

ANALGESIC EFFICACY OF SODIUM SALICYLATE IN AN AMPHOTERICIN B
INDUCED BOVINE SYNOVITIS-ARTHRITIS MODEL

by

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Abstract

Lameness is a common, costly, and painful affliction in cattle at all production levels. There are currently no compounds specifically approved for analgesia in cattle in the United States. We hypothesized that intra-articular amphotericin B produces a controlled, transient synovitis-arthritis in cattle and that this model would allow characterization of the analgesic effects of intravenous sodium salicylate.

This study examined the efficacy of sodium salicylate for providing analgesia in an amphotericin B-induced bovine synovitis/arthritis model utilizing ten male Holstein calves, 4-6 months old, and weighing approximately 250 kg. The study used a repeated measures partial cross-over design with 2 phases consisting of 3 treatment periods within each phase. Calves were blocked by weight and randomly assigned to sodium salicylate (50mg/kg intravenously) or placebo group for phase 1. In period 1, lameness induction was simulated with a needle-prick of the coronary band, followed by drug or placebo administration. At predetermined timepoints, serial blood samples for cortisol and salicylate concentrations, electrodermal activity measurements, heart rates, and pressure mat data were collected. Visual lameness scores were recorded by a blinded observer. In period 2, lameness was induced with injection of amphotericin B into the distal interphalangeal joint followed by drug or placebo administration with sample collection as previously described. In period 3, drug or placebo was administered to the respective calves with sample collection. After a 10-day washout, Phase 2 was conducted with treatments crossed over between groups. Cortisol and salicylate samples were analyzed by competitive chemiluminescent immunoassay and fluorescence polarization immunoassay,

respectively. The pharmacokinetic data were analyzed using compartmental analysis. Mean intravenous salicylate apparent volume of distribution (V_d) was 0.2 ± 0.005 L/kg, total body clearance (CL) was 4.3 ± 0.2 mL/min*kg, and elimination half life ($t_{1/2}$ el) was 36.9 ± 1.2 minutes. The repeated measures data were analyzed based on a univariate split-plot approach with a random effects-mixed model. Differences in stance phase duration and serum cortisol concentration values were seen between both periods and treatment group*periods; differences in heart rate, contact surface area, and contact pressure values were seen between periods, suggesting that our lameness model was effective. No differences were seen between treatment groups. When analyzed by visual lameness score, differences were seen in heart rate, contact surface area, contact pressure, and cortisol concentrations. Area under the time-effect curves, determined using the trapezoidal rule, had results similar to the repeated measures data, except for a difference in period for electrodermal activity. This amphoterecin B-induced synovitis/arthritis model is a useful tool for studying changes associated with lameness in cattle. Sodium salicylate was not effective in providing analgesia following lameness.

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Dedication

This thesis is dedicated to my husband Nathan and our daughter Sierra. Without their love, understanding and hard work, this work would not have been possible. I thank God for them every day.

CHAPTER 1 - Literature Review

Food Animal Welfare and Pain Management

Animal welfare is a controversial and ill-defined topic, even among veterinarians (Morrow-Tesch, 2001). Animal welfare is defined by the American Veterinary Medical Association (AVMA) as “including proper housing, management, nutrition, disease prevention and treatment, responsible care, humane handling, and euthanasia” (Morrow-Tesch, 2001).

Animal welfare is an issue that has increasingly become more important in the past decade in food animal production medicine in part due to increased attention from the media and public sectors (de Grassi, 2001). However, animal welfare has long been consciously practiced by food animal producers to increase health, comfort, and production efficiency in livestock.

Animal welfare policy changes range from voluntary programs by producer organizations to recent publically determined ballot initiatives in Florida, Arizona, and California (Underwood, 2002). Historically, government has had little influence on mandating animal welfare in the United States. This, however, does not hold true in European countries, where mandates and regulations are stricter (Andreasen, et al., 2005). Sweden, which has long led the cry for animal welfare legislation, is a striking representation for analyzing such actions. The current regulations in Sweden, the Swedish Animal Welfare Act and Swedish Animal Welfare Ordinance, were established in 1988 to address consumer and producer concerns of cruelty prevention and provision of basic needs, as well as dealing with housing, restraint, health care, and transportation. These regulations also have provisions for enforcement and penalties for

nonconformity. In striking contrast to the United States, some examples of differences include allowing natural behaviors (eg, cattle must be allowed to graze for a certain amount of time), provision of natural daylight, a strict ban of devices to drive animals, mandated expedient administration of medical treatment or euthanasia for ill or injured animals, anesthetizing animals prior to slaughter, and regulation of treatments and surgical procedures to be performed solely by veterinarians. Also, numerous provisions for transportation are required, with education of drivers, food, shelter, and avoidance of injuries and distress necessary. Examples of requirements in swine production include castration by lay people only when the piglets are under 1 week old and after that only by a veterinarian under anesthesia, clipping of needle teeth only when there is evidence of trauma to the sow, no early weaning (four weeks or older), deeply bedded housing, and no tail docking with the exception of good medical reason.

A nation-wide telephone survey was conducted in the United States by Lusk and Norwood (2008) with an objective to “determine the attitude of the public toward farm animal welfare and identify beliefs regarding how decisions about farm animal welfare should be made.” They also evaluated if a participant’s social values had an effect on their beliefs of treatment of farm animals. From this, they found that 56.4% of respondents believed that decisions about animal welfare should be made by experts and should not be influenced by public opinion. These respondents were less likely to feel that farm animal welfare should be regulated by government. Also, 54.3% thought that animal welfare decisions should be based on scientific measures of animal wellbeing and not moral or ethical considerations. Participants who believed that farm animal welfare should be made by experts and should be based on scientific measures were found to be the least concerned about farm animal welfare issues. An email survey regarding attitude of United States veterinary college faculty with large or food animal

emphasis toward farm animal welfare was conducted by Heleski and others (2005). They found that 71% of participants characterized their attitude as “we can use animals for the greater human good but have obligations to provide for the majority of the animals’ physiologic and behavioral needs,” with 19% of respondents more concerned than this statement and 10% less concerned. They found the following demographics to increase concern: females, those characterized with more liberal political views, and those with lower religiosity. A similar survey conducted by the same group (2004) targeting animal science faculty found that greater than 90% of participants support general principles of animal welfare, an example being keeping animals free from unnecessary pain or fear. Only 32% of participants felt that practices such as castration without an anesthetic were not of concern. Similar demographic effects relating to gender and political views were established as found in the survey of United States veterinary college faculty.

In 2001, the AVMA presented “Bovine Welfare” at the eleventh annual Animal Welfare Forum (Animal Welfare Forum: Bovine Welfare, 2001). Topics included in the discussion included the impact of management practices on welfare, slaughter cattle welfare, feedlot welfare, rodeo and show cattle welfare, and various dairy cattle issues including veal calves, replacement heifers, and cow comfort. An example of producer efforts to address welfare issues noted at this forum was quality assurance programs (de Grassi, 2001). Quality assurance programs usually consist of formal training, documentation, and verification by a third party to ensure proper procedures are being followed. Groups as the National Institute for Animal Agriculture (NIAA), National Cattlemen’s Beef Association (NCBA), National Milk Producers Federation, and the American Association of Bovine Practitioners (AABP) are advocates through educational and publication materials to animal welfare issues. Current welfare challenges noted include standard practices such as castration, dehorning, and branding (Morrow-Tesch, 2001).

Other areas of need that are currently manageable include facilities and equipment, which is most important in slaughter facilities (Grandin, 2001). Significant progress in this field has been made by Dr. Temple Grandin. Easily employed practices advocated by Dr. Grandin such as improving lighting, removing visual and audible distractions, and calm behavior of the handler go far in increasing animal welfare and decreasing excess force needed such as electric prods. Dr. Grandin has worked with several restaurants to audit meatpacking facilities for handling quality. Her simple objective scoring system for handling and stunning at slaughter facilities, scored on a yes/no basis, is:

- 1) Percentage of animals stunned correctly on the first attempt
- 2) Percentage of animals insensible on the bleed rail
- 3) Percentage of cattle that vocalize (moo or bellow) during movement through the chute and restrainer
- 4) Percentage of animals for which an electric prod is used, and
- 5) Percentage of animals that slip or
- 6) Percentage that fall

Non-ambulatory (downer) cows are an exceedingly important portion of the animal welfare concerns in food animals (de Grassi, 2001). A review by Stull and colleagues (2007) thoroughly detailed current knowledge of downer cattle, from definition, incidence, history and policy, to prognosis and prevention. They found that the estimated incidence of downer beef cattle ranged from 0.7% to 1.1%, and downer dairy cattle incidence ranged from 1.1% to 1.5%. Legislation to limit the slaughter of non-ambulatory cattle struggled in the late 1990's and early 2000. After the discovery of a BSE infected cow in the United States in late 2003, an immediate ban on slaughter of all non-ambulatory cattle was put into place. A non-ambulatory animal at

that time was defined as “animals that cannot rise from a recumbent position or that cannot walk, including but not limited to those with broken appendages, severed tendons or ligaments, nerve paralysis, a fractured vertebral column, or metabolic conditions.” The AVMA’s current policy statement on non-ambulatory cattle states that cattle should be euthanized if in distress, non-ambulatory cattle should never be dragged, and a veterinarian should be recruited to aid if the cow is otherwise eating and drinking and is not suffering. General pathological causes cited for loss of ability to walk include musculoskeletal and neural injuries, metabolic abnormalities, and infectious diseases. Prognosis for non-ambulatory cattle depends heavily on the cause. Crucial components of downer cow prevention are incorporation of strong management practices that include culling, selecting for good conformation, and proper facilities to prevent lameness.

