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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List of Symbols

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
g. Anased, Lloyd Lab, Shenandoah, IA.
h. Ketaset, Fort Dodge, Fort Dodge, IA.
i. Torbugsic, 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.
s. Public Health Information Systems, Inc, Dublin, OH.
t. Fisherbrand, Pittsburg, Penn.
u. Immulite 1000 Cortisol, DPS, Los Angeles, Calif.
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.

c. 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
\lambda_z Slope of the terminal portion of the time-concentration curve
T_{1/2\lambda_z} Terminal elimination half-life time
Cl_F      Total body clearance per fraction of drug absorbed
V_{z,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
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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.

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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 testicles 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, pharmacokinetics, 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 $\alpha$ and unmyelinated $C$ afferent nerve fibers to the
dorsal horn of the spinal cord (Muir and Woolfe, 2001). These $\alpha$ 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.
Unmyelinated $C$ 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 \( C_{\text{max}} \) was 572 ng/mL, \( T_{\text{max}} \) 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: \( T_{\text{max}} \) 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 · kg⁻¹ · 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
predominantly 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 reported 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
hypermolarization 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 TxA2, PGE2, LTB4, 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 TxB2 for 12 hours, PGE2 for 24 hours, and bradykinin for 25 hours after administration however did not significantly change LTB4...
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 $\alpha$ (TNF$\alpha$), and COX-2 production in cattle to determine its anti-inflammatory effects. This study found that a 300 $\mu$M concentration of aspirin in culture media significantly decreased PGE$_2$ production as compared to control and that aspirin significantly decreased bradykinin and TNF$\alpha$ 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 $\mu$g/mL (50 mg/kg dose) and 45 to 65 $\mu$g/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 $\mu$g/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\textsubscript{max}, T\textsubscript{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 \textit{et al.} (1997) found that surgical castration significantly elevated cortisol C\textsubscript{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 \textit{et al.} (2008) found no significant difference in cortisol C\textsubscript{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 \textit{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 \textit{et al.} (2007) evaluated cortisol response at time periods, AUC, and C\textsubscript{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\textsubscript{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 \textit{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\textsubscript{max} between Buddex (57.1 nmol/L) and cautery (60.4 nmol/L) methods. A study using scoop versus cautery dehorning by Petrie \textit{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 \textit{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\textsubscript{max} and integrated cortisol response was not statistically different among treatment groups.

A study by McMeekan \textit{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 \textit{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 bupivacaine 15 minutes prior to procedures and then
again at 1 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 ± 56.7 nmol/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 $C_{\text{max}}$, $T_{\text{max}}$, 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 $C_{\text{max}}$ 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 $T_{\text{max}}$ 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.
Table 1.1 Cortisol and Castration

<table>
<thead>
<tr>
<th>Author</th>
<th>Size</th>
<th>Age</th>
<th>Method</th>
<th>Treatment Groups</th>
<th>Sampling Schedule</th>
<th>Cortisol Concentrations</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fell</td>
<td>19</td>
<td>4 – 11 wks</td>
<td>Rubber ring</td>
<td>a) Rubber ring</td>
<td>Immediately prior, 15, 30, 60 min, 2, 3, 4, and 24 h, 6 d</td>
<td>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</td>
<td>Between 15 min and 2 h, (b) was significantly higher than (a).</td>
</tr>
<tr>
<td></td>
<td>1986</td>
<td></td>
<td>Surgical</td>
<td>b) Surgical</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) Control</td>
<td></td>
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<tr>
<td>Cohen</td>
<td>17</td>
<td>28 – 36 wk</td>
<td>Surgical</td>
<td>a) Control</td>
<td>0, 2, 3, 6, 12, 24, 36, and 48 h, 3, 4, 5, 6, 7, and 8 d</td>
<td>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</td>
<td>AUC for (b) was greater than (c)</td>
</tr>
<tr>
<td></td>
<td>1990</td>
<td></td>
<td>Chemical</td>
<td>b) Surgical</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>c) Chemical with α-hydroxypropionic acid</td>
<td></td>
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<tr>
<td>King</td>
<td>142</td>
<td>Early: 11 wks</td>
<td>Newberry Burdizzo</td>
<td>a) Surgical</td>
<td>Immediately prior and</td>
<td>Early Castration 2 min (nmol/L)</td>
<td>During early castration (c) was</td>
</tr>
<tr>
<td></td>
<td>1991</td>
<td></td>
<td>Early Castration</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>b) Surgical</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>c) Control</td>
<td></td>
<td></td>
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<tr>
<td>Mellor 1991</td>
<td>11 Friesian</td>
<td>&lt; 1 wk</td>
<td>Rubber ring</td>
<td>a) Handling</td>
<td>b) Rubber ring</td>
<td>c) 50 µg ACTH IV</td>
<td>Immediately prior, 0, 15, 30, 60, 90, 120, 180, 240 min.</td>
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</tr>
<tr>
<td>Faulkner 1992</td>
<td>268 Cross</td>
<td>24 – 36 wks</td>
<td>Newberry Knife</td>
<td>a) 0.07 mg/kg butorphanol + 0.02 mg/kg xylazine IV, 90 sec prior to castration</td>
<td>0, 3, and 7 d</td>
<td>3 d (nmol/L): a) 56.5 b) 62.8 c) 38.0</td>
<td>There was not a significant difference between treatment groups for calves</td>
</tr>
<tr>
<td>Study</td>
<td>Cows</td>
<td>Age (wks)</td>
<td>Technique</td>
<td>Pretreatment Sample</td>
<td>Immediate Post Treatment</td>
<td>Castration Status</td>
<td></td>
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<tr>
<td>Robertson 1994</td>
<td>Ayrshire</td>
<td>1.3, and 6</td>
<td>Handling only, Burdizzo, Surgical Banding, Rubber ring</td>
<td>20, -1, 12, 24, 36, 48, 60, 72, 84, 96, 138, and 180 min</td>
<td>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).</td>
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<td></td>
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<td></td>
<td></td>
<td>C$<em>{max}$ (nmol/L), T$</em>{max}$ (min) at 6 d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>a) ~ 40, 12</td>
<td>b) ~ 80, 24</td>
<td>C$_{max}$ for (c) was significantly greater than (a), (b), and (d).</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>b) ~ 55, 24</td>
<td>c) ~ 105, 12</td>
<td>(b) was significantly higher than (a).</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>c) ~ 63, 24</td>
<td>d) ~ 40, 12</td>
<td>C$_{max}$ for 6 and 42 d old calves was significantly higher than 21 d calves.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>d) ~ 58, 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chase 1995</td>
<td>12 Angus Hereford</td>
<td>80 – 88</td>
<td>Uncastrated, 25 mL lidocaine 2% injected into both spermatic chords, surgical castration 3 min later, EZE latex rubber</td>
<td>Pre surgical sample, post surgical 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, 26, 28, and 35 d</td>
<td>Immediately post castration (nmol/L)</td>
<td>(b) was significantly higher than (a) and (c) immediately after.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 54.6</td>
<td>c) 42.5</td>
<td>(b) and (c) was significantly higher than (a) 2 days after.</td>
<td></td>
</tr>
</tbody>
</table>
bands

