehorning studies face: finding a practical, efficient, and economical method to provide the optimum level of analgesia during procedures.

XKB did exert a treatment effect on cortisol levels for the AUC_{0-1} hours as compared to PLACEBO and SAL. This shows that XKB may be helpful in curbing the initial painful response and with adjunctive analgesia, may be a way to adequately address pain during procedures. Furthermore, SAL experienced a curbed cortisol response from AUC_{1-6} suggesting a prolonged analgesic affect of sodium salicylate. Through influences on cortisol concentrations, it could be thought that each treatment did have some effect on pain during some time during the study.

One of the major focuses of this study was to find a reliable method of measuring pain in cattle. Some methods presented such as chute exit speed and electrodermal activity, seemed to reflect more of a response to sedation that actual pain. Therefore these may not be an accurate indicator of measuring pain. For example, there was no period effect between period 1 and period 2 on electrodermal activity. As well, several other methods for measuring pain were used during this study, but not analyzed here in this paper. This data may prove to be useful in determining more treatment effects. It is supported in this study that cortisol may be an good indicator of pain as serum concentrations were significantly higher during the presence of painful stimuli occurring in period 2, which for all treatment groups was significantly greater than period 1.

While treatment with salicylate exerted a significant effect on average daily gain, a prolonged effect on gain and feed intake would be beneficial knowledge for producers. To date, the literature is deficient in effects of castration and dehorning on final market weight and if anesthesia or analgesia performed at these times would have an impact that far down the road. A way the study design could have been improved was to have a control group to compare what normal average daily gain would be in calves not undergoing such procedures. Additionally, measuring average daily gain for a prolonged period of time after castration and dehorning would be beneficial.