Pain has been defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” (Paul-Murphy et al, 2004). Because animals cannot communicate through language or emotions, some have used nociception, or the perception of a damaging or potentially damaging stimulus, to describe animal responsive to noxious stimuli. In 2002, experts in animal and human pain gathered in an international workshop, summarized by Paul-Murphy and others (2004). From this, a collective consensus statement was formed which established that animals feel pain, although it was uncertain at that time if all vertebrates and invertebrates feel pain in the same amount and what effect taxonomic level had in respect to nociception associated with pain. The purpose of this vague consensus statement was to provide a rousing call to action by veterinary and human medical professionals to encourage both sides to work together in the study of pain. Also, workshop contributors worked to identify important deficiencies in current knowledge of pain and to develop action

plans to increase what is known about pain. Essential gaps of current knowledge of pain identified included basic mechanisms of pain, lack of information regarding analgesics across species, lack of veterinary medical training in animal analgesia, few resources available for research and education in animal pain and analgesia, and finally lack of a universally recognized assessment of pain. Suggested action plans included meaningful pain scales, development of a multidisciplinary approach to treatment of pain, creation of a special interest group in IASP to encourage exchange of research, improve funding, and educate the public about animal pain to generate support.

One of the concerns expressed by Lascelles and Main (2002) at the 2002 AVMA Animal Welfare Forum: Pain Management was for animal welfare considerations in relation to surgical trauma and chronic conditions. Recognized causes of pain in food animals include lameness in cattle, degenerative joint disease in poultry, systemic illness, mastitis, castration, dehorning, branding, beak trimming, tail docking, transportation, handling, conditions associated with cattle feedlot confinement, dairy cattle tie stalls, swine gestation crates, poultry batteries, and induced molting (Underwood, 2002). Many of these are currently necessary management practice in food animal production systems. However, excess stress can be minimized by proper housing, handling, movement, and animal husbandry.

Lameness in Cattle

Lameness is one of the top 3 reasons for premature culling in dairy cows and also is an important cause of loss in beef cattle (Berry, 2001). Important causes of lameness include nutrition, facility design, hygiene, hoof care, and animal husbandry.

The number of cattle affected by lameness varies with production system, housing, and management. Cook (2003) looked at the prevalence of lameness in dairy cattle in Wisconsin by

examining the variables of season, housing type, and stall surface. He found the mean prevalence was 21.1% during the summer and 23.9% during the winter, with statistically significant difference between these values. There was a significantly higher prevalence of lameness in free-stall herds with non-sand stall surfaces (33.7%) compared to free-stall herds using sand stall surfaces (21.2%), tie-stall herds with non-sand stall surfaces (21.7%), and tie-stall herds using sand stall surfaces (12.1%).

Researchers in New Zealand (Laven *et al.*, 2008) performed a study on New Zealand dairy cattle to look at the pain response to lameness as determined by increased locomotion score and decreased nociceptive response, prior to treatment and at the timepoints 3, 8, 28, and 100 days after treatment. They also looked at the influence of 4 treatment interventions: 2 mg/kg tolfenamic acid (a non-steroidal anti-inflammatory drug), a shoe to elevate the lesion, both, or neither. They found improvements in all treated cattle. There was no significant long term advantage of using tolfenamic acid, nor an advantage to using a plastic shoe in cases determined not to be necessary. At all time-points after treatment, lameness scores and nociceptive threshold was significantly better compared to the previous time-point. The effects of lameness were found to persist beyond the 28 day time-point.

The 1999 National Market Cow and Bull Beef Quality Audit was conducted to examine the quality of market cows and bulls and to compare to the data collected from the 1994 National Non-Fed Beef Quality Audit. (Roerber *et al.*, 2001) Data were derived from interviews with industry representatives, in-depth evaluation at slaughter houses and holding pens, and a strategy workshop. After analysis, the incidence of lameness was 31.4% of all cattle audited and losses due to lameness were significantly increased compared to the previously conducted audit (Table 1.1). Of all cattle, 7.4% of carcasses had one arthritic joint that required removal, and 4.0% of

carcasses had two arthritic joints that were removed. This required removal in an estimated mean loss of 17.9 kg of product for each arthritic joint, which is increased compared to 11.4 kg per joint found in the previous audit.

Economic Effects of Lameness

The economic effect of lameness is difficult at best to quantify. Clearly, the cost of lameness is not in treatment cost alone, but also in production cost of fertility, milk yield, days to slaughter, and in opportunity cost of culling and replacement. An estimate by Esslemont and Kossaibati in 1997 for total cost of lameness per case in the United Kingdom was £245.22 (US \$446) (Booth *et al.*, 2004).

In a study conducted to look at the effect of lameness on culling, Booth *et al.* (2004) looked at lactation data from 2,520 cows in 2 New York state dairy herds which were analyzed by Cox's proportional hazards regression models. They found that for all causes of lameness, survival in the herd decreased for cows becoming lame within the first half of lactation with a hazards ratio of up to 2 times compared to a non-lame cow. When broken down by cause of lameness, foot rot at the second or third month of lactation decreased survival (hazard ratio of 5.1), sole abscesses in the first 4 months of lactation decreased survival (hazard ratio of 2.7), and there was no association of foot warts with decreased survival in this study. Universally, they found lameness never to be associated with increased survival in any models.

In a study designed to test whether lameness in dairy cows decreased milk production compared to healthy cows, Bicalho, Warnick and Guard (2008) retrospectively evaluated lame cattle afflicted with laminitic disorders. From this study, they demonstrated that the estimated losses in a 305 day lactation were 314 to 424 kg/cow. They also demonstrated a risk factor of high milk yield in the beginning of lactation.

Hernandez *et al.* (2002) performed a study to evaluate the effect of lameness on milk yield in 531 dairy cows. Cows were classified based on lesions: healthy, foot rot (interdigital phlegmon), foot warts (papillomatous digital dermatitis), or claw lesions. The cows 305 day mature equivalent milk yield data were collected using the Dairy Herd Improvement Association records. Data were then compared between healthy and lame cows. They found that 31% of cows were affected with lameness during lactation, 60% of the cows were afflicted with claw lesions, 31% had foot warts, and 9% had foot rot. The milk yield in cows with foot rot was significantly less (10% decrease) compared to the milk yield in healthy cows. Milk yield in the other categories of lameness was less than that of the healthy cows, but this difference was not significant.

Rajala-Schultz and colleagues (1999) also analyzed the effects of milk fever, ketosis, and lameness on milk loss in 23,416 Finnish Ayrshire milking cows. For cows affected with milk fever, they found a milk loss ranging from 1.1 to 2.9 kg/day lasting from 4 to 6 weeks after calving. Ketotic cows experienced decreasing milk yield 2 to 4 weeks prior to the diagnosis of ketosis, with milk loss the greatest within 2 weeks after diagnosis, ranging from 3.0 to 5.3 kg/day. Lame cattle experienced milk losses from 1.5 to 2.8 kg/day within the first 2 weeks after diagnosis.

Hernandez *et al.* (2005) compared the time from calving to conception in 499 Holstein cows with varying degrees of lameness: non-lame (31% of cows), moderately lame (43% of cows), or lame (26% of cows), classified by use of a 6 point locomotion scoring system based on the system described by Sprecher *et al.* (1997). Cows that were classified as “lame” or having scores of 4 or 5 were examined for diagnosis and treatment of lameness. The majority (54%) of cows categorized as lame were diagnosed with laminitis or disorders of the claw (33%). They

found that the median calving to conception interval in lame cows was 66 days longer in cows with high cumulative locomotion scores compared to the cows with low cumulative locomotion scores.

Lameness Models in Large Animals

Limited research on induced lameness exists in food animals. However, there has been extensive research using lameness models in horses.

Francoz *et al.* (2005, 2008) used a bacterial-induced septic arthritis model to evaluate changes over time in relative expression of matrix metalloproteinase (MMP)-2 and -9 in Holstein calves. Seven experimental calves were injected in the tarsal joint with colony forming units (CFU) of a live culture of *Escherichia coli*. Calves were evaluated for lameness, joint pain and swelling (Table 1.2) 4 times daily for the first 6 days, then once daily for the remainder of the study period. Calves were given a caudal epidural injection of morphine and intravenous butorphanol every 4 hours starting 12 hours after inoculation until the pain score was judged to be mild. Synovial content changes by day are summarized in Figures 1.1-1.4, with a significant increase in mean values of white blood cell count, neutrophil count, neutrophil percentage, and total protein on day 2 compared to day 1, and a decline in these values through the duration of the experiment. Experimental calves and control calves were treated with through and through joint lavage with 1 liter of lactated ringer's solution. Experimental calves also received intravenous antibiotic (ceftiofur, at 1.1 mg/kg every 12 hours for 19 days). Synovial fluid collected prior to daily joint lavage was compared between the 2 treatment groups. The experimental calves developed lameness, joint pain and swelling associated with the intra-articular injection of bacteria that were clinically apparent for 9 days. This experimental septic arthritis model experienced good recovery with early and aggressive treatment.