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Breed</th>
<th>Age</th>
<th>Treatment</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (nmol/L), T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molony 1995</td>
<td>40 Ayrshire</td>
<td>1 wk</td>
<td>Burdizzo Surgical Rubber ring</td>
<td>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.</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; 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.</td>
</tr>
<tr>
<td>Fisher 1996</td>
<td>56 Friesian</td>
<td>22 wks</td>
<td>Burdizzo Surgical</td>
<td>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 e) Surgical</td>
<td>Mean cortisol calculated from: -2-0, 0.25-1.5, 2-6,8-24,48, &amp; 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</td>
</tr>
</tbody>
</table>
f) Surgical w/ local anesthetic

<table>
<thead>
<tr>
<th>C_{max} (nmol/L)</th>
<th>AUC 0-10 h (nmol·L^{-1}·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 40.3</td>
<td>a) 15.7</td>
</tr>
<tr>
<td>b) 63.5</td>
<td>b) 134.9</td>
</tr>
<tr>
<td>c) 86.6</td>
<td>c) 128.0</td>
</tr>
<tr>
<td>d) 73.1</td>
<td>d) 111.2</td>
</tr>
<tr>
<td>e) 126.9</td>
<td>e) 291.4</td>
</tr>
<tr>
<td>f) 97.7</td>
<td>f) 227.3</td>
</tr>
</tbody>
</table>

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.

\[
\begin{array}{ccc}
\text{Fisher 1997} & 40 \text{ wks} & \text{Surgical} \\
\text{Friesian} & 20 \text{ wks} & \text{Surgical} \\
\end{array}
\]

Mean cortisol calculated from:
- 2 – 0, 0.25 – 1.5, 2 – 6, and 8 – 12 h

<table>
<thead>
<tr>
<th>Mean cortisol</th>
<th>Mean cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nmol/mL)</td>
<td>(nmol/mL)</td>
</tr>
<tr>
<td>a) 16.6</td>
<td>0.25 - 1.5 h</td>
</tr>
<tr>
<td>b) 10.5</td>
<td>0.25 - 1.25 h</td>
</tr>
<tr>
<td>c) 82.5</td>
<td>(c) was</td>
</tr>
<tr>
<td>d) 46.4</td>
<td>significantly</td>
</tr>
<tr>
<td>2 – 6 h</td>
<td>greater than</td>
</tr>
<tr>
<td>(nmol/L)</td>
<td>(a), (b), and</td>
</tr>
<tr>
<td>a) 14.1</td>
<td>(d). (d) was</td>
</tr>
<tr>
<td>b) 10.8</td>
<td>significantly</td>
</tr>
<tr>
<td></td>
<td>higher</td>
</tr>
</tbody>
</table>

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·L^{-1}·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)
<table>
<thead>
<tr>
<th>Fisher</th>
<th>30</th>
<th>22 wks</th>
<th>Surgical</th>
<th>a) Untreated control</th>
<th>b) IV cortisol administration of 12 mg (0 hr), 6 mg (30, 60, 70, 100, 130, &amp; 160), 2 mg every 30 min until 430 min, &amp; 1 mg at 460 min</th>
<th>c) Surgical castration</th>
<th>C\textsubscript{max} (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Friesian</td>
<td></td>
<td></td>
<td>-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</td>
<td>-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</td>
<td>a) 54.4</td>
<td>b) 114.0</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Fisher</th>
<th>52</th>
<th>56 wks</th>
<th>Surgical</th>
<th>a) 6 – 7 mL lidocaine 0, 1, 2, 4, 7, in each testis, 6 mL SC</th>
<th>b) Surgical, 6 – 7 mL lidocaine in each testis, 6 mL SC along incision line, 15 min prior to castration</th>
<th>c) Banding, 6 – 7 mL lidocaine in each testis, 6 mL SC along banding area, 15 min prior to banding</th>
<th>Day 0 (nmol/L)</th>
<th>Day 1 (nmol/L)</th>
<th>Day 2 (nmol/L)</th>
<th>Day 4 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Angus, Angus cross and Simment al</td>
<td></td>
<td>Banding</td>
<td></td>
<td></td>
<td></td>
<td>a) 54</td>
<td>b) 46</td>
<td>c) 51</td>
<td>a) 49</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Day 1 (nmol/L)</td>
<td>Day 2 (nmol/L)</td>
<td>Day 4 (nmol/L)</td>
<td>Day 4 (nmol/L)</td>
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<td></td>
<td></td>
<td></td>
<td>a) 40</td>
<td>b) 52</td>
<td>c) 41</td>
<td>No significant difference between groups (b) and (c) at any of the time points</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>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.</td>
<td></td>
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</tr>
</tbody>
</table>
| 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  
Cmax (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 ± 11  
Tmax (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).  
Cmax (P < 0.05) was greater in (b) than (c), (d), and (e)  
Tmax 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  | Cmax, Tmax (nmol/L, h)  
a) not sig  
<p>| Attenuation in cortisol response for  |</p>
<table>
<thead>
<tr>
<th>Emasculator Clamp</th>
<th>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</th>
<th>b) not sig</th>
<th>c) not sig</th>
<th>d) 99 ± 3, 2</th>
<th>e) 76 ± 11, 1.5</th>
<th>f) 24 ± 3, 0</th>
<th>g) 31 ± 4, 0</th>
<th>h) 101 ± 6, 1</th>
<th>i) 28 ± 6, 0</th>
<th>j) 26 ± 5, 0</th>
<th>k) 68 ± 7, 0.5</th>
<th>l) 66 ± 14, 2</th>
<th>m) 30 ± 14, 1.5</th>
<th>n) 56 ± 12, 2.5</th>
<th>o) 84 ± 4, 1</th>
<th>p) 31 ± 6, 0.5</th>
<th>q) 64 ± 7, 0.5</th>
<th>r) 53 ± 5, 0.5</th>
<th>s) 21 ± 2, 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emasculation</td>
<td>each testicle, control b + 3 mg/kg IV ketoprofen, control 28µg/kg ACTH IV 2 rubber rings on the scrotal neck 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</td>
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</tr>
</tbody>
</table>

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) \( q + r + 3 \text{ mg/kg IV ketoprofen 20 min prior} \)

| Ting 2003a | Holstein x Friesian | 50 | 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 | 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) 15.25 | 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... |
For C<sub>max</sub>, (c), (d), and (e) were significantly lower than (b) but significantly higher than (a).

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<sub>max</sub> for all treatment
<table>
<thead>
<tr>
<th>Schwartzkopf</th>
<th>Holstein</th>
<th>7.5 – 11 wks</th>
<th>Surgical castration</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Day 1: control</td>
<td>0, 15, 30, 60, 120 and 240 min</td>
<td>a) 0, 15, 30, 60, 120 and 240 min</td>
<td>15 min (nmol/L)</td>
</tr>
<tr>
<td>b) Day 2: sham castration</td>
<td>15, 30, 60, 120, and 240 min</td>
<td>b) 15, 30, 60, 120, and 240 min</td>
<td>30 min (nmol/L)</td>
</tr>
<tr>
<td>c) Day 3: castration</td>
<td>15, 30, 60, 120, 240 min, 24 and 48 h</td>
<td>c) 15, 30, 60, 120, 240 min, 24 and 48 h</td>
<td>120 min (nmol/L)</td>
</tr>
</tbody>
</table>

### Results

- **Cmax (nmol/L), Tmax (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

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)).

AUC was greatest for (b) which was significantly greater than all other treatment groups. (c), (d), and (e) were also significantly greater than (a).
<table>
<thead>
<tr>
<th>Pang 2006</th>
<th>50 Holstein x Friesian</th>
<th>Banding Burdizzo</th>
<th>a) Untreated control</th>
<th>b) Banding</th>
<th>c) 1.4 mg/kg carprofen IV 20 min prior to banding</th>
<th>d) Burdizzo</th>
<th>e) 1.4 mg/kg carprofen IV 20 min prior to burdizzo</th>
<th>240 min(nmol/L)</th>
<th>a) 18.8 ± 3.3</th>
<th>b) 15.2 ± 1.9</th>
<th>c) 25.4 ± 4.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-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</td>
<td>AUC 0 – 2 h (nmol · L⁻¹·h)</td>
<td>(a) 39.2 ± 11.0</td>
<td>(b) 147.6 ± 11.0</td>
<td>(c) 123.9 ± 11.6</td>
<td>(d) 92.2 ± 11.3</td>
<td>(e) 81.7 ± 10.9</td>
<td>AUC 2 – 6 h (nmol · L⁻¹·h)</td>
<td>(a) 60.7 ± 12.9</td>
</tr>
</tbody>
</table>

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<sub>max</sub>, however it was only significantly different from (b). There were no other significant differences among treatment groups.

T<sub>max</sub> was the different between (b) and (c) versus (d).
<table>
<thead>
<tr>
<th>Coetzee 2007</th>
<th>20 Angus cross</th>
<th>16 – 26 wks</th>
<th>Newberry knife Henderson castration tool</th>
<th>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</th>
<th>Immediately prior and after, 10, 20, 30, 40, 50 min, 1, 1.5, 2, 4, 6, 8, 10, 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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thuer 2007</td>
<td>70 Simment al or Simment al x Holstein</td>
<td>3 – 4 wks</td>
<td>Rubber ring Burdizzo</td>
<td>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</td>
<td>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</td>
</tr>
</tbody>
</table>
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

Boesch 2008

<table>
<thead>
<tr>
<th>30 Cross</th>
<th>&lt; 1wk</th>
<th>Burdizzo</th>
<th>Boesch 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 10 mL 2% lidocaine (2 mL/spermatic, 3 mL in spermatic neck) 20 min prior</td>
<td>10 mL 2% lidocaine (2 mL/spermatic, 3 mL in spermatic neck) 20 min prior</td>
<td></td>
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</tr>
<tr>
<td>b) 10 mL 0.5 % bupivicaine 20 min prior</td>
<td>14.0, 15, 16, 17 h</td>
<td></td>
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<tr>
<td>c) 10 mL saline 20 min prior</td>
<td>a) ~ 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) ~ 75</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>c) ~ 90</td>
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<td></td>
</tr>
</tbody>
</table>

Immediately post (nmol/L)

| a) ~ 120 |
| b) ~ 75 |
| c) ~ 90 |

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).
| Coetzeet al. 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$_{\text{max}}$ (nmol/L):  
a) 128.80 ± 9.06  
b) 36.58 ± 31.94  
Both decreased to less than baseline by 2.5 hours  
T$_{\text{max}}$ (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

-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.

<table>
<thead>
<tr>
<th>Marti 2010</th>
<th>47 Holstein</th>
<th>12 wks</th>
<th>Rubber ring</th>
<th>a) Intact</th>
<th>b) 3 mL in each testis, 60, 90, and 180 min</th>
<th>30 – 180 min (nmol/L)</th>
<th>AUC 0 – 180 (nmol/L/h)</th>
<th>a) 13.2 ± 1.56</th>
<th>b) 5.6 ± 1.56</th>
<th>(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)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez 2010</td>
<td>43 steers 30 wks</td>
<td>46 bulls ± 3 wks</td>
<td>Banding</td>
<td>a) Sham</td>
<td>b) 0.07 mg/kg xylazine epidural, then 1.1 mg/kg flunixin meglumine IV 30 min prior to sham</td>
<td>-0.5, 0.5, 1, 2, 4, 24, and 48 h, 7 and 14 d</td>
<td>Salivary cortisol 4 h (nmol/L)</td>
<td>a) 4.6</td>
<td>b) 3.4</td>
<td>c) 10.0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>c) Banding</td>
<td>d) 0.07 mg/kg xylazine epidural, then 1.1 mg/kg flunixin meglumine</td>
<td></td>
<td></td>
<td>a) 3.1</td>
<td>b) 2.5</td>
<td>c) 4.7</td>
</tr>
</tbody>
</table>
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

-20, -10, 15, 20 min

Castration increased cortisol in (b) significantly greater than (d)

Table 1.2 Dehorning and Cortisol

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size</th>
<th>Age</th>
<th>Method</th>
<th>Treatment Groups</th>
<th>Sampling Schedule</th>
<th>Cortisol Concentrations</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laden 1985</td>
<td>18 Holstein</td>
<td>8 wks</td>
<td>Electric</td>
<td>a) Dehorned at 8 wks</td>
<td>-15, 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 72 h</td>
<td>5 min: (nmol/L) a) 0.56 b) 0.15 15 min: (nmol/L) a) 0.75 b) 0.24</td>
<td>Plasma cortisol elevated (P &lt; 0.01) above baseline at 15, 30 minutes for (b) and at 5, 15, 30, and 60 min for</td>
</tr>
<tr>
<td>Year</td>
<td>Breed</td>
<td>Age</td>
<td>Treatment</td>
<td>Cmax (nmol/L)</td>
<td>Description</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Boandl 1989</td>
<td>Holstein</td>
<td>7–16 wks</td>
<td>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)</td>
<td>a) 26.5 b) 78.1 c) 46.4 d) 82.5</td>
<td>(b) and (d) not significantly different from each other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wohlt 1994</td>
<td>Holstein</td>
<td>3–4 wks</td>
<td>Cauterized on day Buddex the next day a) Sham b) Electric cautery c) Buddex</td>
<td>1-2, 5 min post restraint, 5, 15, 30, and 45 min, 1, 2, 3, 4, 8, and 12h</td>
<td>Cmax (nmol/L): a) 30.3 b) 60.4 c) 57.1</td>
<td></td>
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</tr>
<tr>
<td>Morrisse 1995</td>
<td>Montbeliarde</td>
<td>4–8 wks</td>
<td>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</td>
<td>Prior, 1, 4, and 24 h post treatment</td>
<td>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</td>
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</tbody>
</table>