Chemically induced synovitis-arthritis using Amphotericin B has been used extensively in equine lameness research, resulting in lameness, swelling, joint pain and heat, pain, osteoarthritis, and edema (Bowman *et al.*, 1983; Crawford *et al.*, 1989; Fahmy *et al.*, 1994; Hegazy *et al.*, 1994; Marttinen *et al.*, 2006). Intra-articular doses ranging from 10-20 mg have been effective in inducing arthritis in these studies. From these studies, Amphotericin B has been found to induce acute arthritis by inciting secretion of inflammatory mediators such as interleukin-1 and degradative enzymes such as lysosomal enzymatic activity and collagenase matrix metalloproteinases, which have been found to remain elevated in concentration in the joint for up to 5 weeks. Intra-articular Amphotericin B has also been found to increase acid phosphatase activity in joints, and increased neutrophils and mononuclear lymphocytes. The degenerative joint disease that resulted from intra-articular injections has been found to produce an acute inflammatory phase followed by a degenerative phase (Fahmy *et al.*, 1994) resulting in radiographic evidence of osteoarthritis in one study in horses that did not receive treatment (Bowman *et al.*, 1983).

Analgesic Drugs for Lameness

Veterinarian's attitudes toward routine analgesic drug use in large animal species remains divided. In a random survey of Canadian veterinarians, Hewson *et al.* (2007) found that 90% of veterinarians surveyed used analgesic drugs for equine surgeries, caesarean sections in bovine and swine, and claw amputations and omentopexies in cattle. However, the analgesics used were considered by the authors to be inadequate and analgesics often were not given to young animals. A majority of respondents cited a lack of available long-acting, cost-effective analgesics and the long or unknown withdrawal periods of other drugs outweighing the benefit of using them.

Figure 1.5 details the distribution of veterinarians in this study administering analgesic drugs to cattle with acute or chronic lameness. The drugs most commonly used by the surveyed veterinarians in these lameness cases were ketoprofen and aspirin.

Few published studies concerning analgesic options for lameness exist. One such study was conducted by Flower *et al.* (2008), in which he conducted 3 separate studies on lactating Holstein cows (n=20, n=21, n=27) with lameness. These cows were given intramuscular (Study 1 and Study 2) or intravenous (Study 3) ketoprofen at 0, 0.3, 1.5, or 3.0 mg/kg once daily. The gaits of the cattle were subjectively scored using a numerical system ranging from 1 to 5 with specific gait attributes that included back arch, tracking up, joint flexion, asymmetric steps, head bob, and reluctance to bear weight. Cattle were evaluated before treatment, during treatment, and after treatment, each of which lasted 3 days. They found a slight effect of the highest dose of ketoprofen in Study 1, with increases in symmetry and evenness of weight distribution. The other 2 studies resulted in no significant effects of ketoprofen.

Currently, no drugs are specifically approved for analgesia in cattle by the U.S. Food and Drug Administration (Smith *et al.*, 2008). The only approved non-steroidal anti-inflammatory drug labeled for its anti-inflammatory properties in cattle is flunixin meglumine.

Despite the void of published data or approved drugs for analgesic therapy for lameness in cattle, several empirical and multi-modal treatment options are available to veterinarians for relief of pain associated in cattle. Anderson and Muir (2005) published a review of pain management in cattle. In this review, they gave several options for analgesia in cattle. The most commonly used administration options for analgesia in food animals are systemic administration of nonsteroidal anti-inflammatory drugs, local anesthesia, epidural anesthesia, transdermal analgesia, and continuous infusions. Local anesthesia, mostly commonly injectable lidocaine, is

used to prevent sensation during incisions during surgery. Epidurals are also most commonly used in surgical situations for analgesia using lidocaine, opioids, and certain alpha-2 agonist drugs for standing surgeries. Continuous rate infusion analgesia using alpha-2 agonists, opioids, or lidocaine has most recently become available and is most ideal for a hospital setting. The most promising avenue cited in the review for practical application of analgesia in cattle is systemic administration of nonsteroidal anti-inflammatory drugs (NSAIDs). The mechanism of action of NSAIDs is based on their inhibition of cyclo-oxygenase enzyme (COX). Two isoforms of COX exist: COX-1 and COX-2 (Papich, 2002). COX-1 is thought to be responsible for maintaining healthy mucosa, renal function, and platelet function, among others. COX-2 is responsible mainly for mediation of pain and inflammation. However, there is some evidence of a cross-over of effects in some instances. COX inhibition in turn prevents production of arachidonic acid and its product, prostaglandins and other mediators of inflammation. Examples of nonselective COX inhibitors included in the review include flunixin, ketoprofen, and phenylbutazone. COX-2 preferential, but not specific, inhibitors listed in the review, which are thought to be better candidates for prolonged use due to decreased risk of nephrotoxicity or abomasal ulcers, include etodolac and carprofen. Dosages from the review for the most commonly used NSAIDs in ruminants are included in Table 1.3. Meloxicam is another COX-2 preferential drug that has just recently become available in the US (Smith et al., 2008). Phenylbutazone is currently prohibited from extralabel drug use in female dairy cattle 20 months of age or older, with no approved use in food-producing animal (CVM Update, 2003).

The most promising aspect of current pain research has shown that pre-emptive analgesia using NSAIDs is beneficial, with minimal effect of analgesia when given after the painful procedure. Ting *et al.* (2003) compared the effects of burdizzo castration alone or in combination

with systemically administered NSAID (ketoprofen), local anesthesia using lidocaine, or a combined xylazine-lidocaine caudal epidural on cortisol, acute phase proteins, average daily gain (ADG) and behavior. They found that ketoprofen was more effective in decreasing the cortisol response and decreasing loss in ADG. Ketoprofen or epidural was found to decrease pain-related behavior in the first 6 hours after treatment.

From this review of the literature, it is easily seen that there is a dire need for further research for effective, systemically administered analgesics for cattle.

Figures and Tables

Figure 1.1 Changes in synovial fluid white blood cell count for each calf during the experiment. A star indicates a result that is statistically different from day 1.

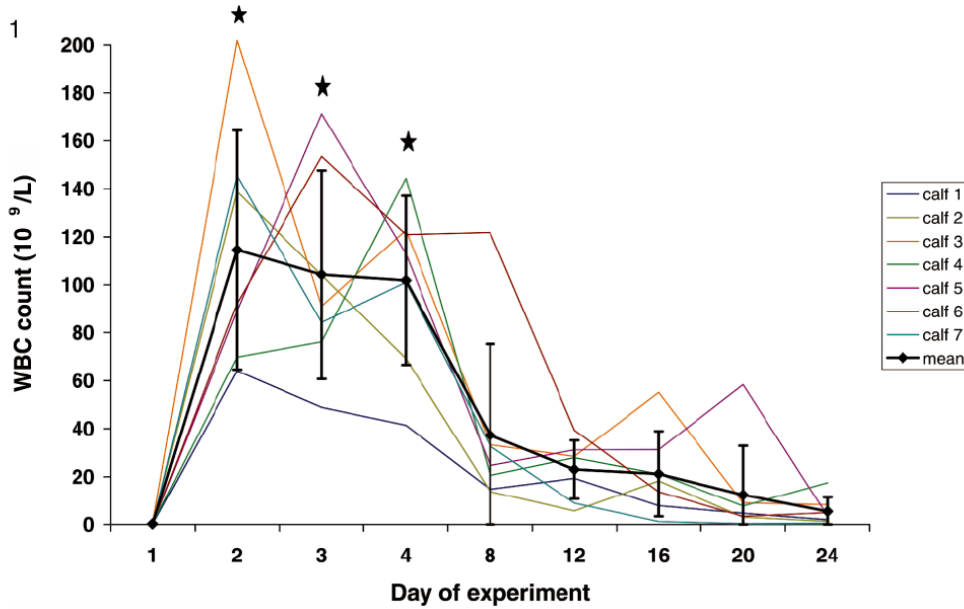


Figure from Francoz *et al.*, 2005

Figure 1.2 Change in synovial fluid neutrophil count for each calf during the experiment. A star indicates a result that is statistically different from day 1.

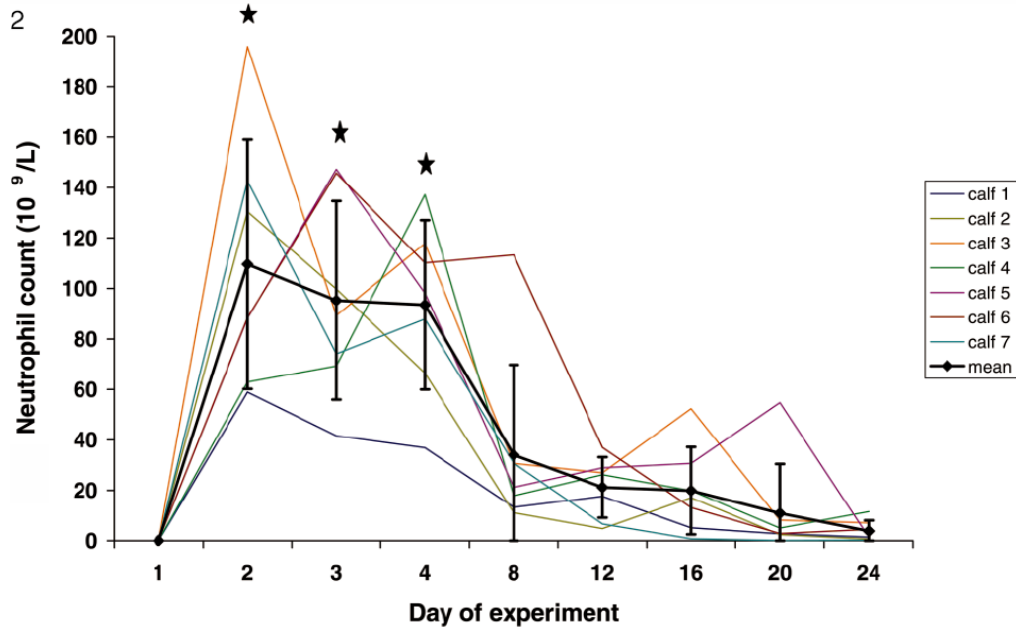


Figure from Francoz *et al.*, 2005

Figure 1.3 Changes in synovial fluid neutrophil percentage for each calf during the experiment. A star indicates a result that is statistically different from day 1.

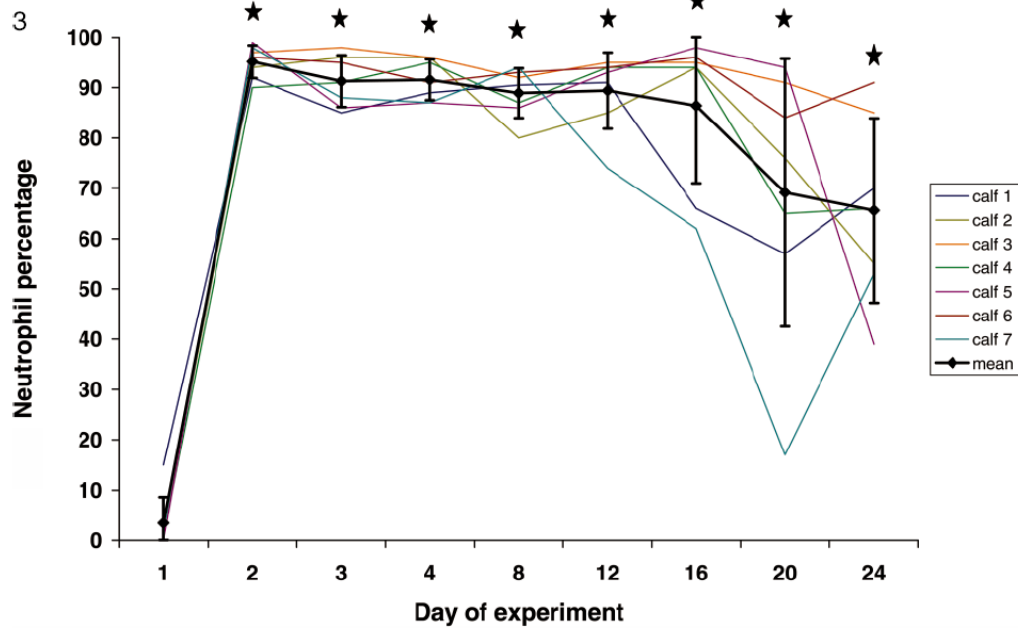


Figure from Francoz *et al.*, 2005

Figure 1.4 Changes in synovial fluid total protein concentration for each calf during the experiment. A star indicates a result that is statistically different from day 1.

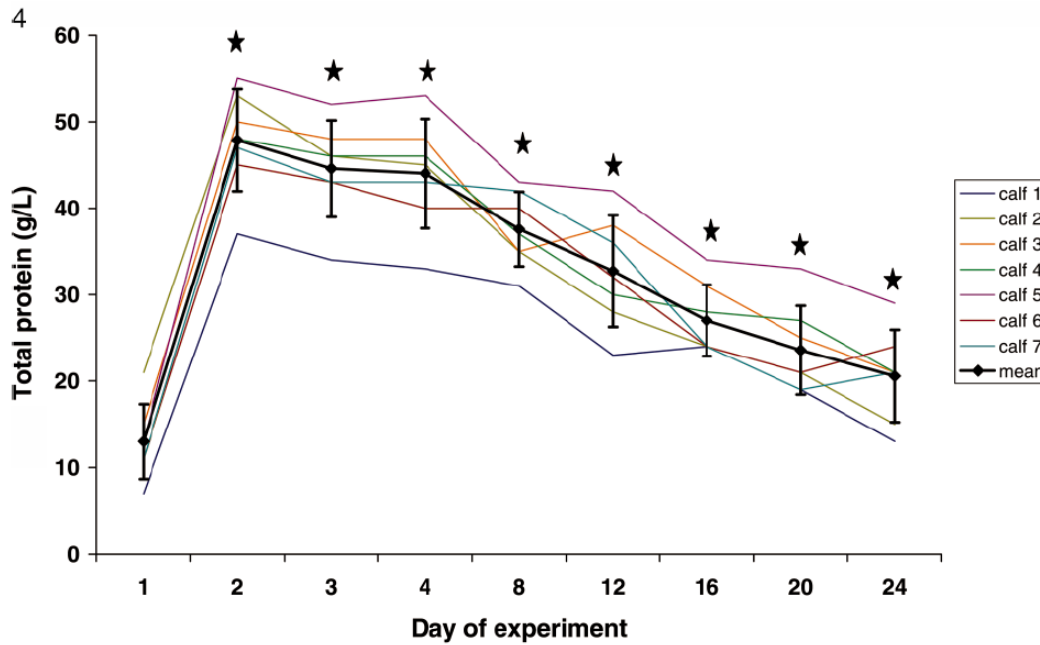


Figure from Francoz *et al.*, 2005

Figure 1.5 Distribution of Canadian veterinarians according to the percentage of dairy cattle with acute or chronic lameness that received analgesic drugs.

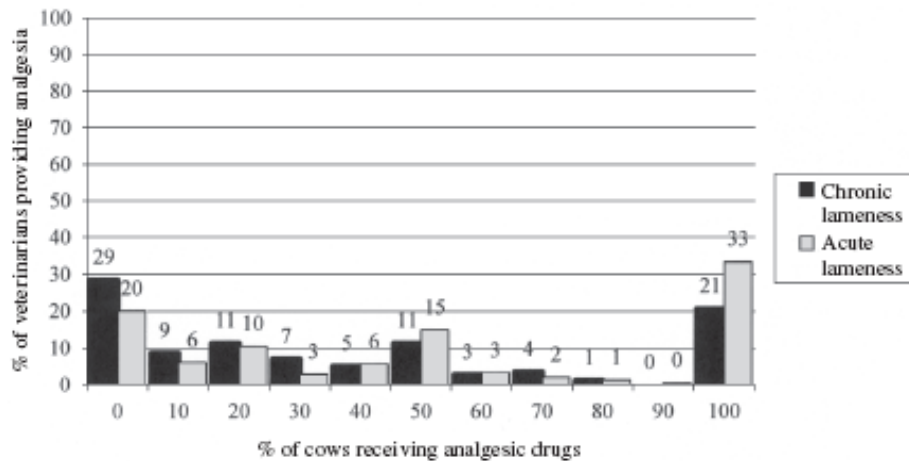


Figure from Hewson *et al.*, 2007

Table 1.1 Percentage of live cattle in 1999 National Market Cow and Bull Beef Quality Audit that were not lame compared to the percentage in 1994

Cattle type	1994 % not lame	1999 % not lame
Beef cows	88.6	73.4
Dairy cows	76.5	60.8
Beef bulls	72.8	63.7
Dairy bulls	76.3	70.9

Table adapted from Roeber *et al.*, 2001.

Table 1.2 Lameness, pain, and joint swelling grade scale used for clinical assessment of calves

Grade	Lameness	Pain	Joint Swelling
0	Normal	Normal	Normal
1	Mild lameness	Head movement during leg manipulation	Mild swelling compared to the normal joint
2	Easily detectable lameness making ambulation difficult	Withdrawal of the leg during leg manipulation	Easily detectable swelling
3	Moderate lameness making ambulation difficult	Head movement during joint palpation	Easily detectable swelling and joint capsule under tension
4	Severe lameness with reluctance to bear weight on the affected limb	Withdrawal of the leg during joint palpation	Grade 3 including edema

Table adapted from Francoz *et al.*, 2005.

Table 1.3 Nonsteroidal anti-inflammatory drug dosages commonly used in ruminants

Drug	Dose	Route	Frequency
Salysilic acid (aspirin)	100 mg/kg	PO	q 12 h
Flunixin meglumine	1 mg/kg	IV	q 12 h
Ketoprofen	2 mg/kg	IV	q 12 h
Phenylbutazone	5 mg/kg	PO	q 24 h
	10 mg/kg	PO	q 48 h
Abbreviations: IV, intravenous; PO, per OS (by mouth); q, every.			

Table adapted from Anderson and Muir, 2005.

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CHAPTER 2 - Analgesic Efficacy of Sodium Salicylate in an Amphotericin B Induced Bovine Synovitis-Arthritis Model

Introduction

Over the past decade, the issue of food animal welfare has come to the forefront of veterinary medicine (AVMA Animal Welfare Forum, 2001). Much of this interest has centered on pain management both in relation to routine production practices and pain involving diseases encountered in production environments.

Lameness is one of the most common reasons for premature culling in dairy cows and is also an important cause of loss in beef cattle (Berry, 2001). Cook (2003) found the prevalence of lameness in dairy cows to be as high as 33.7%. The 1999 National Market Cow and Bull Beef Quality Audit was conducted to examine the quality of market cows and bulls and to compare to the data collected from the 1994 National Non-Fed Beef Quality Audit. Roeber et al. (2001) found that 31.4% of all cattle audited were lame and that losses due to lameness were significantly greater than reported in the 1994 audit. Of all cattle, 7.4% of carcasses had one arthritic joint that required removal at slaughter, and 4.0% of carcasses had two arthritic joints that were removed.