(a). Significant difference between (b) and (a) at 5, 15, 30, and 60 min.
<table>
<thead>
<tr>
<th>Petrie 1995</th>
<th>55 Friesian</th>
<th>6 – 8 wks</th>
<th>Scoop</th>
<th>Cautery</th>
<th>a) Sham</th>
<th>b) 3 mL 2% lidocaine 20 min prior to sham</th>
<th>c) Scoop</th>
<th>d) 3 mL 2% lidocaine 20 min prior to scoop</th>
<th>e) Cautery</th>
<th>f) 3 mL 2% lidocaine 20 min prior to cautery</th>
<th>g) 0.31 mg ACTH IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Total AUC (nmol/L·min)</td>
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<td>Total AUC (nmol/L·min)</td>
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<td></td>
<td></td>
<td>a) 4,386.8 ± 1,426.4</td>
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<td>a) 786.3 ± 281.4</td>
</tr>
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<td></td>
<td>b) 5,024.1 ± 1,296.7</td>
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<td></td>
<td></td>
<td></td>
<td>(c): mean cortisol concentration returned to control values by 6.5 h</td>
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<td></td>
<td>c) 15,210.4 ± 3,327.4</td>
<td></td>
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<td></td>
<td></td>
<td>AUC (-70 min – 2 h) of (c) was significantly greater than (a), (b), (d), (e), and (f)</td>
</tr>
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<td>d) 16,871.3 ± 3,975.7</td>
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<td>e) 8,467 ± 1,591.9</td>
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<td>f) 8,660.5 ± 2,041.7</td>
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<td>g) 18,639.8 ± 2,276.2</td>
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<td>AUC -70 min – 2 h (nmol/L)</td>
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<td>a) 786.3 ± 281.4</td>
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**Note:**
- **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
<table>
<thead>
<tr>
<th>McMeekan 1997</th>
<th>Scoop</th>
<th>McMeekan 1998a</th>
<th>Scoop</th>
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<tbody>
<tr>
<td>30 Friesian</td>
<td>14 – 16 wks</td>
<td>70 Friesian</td>
<td>12 – 16 wks</td>
</tr>
<tr>
<td>a) Control – not dehorned</td>
<td>b) Shallow scoop dehorned</td>
<td>a) Handling</td>
<td>b) Cornual nerve block: 6 mL, 0.25% bupivicaine 20 min prior to (a)</td>
</tr>
<tr>
<td>b) 1,616.8 ± 427.7</td>
<td>c) 6,100.2 ± 1,161.5</td>
<td>-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, 4.66, 5.33, 6, 7, 8, and 9 h</td>
<td>c) b + cornual nerve</td>
</tr>
<tr>
<td>c) 3,732.9 ± 769.8</td>
<td>d) 2,827 ± 102.1</td>
<td>AUC 0 – 3.83 h (nmol/L · min)</td>
<td>AUC 0 – 3.83 h (nmol/L · min)</td>
</tr>
<tr>
<td>d) 2,858.3 ± 789.1</td>
<td>e) 3,732.9 ± 769.8</td>
<td>a) 2,195 ± 853</td>
<td>a) 2,195 ± 853</td>
</tr>
<tr>
<td>e) 9,731.0 ± 1,409.9</td>
<td>f) 2,858.3 ± 789.1</td>
<td>b) 2,562 ± 824</td>
<td>b) 2,562 ± 824</td>
</tr>
<tr>
<td>g) 3,332.9 ± 1,247.1</td>
<td>g) 9,731.0 ± 1,409.9</td>
<td>c) 1,358 ± 375</td>
<td>c) 1,358 ± 375</td>
</tr>
<tr>
<td>AUC 2 – 9.5 h (nmol/L)</td>
<td>AUC 2 – 9.5 h (nmol/L)</td>
<td>d) 1,601.5 ± 3,327.4</td>
<td>d) 1,601.5 ± 3,327.4</td>
</tr>
<tr>
<td>a) 3,404.6 ± 935.3</td>
<td>b) 9,110.2 ± 2,466.6</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>c) 2,827 ± 102.1</td>
<td>d) 2,827 ± 102.1</td>
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</table>

- AUC: Area Under the Curve
- Cmax: Maximum Concentration
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

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 | C<sub>max</sub> (nmol/L) (a,b,c,d,e) all between 5 - 10  
-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  
f) 77.25  
h) 60.7  
i) 13.8 |
<table>
<thead>
<tr>
<th><strong>Sylvester</strong> 1998</th>
<th>60 Friesian 20–26 wks</th>
<th>Scoop Cautery</th>
<th>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</th>
<th>AUC (nmol/L · hr)</th>
<th>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)</th>
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<tbody>
<tr>
<td>51.3 ± 10.8</td>
<td>59.4 ± 14.5</td>
<td>70.02 ± 19.0</td>
<td>140.4 ± 40.5</td>
<td>210.6 ± 56.7</td>
<td>283.5 ± 48.6</td>
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<tr>
<td>59.4 ± 14.5</td>
<td>70.02 ± 19.0</td>
<td>140.4 ± 40.5</td>
<td>210.6 ± 56.7</td>
<td>283.5 ± 48.6</td>
<td>36 h post (nmol/L)</td>
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<tr>
<td>10.3 ± 3.2</td>
<td>9.2 ± 2.4</td>
<td>21.3 ± 4.3</td>
<td>29.2 ± 9.2</td>
<td>26.0 ± 6.0</td>
<td>28.6 ± 6.2</td>
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<tr>
<td>9.2 ± 2.4</td>
<td>21.3 ± 4.3</td>
<td>29.2 ± 9.2</td>
<td>26.0 ± 6.0</td>
<td>28.6 ± 6.2</td>
<td>5 min (nmol/L)</td>
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<tr>
<td>~21</td>
<td>~42</td>
<td>~22</td>
<td>10 min(nmol/L)</td>
<td>~30</td>
<td>Plasma cortisol concentrations were significantly elevated for (b) and (c) as compared to (a) at 20 through 90 min.</td>
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<td>~42</td>
<td>~22</td>
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<td>20 min(nmol/L)</td>
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<tr>
<th>Graf 1999</th>
<th>53 calves 4–6 wks</th>
<th>Cautery</th>
<th>a) Cornual nerve block with 2% lidocaine 20 min prior b) Saline injection 20 min prior c) none</th>
<th>5 min (nmol/L)</th>
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<tr>
<td>~21</td>
<td>~42</td>
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<td>10 min(nmol/L)</td>
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<td>20 min(nmol/L)</td>
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<td>Study</td>
<td>Species</td>
<td>Age (weeks)</td>
<td>Method</td>
<td>Plasma Cortisol Response</td>
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| Grondahl-Nielson 1999 | Friesian | 6           | 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 butorphanol IM 20 min prior  
  d) a + c, 15 min prior  
  e) Hot iron dehorned  
  f) control  
  60 min (nmol/L): a) ~ 10  
  b) ~ 36  
  c) ~ 33  
  90 min (nmol/L): a) ~ 10  
  b) ~ 26  
  c) ~ 25  
  -25 to 25 min (nmol/L): a) ~ 0.2  
  b) ~ 0.4  
  c) ~ 0.5  
  -10 to 30 min (nmol/L): a) ~ -0.25  
  b) ~ -0.3  
  c) ~ 0.3  
  d) ~ -0.1  
  e) ~ 1.25  
  Plasma cortisol in group (e) increased significantly more immediately after dehorning than all other groups.  
  No other statistical differences

Plasma cortisol in
  group (e) increased
  significantly more
  immediately after
  dehorning than all
  other groups.