In order to evaluate pain and then evaluate interventions, an objective method of measurement is required. Pain assessment is difficult in stoic species such as cattle. Most research has focused on behavioral or physiological changes associated with acute pain (Anil et al., 2002; Ting et al., 2003; Stilwell et al., 2008), but these changes can be complex, with natural variation between animals complicating the differentiation of pain from other factors such as stress (Anderson and Muir, 2005).

In addition to variations in physiologic responses, variations in disease severity also complicate the study of pain. Induction of lameness allows for controlled evaluation of pain in animals because pre and post-lameness measurements can be taken from the same animal, thereby reducing the confounding effects of individual differences. Well-controlled bovine lameness induction models are scarce in the published literature. Models based on intra-articular inoculation of bacteria are difficult to standardize and control and often cause intense and prolonged pain (Francoz et al., 2005). Amphotericin B, a polyene antibiotic, produces a controlled, mild to moderately severe synovitis/arthritis of short-duration. (Fahmy et ., 1994). Thus, an amphotericin B-induced arthritis model is ideal for evaluating analgesic compounds such as sodium salicylate.

Currently, there are no drugs approved by the U.S. Food and Drug Administration to specifically provide analgesia in cattle (Smith et al., 2008). This absence of approved compounds leaves veterinarians to make extrapolations about the proper clinical use of drugs applied for these purposes from data developed in other species. Presently, flunixin meglumine is the only approved non-steroidal anti-inflammatory drug (NSAID) labeled for use in cattle and its use is limited to the treatment of fever and inflammation. This drug is undesirable in many lameness cases because prolonged use is associated with abomasal ulcers and nephrotoxicity (Anderson and Muir, 2005). Therefore, it is important to identify other analgesic compounds with fewer undesirable side-effects for providing relief of chronic pain in cattle. Sodium salicylate is a commonly used anti-inflammatory drug in multiple species (USP Veterinary Pharmaceutical Information Monographs, 2004), but the efficacy of sodium salicylate for analgesia related to arthritis in cattle has not been determined. The FDA Center for Veterinary Medicine guidance for the development of effectiveness data for NSAIDS indicates that

validated methods of pain assessment must be used for a drug to be indicated for pain relief in the target species.

Several tools have been described to assess pain associated with lameness in cattle. No single lameness grading system has been universally accepted for lameness scoring in cattle. One of the most common lameness scoring systems is based on visual gait analysis based on behavior and posture (Sprecher et al., 1997). This system is most useful in categorizing general lameness for serial monitoring, setting intervention points for herd level decisions, and identification of individuals for immediate treatment. Although visual scoring systems do allow for non-invasive categorization of lameness that can be correlated with the other diagnostic tools they are limited by inter- and intra-observer variability. Wells et al. (1993) found a 91.3% inter-observer agreement between 2 investigators using a standardized visual lameness scoring system. However, objective assessment tools in support of drug approvals are needed and must be validated for use in cattle.

Multiple assessment tools for pain or loss of function have been proposed. Pressure mat technology provides objective data through determination of weight distribution and temporality. This allows for non-invasive analysis of lameness that can be correlated with the other diagnostic tools. Electrodermal activity is a non-invasive technique that is advocated as an objective measurement of pain. The proposed mechanism is that afferent neurons from the sympathetic axis of the autonomic nervous system innervate eccrine sweat glands, resulting in modulations of skin electrical conductance known as electrodermal activity (EDA) (Anderson and Muir, 2005). The determination of serum cortisol concentration is a physiologic measurement to allow for minimally invasive measurement of stress in animals (Coetzee et al., 2007). Heart rate monitors

use a non-invasive method to evaluate changes in symphathovagal tone related to stress due to emotional, pathological, or physiological causes (von Borell et al., 2007).

We hypothesized that intra-articular amphotericin B produces a controlled, transient synovitis/arthritis in cattle and that this model could describe the analgesic effects of intravenous sodium salicylate. Amphotericin B-induced lameness in cattle was quantified using subjective visual gait assessments and objective pressure mat, EDA, cortisol, and heart rate measurements.

Materials and Methods

Study Design and Procedures

All experimental procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) under the supervision of the University Veterinarian (Protocol #2726).

Ten Holstein bull calves aged 2 to 4 months were acquired from a Kansas livestock producer in June 2008. Weights obtained approximately 20 days prior to commencing the study in September ranged from 231 to 286 Kg. On arrival, the calves received a single subcutaneous (SC) dose of an 8-way clostridial vaccine (Covexin 8, Schering Plough, Summit, NJ), a single intramuscular dose of a 4-way modified live viral respiratory disease vaccine (Bovi-shield Gold 4, Pfizer, New York, NY), and a single SC injection of tulathromycin at 2.5 mg/kg bodyweight (Draxxin, Pfizer, New York, NY). A topical pour-on (Ultra Boss Pour-on, Schering-Plough Animal Health, Summit, NJ) was applied on arrival and repeated approximately every 7-10 days for fly control. Approximately 2 weeks after arrival, the calves were dehorned and castrated. Approximately 1 month after initial processing, the cattle were given a single dose SC implant at the base of the ear, containing trenbolone acetate and estradiol benzoate, for steers intended for slaughter (Synovex Choice, Fort Dodge Animal Health, Fort Dodge, IA), a single dose of topical

doramectin at 500 µg/kg bodyweight (Dectomax Pour-on, Pfizer, New York, NY), and a single SC injection at the base of the ear of ceftiofur crystalline free acid at 6.6 mg ceftiofur equivalents/kg body weight (Excede, Pfizer Animal Health, New York, NY).

Study calves were initially housed in a dry lot confinement facility at KSU Animal Resource Facility (ARF) for approximately 90 days after arrival. Thereafter, calves were acclimated to individual 13.40 indoor stalls in an ARF barn for 2 days prior to study commencement. Each stall was separated by a gate. During the adaptation period, each animal was restrained for at least 10-15 minutes/day using a squeeze chute with head gate, tied with a rope halter to the fence within their pen for at least 10 minutes/day and were trained to walk through an alleyway to allow for restricted access across a pressure mat at least twice daily. For the entire housing period, calves had free access to water and brome hay along with a daily feeding of 3.6 kg/head of a balanced beef feedlot receiving diet composed of cracked corn, whole oats, whole milo, dry distillers grain, and protein/vitamin/mineral supplement.

Approximately 24 hours prior to study commencement, all calves were individually restrained in a squeeze chute using a rope halter and the attached head gate. Following restraint, the area over the right jugular vein in phase 1 and the left jugular vein in phase 2 was clipped and disinfected using povidone iodine and 70% isopropyl alcohol swabs. The catheter site was infiltrated with approximately 0.5 ml of 2% lidocaine injection (Hospira Inc, Lake Forest, IL) and a small skin incision with a # 22 blade was made to facilitate placement of a 14 G x 140 mm catheter (Abbocath-T, Abbott Ireland, Sligo, Rep. of Ireland) which was sutured to the skin using 2-0 nylon suture (Burns Veterinary Supply, Inc. Westbury, NY). Catheter patency was maintained using 3 ml heparin saline flush containing 3 USP units heparin sodium/ml 0.9 % saline (Heparin Sodium Injection, USP, Baxter Healthcare, Deerfield, IL; Baxter Healthcare

Corporation, Deerfield, IL). The catheters were removed immediately after the last blood collection time point in each phase.

The study design was a partial cross-over design with 2 phases. Each phase consisted of 3 periods (Figure 1). Study animals were blocked in pairs according to their weights determined approximately 20 days prior to study commencement. The heaviest 8 of 10 calves were selected for inclusion in Phase 1 of the study, with the remaining 2 serving as replacements if needed. Calves were ranked by ascending weight in kilograms and assigned a computer-generated random number (Microsoft Excel 2007, Microsoft Corporation). Pairs were then assigned based on relative similar weights. In each pair, calves with the highest random number were assigned to the salicylate group for Phase 1, while the remaining calves were designated as placebo controls (n=4 calves/group). These treatment assignments were then reversed for Phase 2. Following randomization, the mean pre-study weights of calves enrolled in the phase 1 treatment and control groups were 262 ± 9.8 kg and 263 ± 18.1 kg respectively. The mean pre-study weights of calves included in the phase 2 treatment and control groups were 260 ± 22.8 kg and 257 ± 22.2 kg respectively.

The study commenced at 6:45 A.M. with mock lameness induction (period 1) and lameness induction (period 2) occurring with 4 minute intervals between calves. Mock lameness induction was performed by applying a needle prick to the coronary band at the site of amphotericin B injection. The purpose of period 1 was to collect non-arthritis baseline data after needle prick alone to compare to the data collected after induction of distal interphalangeal joint arthritis during lameness. The mock lameness induction and lameness induction were completed by 7:15 A.M; all procedures were performed by a single veterinarian (DEA) to avoid inter-operator variation. Period 3 began 24 hours after the induction of lameness for evaluation of

post-emptive analgesia. During this period, there were no further lameness-inducing procedures; the only manipulations were the administration of drug (or placebo) and the collection of data.