No other statistical
differences
<table>
<thead>
<tr>
<th>Sutherland 2002a</th>
<th>Friesian</th>
<th>12 – 16 wks</th>
<th>Scoop</th>
<th>a) Handling</th>
<th>b) Dehorning</th>
<th>c) Cornual nerve block: 6 mL 2% lidocaine 15 min prior to dehorning. 2 h after lidocaine, 6 mL of bupivicaine injected</th>
<th>d) c + cautery</th>
<th>$C_{\text{max}}$ (nmol/L), $T_{\text{max}}$ (h)</th>
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<tr>
<td>28</td>
<td>28</td>
<td>12 – 16 wks</td>
<td>28</td>
<td>12 – 16 wks</td>
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<td>12 – 16 wks</td>
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<td>12 – 16 wks</td>
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<td>1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6.5, 7, 7.5, 8, 9, 9.5, 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</td>
<td>b) 157, 0.5</td>
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<td>c) 150, 7</td>
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<td>d) ~ 84, 4</td>
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<td>e) ~ 1</td>
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<p>| Sutherland 2002b | 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 0.25% bupivicaine 2 h after initial treatment | d) c + 4-5.3 mg/kg phenylbutazone IV + a | $C_{\text{max}}$ (nmol/L), $T_{\text{max}}$ (h), AUC 0 – 24 h (nmol/L h⁻¹) |
|------------------|----------|-------------|-------|-------------|--------------|---------------------------------|-----------------|-----------------|-----------------|
| 93               | 93       | 12 – 16 wks | 93    | 12 – 16 wks | 93           | 12 – 16 wks                     | 93              | 12 – 16 wks     | a) 24,160 (2,629) |
|                  |          |             |       |             | 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 | b) 44,907 (4,171) |
|                  |          |             |       |             | c) 39,006 (6,130) |                                  |                 | d) 29,824 (6,383) |
|                  |          |             |       |             | e) 40,916 (5,268) |                                  |                 | e) 30,748 (5,261) |
|                  |          |             |       |             | f) 35,293 (6,057) |                                  |                 | f) 30,748 (5,261) |
|                  |          |             |       |             | g) 35,293 (6,057) |                                  |                 | g) 35,293 (6,057) |
|                  |          |             |       |             | h) 70,844 (7,357) |                                  |                 | h) 70,844 (7,357) |
|                  |          |             |       |             | i) 61,150 (6,901) |                                  |                 | i) 61,150 (6,901) |
|                  |          |             |       |             | j) 63,738 (6,784) |                                  |                 | j) 63,738 (6,784) |
|                  |          |             |       |             | k) 54,638 (2,438) |                                  |                 | k) 54,638 (2,438) |</p>
<table>
<thead>
<tr>
<th>Stafford 2003</th>
<th>100</th>
<th>12 Scoop Friesian wks</th>
<th>a) Sham</th>
<th>-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.</th>
<th>(C_{\text{max}}) (nmol/L), (T_{\text{max}}) (h)</th>
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<tbody>
<tr>
<td>b) Scoop</td>
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<td>a) (~ 5, 0)</td>
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<tr>
<td>c) Cornual nerve block: 5 mL 2% lidocaine 15 min prior to sham, 3 mg/kg ketoprofen IV</td>
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<td>b) 76, 0.5</td>
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<td>d) c + dehorn</td>
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<td></td>
<td>c) (~ 20, 5.5)</td>
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<tr>
<td>e) 0.1 mg/kg xylazine IV 20 min before sham</td>
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<td>d) 20, 4</td>
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<td>f) e + dehorn</td>
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<td>e) (~ 40, 0)</td>
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<td>g) c + dehorn</td>
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<td>f) (~ 60, 5.5)</td>
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<td>h) d + f + dehorn</td>
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<td>g) (~ 57, 0)</td>
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<tr>
<td>i) c + e + 2 mg/kg tolazoline IV 5 min prior + a</td>
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<td>h) (~ 65, 5.5)</td>
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<tr>
<td>j) d + f + 2 mg/kg tolazoline IV 5 min before dehorn</td>
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<td>i) (~ 90, 0.5)</td>
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<tr>
<td>j) (~ 100, 0.5)</td>
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<tr>
<th>Milligan 2004</th>
<th>40</th>
<th>0.3 Electric Holstein wks</th>
<th>a) Cornual nerve block: 5 mL of 2% lidocaine w/ 0.05 mg/mL Epi</th>
<th>prior, 3 and 6 h</th>
<th>Time 0 (nmol/L):</th>
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<tbody>
<tr>
<td>b) Cornual nerve block + 0.03 mL/kg of 10% ketoprofen IM 10 min prior</td>
<td></td>
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<td>a) 68.4 ± 14.3</td>
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<td>b) 87 ± 12.7</td>
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<td>3 h (nmol/L):</td>
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<td>a) 86.3 ± 18.2</td>
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<td>b) 64.8 ± 12.1</td>
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<td>6 h (nmol/L):</td>
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<td>Study</td>
<td>Breed</td>
<td>Age (wks)</td>
<td>Method</td>
<td>Time Points</td>
<td>Mean ± SD (nmol/L)</td>
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<tr>
<td>Schwartzkopf &amp; Genswein 2005</td>
<td>Holstein</td>
<td>29 – 8.5</td>
<td>Electric</td>
<td>a) Day 1: control – no dehorning; b) Day 2: sham dehorning; b) Day 3: dehorning</td>
<td>a) 96.9 ± 19.6; b) 111.1 ± 10.7</td>
</tr>
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<td></td>
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<td></td>
<td>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</td>
<td>15 min: a) 17.7 ± 2.2; b) 14.6 ± 1.37; c) 46.9 ± 1.93 30 min: a) 14.6 ± 1.37; b) 14.9 ± 1.37; c) 51.3 ± 3.6 60 min: a) 11.9 ± 1.1; b) 12.1 ± 1.37; c) 30.9 ± 2.8 120 min: a) 10.7 ± 1.37; b) 12.4 ± 1.7; c) 18.8 ± 1.9 240 min: a) 16.3 ± 1.9; b) 17.7 ± 1.7; c) 11.0 ± 1.1</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Age</td>
<td>Procedure</td>
<td>Results</td>
<td>Comments</td>
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<tr>
<td>Lepkova 2007</td>
<td>18 Czech Red Pied Adult cows</td>
<td></td>
<td>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</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (nmol/L) a) 110.62 ± 45.86 b) 82.53 ± 6.04 c) 113.86 ± 25.65 T&lt;sub&gt;max&lt;/sub&gt; (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</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; was highest for (c) which was significantly higher than (b) but not significantly different from (a).</td>
</tr>
<tr>
<td>Sylvester 2008a</td>
<td>60 Friesian 20–26 wks</td>
<td>Scoop Cautery</td>
<td>a) Control b) 6 mL of local anesthetic via cornual nerve block</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (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&lt;sup&gt;−1&lt;/sup&gt;) a) 51.3 ± 10.8 b) 59.4 ± 13.5</td>
<td>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 (f) were significantly higher than (a) and (b) but not significantly different from each other.</td>
</tr>
<tr>
<td>Sylvester 2002b</td>
<td>57</td>
<td>Friesian</td>
<td>20 – 26 wks</td>
<td>Scoop Guillotine Shears Saw Embryotomy wire</td>
<td>a) Control, handling b) Barnes scoop c) guillotine shears d) Butcher’s saw e) Embryotomy wire f) 40 mg IV ACTH</td>
</tr>
<tr>
<td>Stillwell 2008</td>
<td>20</td>
<td>Holstein</td>
<td>1</td>
<td>a) Sham, injected with saline -5, 1, 3, 6, and 24 h</td>
<td>1 h (nmol/L) a) 10.18 ± 4.14</td>
</tr>
</tbody>
</table>
### Heinrich 2009

<table>
<thead>
<tr>
<th>Breed</th>
<th>Age</th>
<th>Treatment</th>
<th>Dose</th>
<th>Average Increase (nmol/L)</th>
<th>Cortisol Elevation</th>
<th>Differences</th>
</tr>
</thead>
</table>
| Friesian | 60 Holstein | 6 – 12 wks | Electric | a) Placebo Injection + 0.5, 1, 1.5, corneal nerve block: 5, 2, 4, 6, and 10 min prior b) Single IM 0.5 mg/kg dose of meloxicam + cornual nerve block | 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

<table>
<thead>
<tr>
<th>Breed</th>
<th>Age</th>
<th>Treatment</th>
<th>Average Increase (nmol/L)</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein</td>
<td>32 Holstein</td>
<td>3.5 ± 1 wk</td>
<td>Disbudding</td>
<td>a: 62.64 ± 10.32</td>
</tr>
<tr>
<td>Stillwell 2009 Holstein 3 ± 0.5 wks Caustic Paste</td>
<td>a) saline injection 5 min prior to caustic paste</td>
<td>b) 2% lidocaine prior to caustic paste</td>
<td>c) 2.2 mg/kg flunixin IV + b</td>
<td>d) Saline injection 5 min prior to sham</td>
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</table>

**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.**
<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Age</th>
<th>Procedure Description</th>
<th>Time Points (nmol/L)</th>
<th>Data Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stillwell 2009</td>
<td>16</td>
<td>4 ± 1 wk</td>
<td>Caustic paste</td>
<td>50 min: a) 42.32 ± 14.47, b) 14.73 ± 8.80, c) 19.80 ± 9.67, d) 14.34 ± 8.57</td>
<td>(a) was significantly higher than (b) and (c) at 90 min</td>
</tr>
</tbody>
</table>
|               |         |     | a) Saline injection 5 min prior to caustic paste  
|               |         |     | b) 2% lidocaine prior to caustic paste  
|               |         |     | c) Saline injection 5 min prior to sham  | 90 min: a) 40.5 ± 17.7, b) 23.3 ± 18.6, c) 15.7 ± 9.9  
|               |         |     | 120 min: a) 11.9 ± 16.4  
|               |         |     | b) 5.8 ± 7.6  
|               |         |     | c) 12.8 ± 12.9 | 150 min: a) 20.1 ± 8.5  
|               |         |     | b) 28.1 ± 15.7  
|               |         |     | c) 22.4 ± 6.2 | 180 min: a) 27.2 ± 5.3  
|               |         |     | b) 43.3 ± 9.8  
|               |         |     | c) 16.5 ± 14.3 | (b) was significantly higher than (a) and (b) at 180 min |
| Duffield 2010  | 40      | 4–8 wks | Cautery  
|               | Holstein |     | 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: a) 34  
|               |         |     | 3 h: a) ~34, b) ~32.5, c) ~37  
<p>|               |         |     | 6 h: a) ~37, b) ~33.5 | No differences in serum cortisol concentrations at any time |</p>
<table>
<thead>
<tr>
<th>Stillwell 2010</th>
<th>Holstein</th>
<th>Electric Disbudding</th>
<th>5 min after treatment, 10, 25, 40, 60 min</th>
<th>10 min (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41</td>
<td>5.3 ± 0.5 wks</td>
<td>a) 0.2 mg/kg IM xylazine 10 min prior and saline 8 min prior</td>
<td>a) 94.82 ± 9.54</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>b) 0.2 mg/kg IM xylazine 10 min prior and cornual nerve block with 2% lidocaine 8 min prior</td>
<td>b) 86.34 ± 9.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c) Sham disbudded after xylazine and lidocaine</td>
<td>c) 77.78 ± 9.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d) Sham disbudded after IM saline and lidocaine</td>
<td>d) 18.54 ± 9.54</td>
</tr>
</tbody>
</table>

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.
References


Guidance For Industry: Development of target animal safety and effectiveness data to support approval of non-steroidal anti-inflammatory drugs (NSAIDS) in animals.


Livingston, A. Comparative and Veterinary Pharmacology. pg 159 – 183. 2010


Sylvester 1998a


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

Accepted for publication in the American Journal of Veterinary Research, September 2010.

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...
animal husbandry procedures such as castration and dehorning. This process is especially complex in a prey species, such as cattle, that inherently conceal pain (Underwood, 2002). In previous research (Fisher et al., 2002; Knight et al., 2000; Mellor et al., 2000; Ting et al., 2003, Pang et al., 2006; Gonzalez et al., 2008; Faulkner et al., 2002) biomarkers for the evaluation of pain and distress associated with castration and dehorning have included serum cortisol concentration, heart rate, measurement of the presence of acute phase proteins and in vitro interferon-γ production, behavior scoring, average daily gain, feed intake, chute activity, and vocalization. The magnitude of the increase in serum cortisol concentration (as indicated by the change in peak concentration height \(C_{max}\)) and duration; the integrated response (as indicated by the AUEC), or both has been reported to correspond with the predicted noxious stimulus of these during the procedure (Mellor et al., 2000). The results of studies using ADG as a pain parameter have been equivocal. For example, one study revealed calves undergoing castration have a decrease in ADG when compared to calves not undergoing castration; however, a treatment affect was not observed (Faulkner et al., 1992). Additionally, information is deficient on the use of chute exit speed and EDA for the objective measurement of pain associated with castration and dehorning. Chute exit speed has been used in temperament and reactivity studies in cattle (Mellor et al., 2008; Curley et al., 2006). We therefore hypothesized that evaluation of exit speed could be used to determine the effect of a painful procedure and sedative drug on calf behavior. EDA is a measurement of electrical resistance of a tissue path between two electrodes applied to the skin and can be influenced by changes in sympathetic outflow during times of pain, anxiety, and stress (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 an increase of plasma cortisol to approximately 80 nmol/L after 30 minutes 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\textsuperscript{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\textsuperscript{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.