Prior to sham lameness induction and lameness induction, all calves were restrained in a chute with head gate as conducted through the acclimatization period, and the appropriate hind leg (phase 1: right leg, phase2: left leg) was restrained with ropes at the fetlock and stifle. After restraint, the appropriate hindlimb lateral digit pastern region was prepared with close clipping of hair (No. 40 clipper blade) and aseptic skin preparation using povidone iodine scrub and 70% isopropyl alcohol swabs. Mock induction of lameness occurred in period 1 by introducing a sterile 18 G 1 ½” needle one centimeter proximal to the coronary band and one centimeter abaxial to the tendon of the long digital extensor muscle and angling distally toward the sole. No solutions were injected into the joint, and the needle was immediately removed. Twenty four hours of washout was allowed between periods 1 and 2. In period 2, the above procedure was repeated, with the exception of injecting Amphotericin B (X-Gen Pharmaceuticals, Inc, Big Flats, NY). After the sterile needle was inserted, correct placement into the distal interphalangeal joint was verified by aspiration of synovial fluid back into the syringe. Continued position within the distal interphalangeal joint was verified periodically throughout the injection by ease of injection followed by back-flow of synovial fluid and Amphotericin B into the syringe. This admixture was then fully injected into the distal interphalangeal joint to complete the procedure. In phase 1, the dose was 20 mg, utilizing 2 ml of a 10 mg/ml solution. In phase 2, the dose was decreased to 15 mg, utilizing 3 ml of a 5 mg/ml solution. Concentration of amphotericin B and total dose of drug was reduced in phase 2 in an attempt to lessen the duration of lameness experienced by the calves.

Escape analgesic therapy options for unresolved lameness after final data collection for each phase included flunixin meglumine at 2.2 mg/kg intravenously (IV) once daily, butorphanol tartrate at 0.05 mg/kg SC once daily, morphine at 0.1 mg/kg SC once daily, and lidocaine 2%, with a total dose of 100 mg intra-articular once.

Within each phase of the study, placebo control-arthritis group calves received 50 ml of sterile water (Baxter Healthcare Corporation, Deerfield, IL) IV 4 minutes after lameness induction through the pre-placed jugular catheter in each period, whereas the salicylate treated calves received 50 mg/kg commercially available laboratory grade sodium salicylate (Sigma-Aldrich, St. Louis, MO) dissolved in sterile water (Baxter Healthcare Corporation, Deerfield, IL) for a total volume of 50 ml IV through the jugular catheter 4 minutes after lameness induction in each period. Doses were based on weights obtained 24 hours prior to administration. Immediately after placebo or drug administration, the catheter was flushed using 3 ml of heparin saline flush solution as described above.

Twenty milliliters of whole blood for salicylate concentration in treated calves and cortisol concentration in all calves was collected into syringes using the pre-placed jugular catheter immediately prior to drug or placebo administration, and again at 5, 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours thereafter. Immediately after obtaining the blood sample, 3 ml of heparin saline flush, as described above, was used to maintain patency of the catheter. Blood was immediately transferred to a 7 ml EDTA tube (BD Diagnostics, Franklin Lakes, NJ) containing 350 µl aprotinin (1000 KIU/ml), a serine protease inhibitor, (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) and 7 ml vacutainer tube (BD Diagnostics, Franklin Lakes, NJ) containing no additive. The vacutainer tubes were stored on ice for no more than 60 minutes pending sample processing. Thereafter, blood samples were centrifuged at

1,600 g for 15 minutes at 0°C. Serum and plasma were pipetted from their respective tubes and placed in cryovials identified with calf ID, date, timepoint sample, and treatment group. The samples were stored at -40°C prior to sample analysis. All samples were analyzed within 60 days of sample collection.

Sample Analysis

Serum cortisol concentrations were determined as previously described and validated in bovine plasma (Coetzee et al., 2007) using a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system (Immulite 1000 Cortisol, Siemens Medical Solutions Diagnostics, Los Angeles, CA). A sample volume of 100 µL was used in each assay well. The reported calibration range for the assay is 28 to 1,380 nmol/L with an analytical sensitivity of 5.5 nmol/L.

Plasma salicylate concentrations were determined using a fluorescence polarization immunoassay kit (TDx/TDxFLx, Abbott Laboratories, Abbott Park, IL) validated in our laboratory (Coetzee et al., 2007). The concentration of salicylate in unknown samples was calculated from a calibrations curve using a best fit-curve equation determined using six calibrations points with a detection concentration range between 5 and 800 µg/ml. The limit of quantitation (LOQ) of the assay was 5 µg/ml. The precision of the assay was less than 8% coefficient of variation. The accuracy by recovery at concentrations of 50, 100, 200, 400, and 800 µg/ml was between 98.3% and 102.5%.

A commercially available floor mat-based pressure/force measurement system (MatScan, Tekscan, Inc., South Boston, MA) was used to record and analyze the affected feet of each calf. The pressure mat was calibrated daily and each time the computer software was engaged using a known mass to ensure accuracy of the measurements at each time point. Videosynchronization

was used to ensure consistent gait between and within calves for each time point. Using research grade software (HUGEMAT Research 5.83, Tekscan, Inc., South Boston, MA), contact pressure, contact area, and stance phase duration in the affected feet were measured. Surface area was calculated by area only of the loaded or “contact” sensing elements inside the measurement box. Contact pressure was calculated as force on the loaded sensing elements inside the measurement box divided by the contact area.

Heart rate data were recorded and analyzed using a commercially available heart rate monitor and software (RS800 and Polar Pro Trainer Equine Edition, Polar Electro, Inc, Lake Success, NY). The heart rate product consisted of a transmitter placed over the heart in the left foreflank attached to a girth strap placed around the heart girth of the calves, and a wrist unit attached to the elastic strap which received and recorded the signal from the transmitter. Appropriate conductance for the electrodes on the strap, one positioned on the sternum and one over the right scapula, was facilitated by use of ultrasound gel. The transmitter measured the electric signal (ECG) of the heart every 15 seconds. Prior to study commencement, the heart rate wrist unit time was synchronized with the stopwatches used for all other sample collection. The corresponding heart rate within 15 seconds of each time point was used for analysis.

A commercially available EDA/GSR measurement device (Pain Gauge, PHIS, Inc, Dublin, OH) was used to measure the electrodermal activity on the nasal planum of each of the calves. It converted the level of resistance measured between two electrodes to a 10-point scale with detection at the 0.1 level, with 0 representing “Calm/No Pain” and 9.9 representing “Tense/Severe Pain”.

The degree of lameness was scored using a 1 to 5 scale adapted from Sprecher (Sprecher et al., 1997) (Table 1). Lameness scores were determined twice daily to document presence of

lameness and to visually score severity of lameness. To eliminate inter-observer variation, all lameness scores were assigned by one blinded veterinarian (DEA) with training and expertise in bovine lameness assessment. Intra-observer variability was assessed periodically by randomly selecting calves for repeated assessment to ensure consistency of scoring. All lameness examinations were performed on even, non-sloped concrete floors free of obstructions and debris. Each lameness score was determined by watching the calf walk a minimum of 20 meters in a straight line, turn, and walk 20 meters back to the starting point.

Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis of the salicylate time concentration data was performed using a commercially available software program (WinNonlin, Pharsight Corporation, Cary, NC). A one compartment model with first order elimination was found to fit the data best, based on visual inspection of predicted versus observed data plots and two measures of goodness of fit (Aikake Information Criterion and Schwarz Bayesian Criterion). The data were therefore fit to exponential equation 1 by nonlinear regression analysis.

$$C(t) = \frac{Dose}{V_{area}} \times e^{-k_{10} \times time} \quad (1)$$

Where: V_{area} is the apparent volume of distribution and k_{10} is the elimination rate constant determined by the slope of the terminal elimination phase with the time-concentration data plotted on a semi-logarithmic scale.

The following secondary parameters were also calculated: area under the time-concentration curve (AUC), elimination half-life ($T_{1/2\text{el}}$), concentration at time zero (C_0) and total body clearance (CL), mean residence time (MRT), and volume of distribution at steady state (V_{dss}) (Equations 2-7).

$$AUC = \frac{Dose}{V_{d(area)} \times k_{10}} \quad (2)$$

$$T_{1/2} = \frac{0.693}{k_{10}} \quad (3)$$

$$C_0 = \frac{\text{Dose}}{V_d} \quad (4)$$

$$CL_B = k_{10} \times V_d \quad (5)$$

$$MRT = \frac{AUMC}{AUC} \quad (6)$$

$$V_{ss} = \frac{\text{Dose} \times AUMC}{AUC^2} \quad (7)$$

Statistical Analysis

Hypothesis tests were conducted using JMP 5.1.2 analytical software (SAS Institute, INC, Cary, NC, USA) (Sall et al., 2004). The mean \pm standard error of the means (SEM) were calculated for each outcome variable at each time point. Repeated measures data were analyzed using a univariate split-plot approach. A random effects-mixed model was constructed with treatment, time, time*treatment interaction, period and period*treatment interaction designated as fixed effects. In this model, animal nested in treatment was designated as a random effect to account for the between subject effects. Statistical significance was designated *a priori* as $p < 0.05$. Areas under the time-effect curves (AUEC) were determined as previously described (Leon-Reyes et al., 2008) with the commercially available software program WinNonlin (Pharsight Corporation, Cary, NC) using the linear trapezoidal rule. Analysis of variance (ANOVA), using Tukey's test, was employed to evaluate differences, with statistical significance considered at $p < 0.05$.

Results

Effect of period*treatment group on the parameters is reported in Table 2 for the repeated measures analysis and area under the time-effect curves analysis.

Cortisol mean concentrations were greater in Period 2 as compared with Period 1 or Period 3 both as evaluated by overall period mean ($p < 0.0001$, AUEC $p < 0.0001$) and as evaluated for each treatment between periods (treatment group*period interaction, $p = 0.017$), indicating an acute response to the arthritis induction. No difference was found between treatment ($p = 0.695$, AUEC $p = 0.912$). Differences were seen in time ($p < 0.0001$) and the interaction time*treatment group ($p = 0.018$).

Following intravenous administration, sodium salicylate was not widely distributed and plasma concentrations declined rapidly below the LOQ of the assay ($5 \mu\text{g/ml}$) by 240 minutes (4 hours) after administration. Because a one-compartment model was used, all estimates of the apparent volume of distribution ($V_{d(\text{area})}$ and $V_{d(\text{ss})}$) were equal. The mean V_d was 0.2 ± 0.005 L/kg, Cl_B was 4.3 ± 0.2 mL/min*kg, and elimination half life ($T_{1/2\text{el}}$) was 36.9 ± 1.2 minutes. The average plasma time-concentration curve is illustrated in Figure 1. The other pharmacokinetic parameters for intravenous sodium salicylate are reported in Table 3.

Contact surface area of the affected foot was greater in Period 1 compared with that of Periods 2 and 3 ($p < 0.0001$), and Period 1 compared with that of Period 2 in AUEC ($p = 0.045$). There was also a difference by time ($p = 0.0008$), in which the surface area at 8 hours after lameness induction was less than at 0 minutes and 1.5 hours. No difference was seen in treatment group ($p = 0.430$, AUEC $p = 0.369$), time*treatment group ($p = 0.280$), or treatment group*period ($p = 0.228$).

There was a difference in time ($p < 0.0001$) and treatment group*period ($p = 0.016$), in which the stance phase duration of the affected foot in the salicylate treated calves in period 3 was increased compared to the placebo calves in period 3. There was no apparent effect of period ($p = 0.409$), treatment group ($p = 0.741$, AUEC $p = 0.716$), or time*treatment group ($p = 0.431$).

There was no difference in time ($p=0.136$), treatment group ($p=0.800$, AUEC $p=0.716$), time*treatment group ($p=0.428$), or period*treatment group ($p=0.524$). However, there was a difference in period ($p<0.0001$, AUEC $p<0.0001$), in which period 2 and 3 contact pressure was increased compared to period 1.

There was a difference ($p<0.0001$) between all periods, with mean heart rate in period 3 increased compared to that of periods 1 and 2., and with mean heart rate in period 2 increased compared to that of period 1 (AUEC $p=0.004$). There was also an effect of time ($p<0.0001$). No difference was seen in treatment group ($p=0.512$, AUEC $p=0.104$), time*treatment group ($p=0.348$), or treatment group*period ($p=0.258$).

There was no effect by period ($p=0.086$), time*treatment group ($p=0.431$), treatment group ($p=0.976$, AUEC $p=0.761$), or treatment group*period ($p=0.054$). There was a difference by period using the area under the time-effect curve (AUEC $p=0.016$), in which Period 1 was increased (an indication of increased pain) compared to periods 2 and 3.

When the variables were analyzed by lameness score (LS), some associations were apparent. Mean heart rate was less in lameness score 1 calves compared to that of LS 2, 3, and 4 calves (Figure 3). Mean serum cortisol concentrations were less in LS 1 calves compared to that of LS 3 calves ($p=0.004$) (Figure 4). Contact surface area by lameness score was different ($p=0.018$), with LS 1 calves having greater surface area compared to LS 3 and 4 calves (Figure 5). There was a difference in contact pressure ($p=0.02$), in which LS 3 calves exerted greater ground contact pressure compared with that of LS 1 calves (Figure 6). No difference was seen in stance phase duration by lameness score ($p=0.16$), treatment groups ($p=0.68$), or electrodermal reading ($p=0.29$). The number of calves within each lameness score for the entire study is

contained in Table 4. Three calves (two salicylate treated and one placebo control calf) had unresolved lameness between phases and subsequently were removed from the study.

Discussion

Improved knowledge of the mechanisms of pain, new veterinary technology and an increase in concern among consumers about the welfare of livestock has led to a push within the agricultural community to redefine animal welfare (Heleski et al., 2004). Economic loss in all sectors of the cattle industry result due to pain and consequent decreased feed intake, poor productivity and feed conversion, weight loss, reproductive inefficiency, premature removal from the herd, and death or euthanasia (Bicalho et al., 2008; Hernandez et al., 2005; Hernandez et al., 2002). The objective of this study was two-fold: 1) to determine if the model induced lameness in cattle, and 2) to determine if salicylate-treated calves were less affected by the model than controls. The results of this study, when comparing differences in variables, pre- and post-lameness, demonstrate that amphotericin B was an effective model to induce lameness. Intravenous sodium salicylate administered once daily at 50 mg/kg immediately after lameness induction had no effect on cortisol response, electrodermal activity, stance phase duration, contact pressure, contact surface area, or heart rate. To our knowledge, this is the first time this amphotericin B model has been reported in the bovine and the first time pharmacokinetic-pharmacodynamic data for sodium salicylate in an induced lameness model has been studied.

Amphotericin B has been extensively used to induce lameness in horses in studies evaluating lameness, pain, and the effectiveness of certain non-steroidal anti-inflammatory agents and other pain alleviating agents (Bowman et al., 1983; Crawford et al., 1991; Fahmy et al., 1994; Hegazy et al., 1994; Marttinen et al., 2006). In these studies, a single intra-articular injection of 10-20 mg amphotericin B was effective in inducing lameness. There are no

published studies of intra-articular injections of amphotericin B to induce lameness in cattle. Having a model to evaluate parameters prior to and after lameness induction is very important for evaluation of pharmacological agents and establishment of dosing regimens. Our results indicate that amphotericin B at 15 and 20 mg administered in a single intra-articular injection is an effective agent to induce lameness in cattle.

The area under the effect curve is an expression of the duration and intensity of an effect. AUEC has recently been used to describe the pharmacodynamics of diclofenac, a nonsteroidal anti-inflammatory drug, in rats (Leon-Reyes et al., 2008). In this study, researchers found a dose-dependent reduction of analgesic response of orally administered diclofenac by glibenclamide, a sulfonylurea anti-diabetic medication. In the present study, the AUEC analysis was similar to the repeated measures analysis. The only difference was in an effect of period on electrodermal activity.

Although salicylates are not specifically approved for analgesic use in cattle, the absence of labeled analgesic drugs in this species lends to its use as a pain mediation drug. Recently, it has been shown that sodium salicylate can be used to moderate pain associated with castration in calves (Coetzee et al., 2007). The castration model may be characterized as acute surgical pain followed by pain due to inflammation from the surgical site. The arthritis model reported here is based on induced inflammation, and pharmacokinetic/pharmacodynamic modeling of analgesic drugs is likely different from that associated with the pain of castration. The pharmacokinetics of salicylate (50mg/kg IV) were studied by Coetzee et al. (2007) when given immediately prior to castration of Angus crossbred bulls of similar age and weight to the Holstein calves in our study. The pharmacokinetic results from that study (mean V_d of 0.18 ± 0.01 L/kg, CL_B 3.36 ± 0.25 ml/min*kg, $t_{1/2el}$ of 0.63 ± 0.04 hours) were similar to ours. Additionally, their study showed

drug concentrations quickly decreasing below the LOQ at 4 hours. At this same timepoint, plasma cortisol concentrations in the salicylate-treated group were similar to the castrated control calves. This information, paired with the results of our present study, suggest that the lack of analgesia provided by sodium salicylate in our research most likely is due to the drug's very short half life.

The one-compartment model used here for the salicylate pharmacokinetics data produced the best fit despite a bi-exponential curve that was apparent due to only one sample being taken during the distribution phase. Because the distribution phase was so short, it is considered to be clinically insignificant.

There are no published articles concerning the use of electrodermal activity in cattle during lameness. Our research did not find a difference in the treatment groups using electrodermal activity. Studies have used electrodermal activity as a measurement of pain, anxiety, and stress levels in humans, to test new pharmaceutical preparations and to determine the reliability and validity of more subjective tools (Tuschen-Caffier and Vogele, 1999; Schellenberg et al., 2004; Hoferl et al., 2006). One of these studies found changes in electrodermal activity in individuals suffering from psycho-autonomic imbalances under an herbal treatment, but not under benzodiazepine oxazepam (Shellenberg et al., 2006). This is an assessment in need of further research.

The cortisol response seen in our research was not affected by administration with sodium salicylate. Serum cortisol measurements have been used extensively in pain research to ascertain the presence of pain (Anil et al., 2002; Ting et al., 2003; Coetzee et al., 2007; Stilwell et al., 2008). Although several studies have evaluated acute cortisol response as a physiological measure of the extent and duration of stress associated with castration in cattle, it is important to

recognize that cortisol is also involved in homeostasis and is therefore not a specific indicator of pain and distress (Anil et al., 2002). There are several castration studies published that evaluated cortisol response. In a study using 50 mg/kg intravenous sodium salicylate immediately prior to castration (Coetzee et al., 2007), a plasma salicylate concentration above 25 µg/ml was found to attenuate peak cortisol concentrations. Ting et al. (2003) found ketoprofen in association with castration decreased cortisol concentrations compared to local anesthesia or a caudal epidural. In another castration study by Stilwell et al. (2008), cattle were treated with an epidural injection of lidocaine alone or in addition to subcutaneous injection of carprofen or flunixin meglumine. They found that control calves had higher concentrations of cortisol at 6 hours post castration compared to the epidural plus carprofen or flunixin group. The epidural plus carprofen calves had lower cortisol compared to control at 24 hours post castration and lower at 48 hours post castration compared to epidural alone and epidural plus flunixin treated calves.