\textbf{Study Design}

A 2-period, parallel design study (\textbf{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\textsuperscript{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\textsuperscript{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\textsuperscript{g} + 0.1
mg/kg ketamine\textsuperscript{h} + 0.025 mg/kg butorphanol\textsuperscript{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.\textsuperscript{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. 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. 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. 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]) 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. Hemostasis was achieved through thermocautery by use of a hot iron. 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 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 equipped with a printer.

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 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. 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 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 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 in human serum after 42 years of storage at -20°C. 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\(_2\)O: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\(_2\)O (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\(^v\) maintained at 40°C. The method was accurate and

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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 -20°C as compared with fresh plasma samples. 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 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\textsubscript{max}, C\textsubscript{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.\textsuperscript{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 (\(\lambda_z\)), terminal elimination half-life (T\(\frac{1}{2}\lambda_z\)), time to maximum drug concentration (T\textsubscript{max}), maximum drug concentration (C\textsubscript{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:

\[ T_{\frac{1}{2}} e^{-1} = \frac{0.693}{\lambda_z} \]  \hspace{1cm} [1]  
\[ Cl/F = D/AUC \]  \hspace{1cm} [2]  
\[ Vz/F = \frac{Dose}{AUC \times \lambda z} \]  \hspace{1cm} [3]  
\[ MRT = \frac{AUMC}{AUC} \]  \hspace{1cm} [4]
\[ \text{AUC}_{0-\infty} = \text{AUC} + \frac{C_{\text{last}}}{\lambda_z} \]

where \( C_{\text{last}} \) is the last measured concentration and \( \text{AUMC} \) 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. 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. Statistical significance was designated \textit{a priori} at $P < 0.05$.

\textbf{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.

\textbf{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 $1.187 \pm 0.275$ kg/day and $1.172 \pm 0.305$ kg/day, respectively, as compared with $0.098 \pm 0.336$ 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\(_{\text{max}}\), T\(_{\text{max}}\), AUEC\(_{0 \text{ to } 1 \text{ h}}\), AUEC\(_{1 \text{ to } 6 \text{ h}}\), and AUEC\(_{6 \text{ to } 24 \text{ h}}\)) for
serum cortisol concentration results were significantly (P < 0.001) different in Period 2
versus Period 1. Cortisol T\(_{\text{max}}\) was significantly (P < 0.001) shorter in Period 2, while
cortisol C\(_{\text{max}}\), AUEC\(_{0 \text{ to } 1 \text{ h}}\), AUEC\(_{1 \text{ to } 6 \text{ h}}\), and AUEC\(_{6 \text{ to } 24 \text{ h}}\) were significantly (P < 0.001)
greater in Period 2 compared with Period 1.

A comparison of T\(_{\text{max}}\) and C\(_{\text{max}}\) 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\(_{\text{max}}\)
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\(_{\text{max}}\) 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\(_{\text{max}}\) 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\textsubscript{0 to 1 h}, AUEC\textsubscript{1 to 6 h}, and AUEC\textsubscript{6 to 24 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\textsubscript{0 to 1 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\textsubscript{0 to 1 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\textsubscript{1 to 6 h} \((P = 0.389)\) and AUEC\textsubscript{6 to 24 h} \((P = 0.208)\) between the calves that received XKB and those that did not. A significant difference \((P = 0.872)\) for AUEC\textsubscript{0 to 1 h} in calves receiving salicylate was not detected during Period 2, compared with those not receiving salicylate; however, AUEC\textsubscript{1 to 6 h} was significantly \((P = 0.024)\) less during Period 2 for those calves receiving salicylate. Additionally, AUEC\textsubscript{1 to 6 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\textsubscript{6 to 24 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 \((T_{\text{max}}, C_{\text{max}}, \text{AUC}, V_z,F, \text{Cl}_F, \text{MRT}, \text{and } T_{1/2\lambda z})\) 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 $T_{\text{max}}$, $C_{\text{max}}$, 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.*, 1999).
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 
\(\alpha-2\) 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).
Unmyelinated 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 \(\kappa\) (partial agonist) and \(\mu\)(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 \(\alpha-2\) adrenergic agonist with sedative and analgesic 
effects when administered to cattle at doses ranging from 0.05 to 0.3 mg of xylazine/kg
Antinoceptive 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 to 1 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).
1890 1981), an IV dose of 0.2 mg/kg xylazine was associated with a peak plasma xylazine
1891 concentration of 1.050 µg · mL⁻¹, a $t_{1/2a}$ of 36.48 minutes, and a total body clearance of 42
1892 ml/min/kg. Ketamine administered IV in calves had a $t_{1/2}$ of 60.5 ± 5.4 minutes and a
1893 total body clearance of 40.39 ± 6.6 ml/min/kg in another study (Waterman et al., 1981).
1894 In another study (Sellers et al., 2010), IV administration of ketamine at a dose of 5 mg/kg
1895 demonstrated the following pharmacokinetic parameters; $C_{\text{max}}$ of 18.135 ± 22.720
1896 ng/mL, $T_{\text{max}}$ 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.
1897 Previous studies (Court et al., 1992) in dairy cows administered 0.25 mg/kg IV of
1898 butorphanol showed a $t_{1/2}$ to be 82 minutes, total body clearance to be 34.6 ±
1899 77ml/kg/min, and the mean AUC was 7,567 ± 54 ng · min/mL. In the present study the
1900 $t_{1/2}$ was 109.43 ± 22.62, 81.45 ± 10.44, and 71.28 ± 7.64 minutes respectively for
1901 xylazine, ketamine, and butorphanol. The dosages used in this study were less than doses
1902 used in previously mentioned references. The drugs in the present study a longer $t_{1/2}$ than
1903 previously mentioned studies with the exception of butorphanol which had a shorter $t_{1/2}$.
1904 Total body clearance for all three drugs was also found to be greater than previous
1905 studies. The $T_{\text{max}}$ for ketamine in the present study was also longer than what was
1906 previously reported.
1907
1908 Analysis of the results indicated that there was more variability between Period 1
1909 and Period 2 for the $T_{\text{max}}$ of serum cortisol concentration. These differences in $T_{\text{max}}$ were
1910 most likely the result of individual calf variability in response to treatment with xylazine,
1911 ketamine, and butorphanol. A previous study (Coetzee et al., 2008) with 4 to 6 month old
1912 bull calves found no significant difference in $T_{\text{max}}$ of serum cortisol between calves
surgically castrated versus those undergoing simulated castration. Another study (Ting et al., 2003) found a significantly longer $T_{\text{max}}$ 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 $T_{\text{max}}$ 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 $C_{\text{max}}$ 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 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. 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 $V_z$ 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.
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 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period</th>
<th>AUEC&lt;sub&gt;0 to 1 h&lt;/sub&gt; (h*nmol/L)</th>
<th>AUEC&lt;sub&gt;1 to 6 h&lt;/sub&gt; (h*nmol/L)</th>
<th>AUEC&lt;sub&gt;6 to 24 h&lt;/sub&gt; (h*nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEBO</td>
<td>1</td>
<td>92.560&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.06&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>597.36&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>132.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>342.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>756.28&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAL</td>
<td>1</td>
<td>84.293&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>119.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>434.29&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>119.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>216.36&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>583.64&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>XKB</td>
<td>1</td>
<td>42.102&lt;sup&gt;e&lt;/sup&gt;</td>
<td>123.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>574.37&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>93.993&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>322.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>756.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAL + XKB</td>
<td>1</td>
<td>48.927&lt;sup&gt;e&lt;/sup&gt;</td>
<td>131.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>455.51&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>104.57&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>259.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>637.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Xylazine</th>
<th>Ketamine</th>
<th>Butorphanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XKB</td>
<td>SAL + XKB</td>
<td>XKB</td>
</tr>
<tr>
<td>$T_{1/2x}$ (min)</td>
<td>96.40 ± 20.33 $^a$</td>
<td>122.47 ± 24.90 $^a$</td>
<td>67.43 ± 11.13 $^a$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>9.5 ± 0.50 $^a$</td>
<td>11 ± 1.00 $^a$</td>
<td>10 ± 1.29 $^a$</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>20.95 ± 1.68 $^a$</td>
<td>19.50 ± 2.07 $^a$</td>
<td>14.97 ± 1.91 $^a$</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (hr·ng/mL)</td>
<td>16.68 ± 1.44 $^a$</td>
<td>17.48 ± 1.19 $^a$</td>
<td>12.90 ± 2.4 $^a$</td>
</tr>
<tr>
<td>$V_z/F$ (L/kg)</td>
<td>6.7 ± 1.09 $^a$</td>
<td>8.27 ± 1.54 $^a$</td>
<td>12.11 ± 2.15 $^a$</td>
</tr>
<tr>
<td>$CL/F$ (mL/min/kg)</td>
<td>53.69 ± 4.89 $^a$</td>
<td>49.30 ± 2.72 $^a$</td>
<td>184.28 ± 33.73 $^a$</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>96.31 ± 18.15 $^a$</td>
<td>120.03 ± 22.48 $^a$</td>
<td>67.43 ± 10.46 $^a$</td>
</tr>
</tbody>
</table>

$^a$All reported parameter estimates within a row for each drug with different superscripts differ significantly ($P < 0.05$)
T_{\text{max}} = \text{time to maximum drug concentration. } C_{\text{max}} = \text{maximum concentration of drug. } \text{AUC} = \text{area under the curve.}

V_{z_{\text{F}}} = \text{volume of distribution per fraction of dose absorbed (F). } \text{CL}_{\text{F}} = \text{total body clearance per fraction of dose absorbed.}

\text{MRT} = \text{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.

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>SAL</th>
<th>SAL + XKB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>AUC (min·μg/mL)</td>
<td>4,923.26</td>
<td>856.33</td>
</tr>
<tr>
<td>Sodium salicylate concentration throughout Period 1 (μg/mL)</td>
<td>32.41</td>
<td>12.86</td>
</tr>
<tr>
<td>Sodium salicylate concentration throughout Period 2 (μg/mL)</td>
<td>40.36</td>
<td>12.19</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>41.7</td>
<td></td>
</tr>
<tr>
<td>(C_{\text{max}}) (μg/mL)</td>
<td>61.134</td>
<td>10.312</td>
</tr>
<tr>
<td>Mean sodium salicylate concentration (μg/mL)</td>
<td>32.20</td>
<td>1.59</td>
</tr>
</tbody>
</table>

\(T_{\text{max}} = \text{time to maximum drug concentration}; \ C_{\text{max}} = \text{maximum concentration of drug}; \ AUC = \text{Area under the curve.}\)
**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 $T_{\text{max}}$ 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 $T_{\text{max}}$ of serum cortisol concentrations is indicated by different letters.

Figure 2.8. A comparison of the mean ± SEM $C_{\text{max}}$ 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 to 1 h}), 1st through the 6th hour (■, AUEC_{1 to 6 h}), and 6th through 24th hour (□ AUEC_{6 to 24 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 (—×—) 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

ADG (kg) vs. Treatment Group

PLACEBO  |  SAI  |  XKB  |  SAI + XKB

[Bar chart showing ADG (kg) for different treatment groups: PLACEBO, SAI, XKB, SAI + XKB]
Figure 2.3

![Bar chart showing mean chute exit speed (cc/m) for PLACEBO, SAL, XKB, and SAL+XKB. The chart includes data for Period 1 and Period 2.](image)
Figure 2.5

![Graph showing serum cortisol concentration over time for different groups.](image)
Figure 2.6

- Placebo
- SAL
- XKB
- SAL + XKB

Serum Cortisol Concentration (nmol/L)

Time elapsed after dehorning and castration (min)
Figure 2.8

<table>
<thead>
<tr>
<th></th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XKB</strong></td>
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<td><img src="image" alt="Graph for No Salicylate Period 2" /></td>
</tr>
</tbody>
</table>

C<sub>max</sub> (nmol/L)
Figure 2.9

AUC for Serum Cortisol Concentrations (hr*nmol/L)
Figure 2.10

Log Plasma Drug Concentration (ng/mL)

Time after XKB administration (min)
Figure 2.11

Log Plasma Drug Concentration (ng/mL) vs. Time after XKB administration (min)
Figure 2.12

The graph shows the dose of Sodium Salicylate (mg/kg) over time (h) after initial Sodium Salicylate administration on day -3. The data is represented for two conditions: SAL and SAL + XKB. The graph indicates a scatter plot with time on the x-axis and dose on the y-axis, illustrating the variability in dose over time for each condition.
Figure 2.13

[Graph showing log plasma sodium salicylate concentration (µg/mL) over time from initial sodium salicylate administration (day -3) (h). The graph compares two groups: SAL and SAL + XKB.]
References


Faulkner PM, and Weary DM. Reducing pain after dehorning in dairy calves. 


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I appreciate your help in this matter!
Thanks!

Sarah Baldridge, DVM
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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\textsubscript{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\textsubscript{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.