Unfortunately, there are no studies pertaining to lameness scores and intravenous sodium salicylate in dairy cattle. However, lameness in lactating dairy cows treated with varied doses of ketoprofen was studied by Flower et al. (2008) using a lameness score of 1 to 5 which took into consideration the arch of the back but was not the same lameness scoring system used in our research. In this study, they found that lameness scores improved with the highest dose of 3.0 mg/kg of ketoprofen. This study demonstrates the effectiveness of non-steroidal anti-inflammatory drugs in providing analgesia in lameness. Our research suggested an association between lameness scores and the variables of cortisol concentration, contact surface area, contact pressure, and heart rate. This association warrants further investigation.

Heart rate increase was not affected by salicylate administration in the present study. However, heart rates in horses with lameness induced by an adjustable heart bar shoe were

shown to be decreased with intravenous phenylbutazone (Foreman et al., 2008). This is another area in need of further scrutiny.

Canine and equine trials evaluating non-steroidal anti-inflammatory drugs with objective analysis of lameness utilizing force plate analysis and gait analysis have been published (Erlert et al., 2005; Ishihara et al., 2005; Moreau et al., 2007; Shoonover et al., 2005; Toutain et al., 2001; Vasseur et al., 1995). These studies have found non-steroidal anti-inflammatory drugs to improve force plate readings in treated subjects. These technologies have also been applied in field conditions in cattle (Kujala et al., 2008), but no research has been published using a bovine synovitis/arthritis model to objectively assess lameness. Pressure mat technology can be used to define pressure distribution, stride length, stance duration, and contact area during the stance phase of locomotion after corrective trimming, and during active locomotion. The results of this study demonstrate no overall difference between salicylate and placebo-treated animals in terms of pressure mat readings of contact surface area, stance phase duration, or contact pressure.

In conclusion, amphotericin B at 15 and 20 mg by intra-articular injection provides an effective model for lameness. Intravenous sodium salicylate at 50 mg/kg once daily is ineffective in providing analgesia in an induced lameness due to a very short half-life in cattle. Further studies at higher concentrations and increased frequency are needed to determine the efficacy of sodium salicylate in alleviating the pain associated with lameness.

Figures and Tables

Figure 2.1 Flowchart of study design

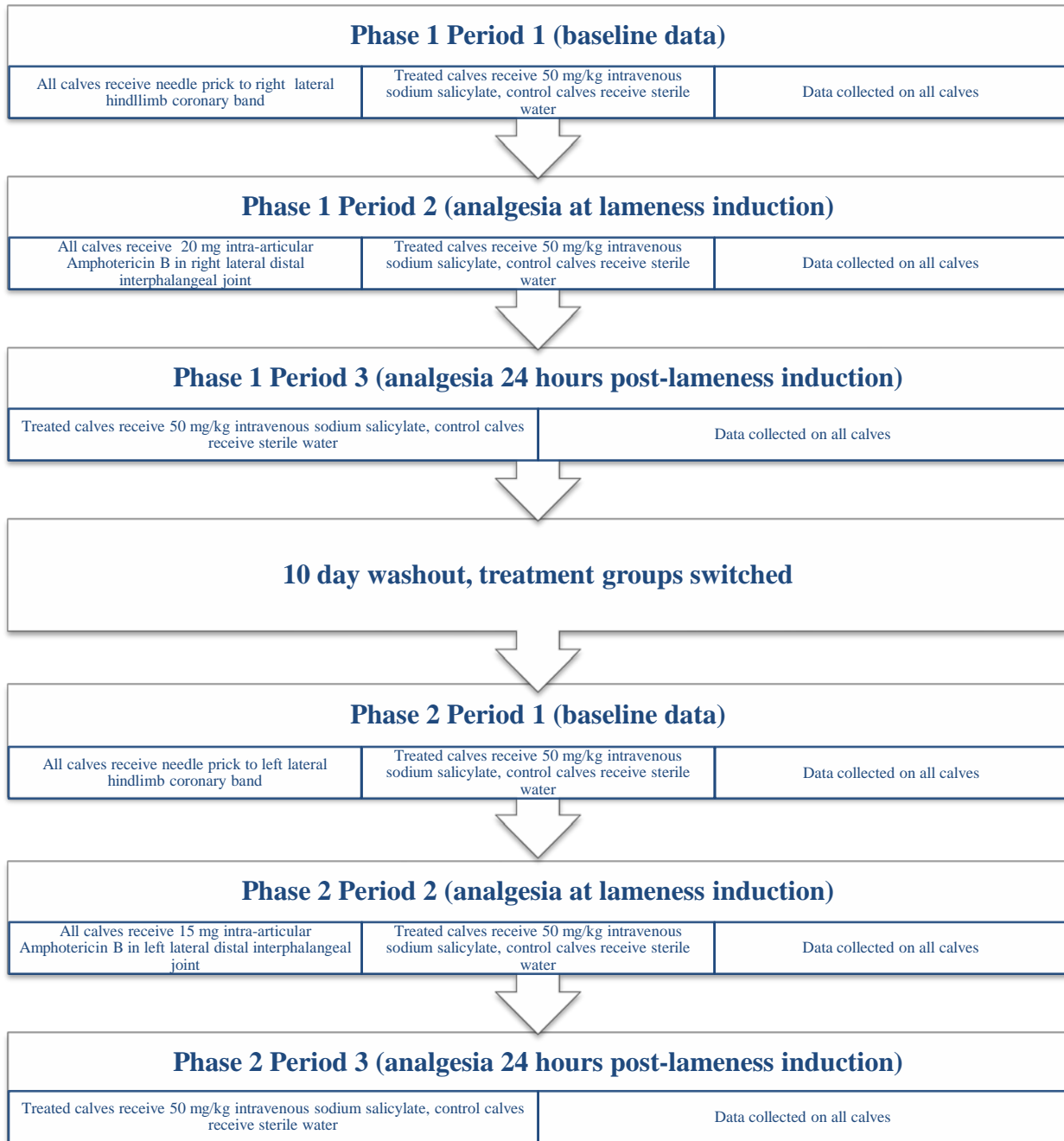


Figure 2.2 Mean heart rate \pm SEM by lameness score for all treatment groups. Data points with different symbols indicate $p < 0.05$.

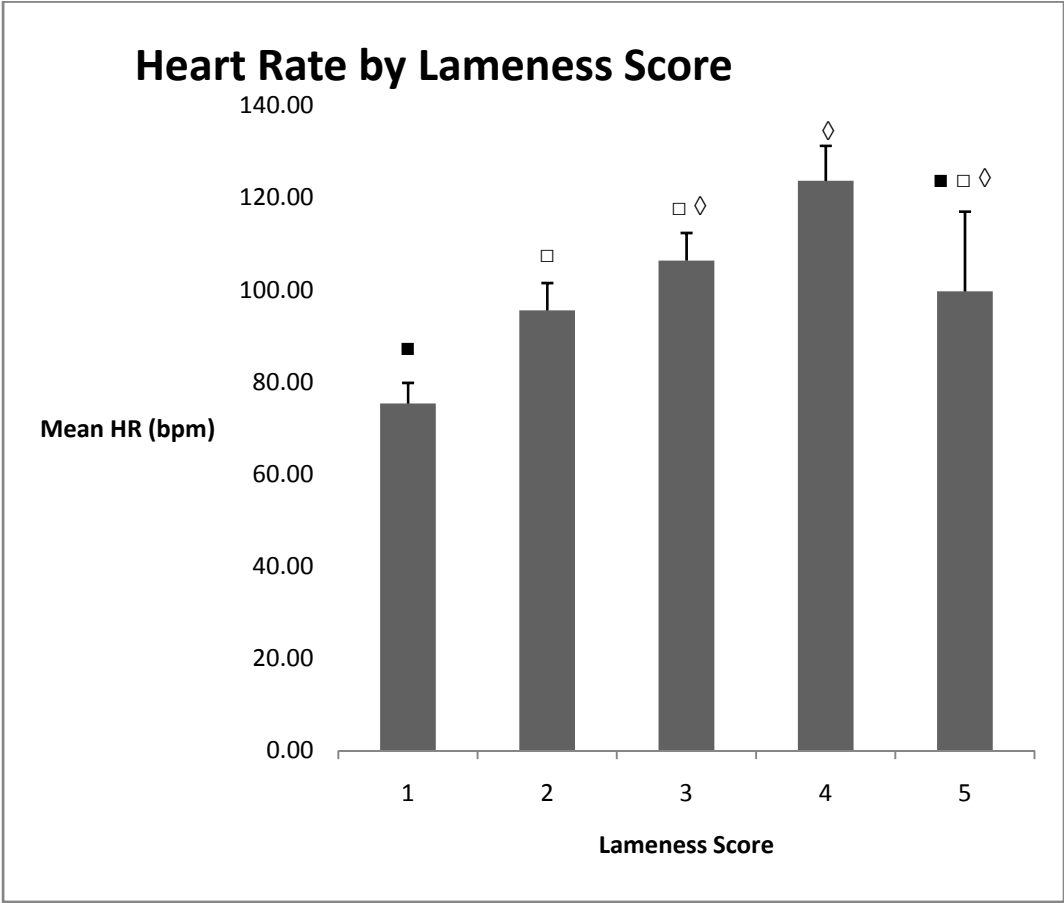


Figure 2.3 Mean cortisol concentration \pm SEM for all treatment groups. Data points with different symbols indicate $p < 0.05$.

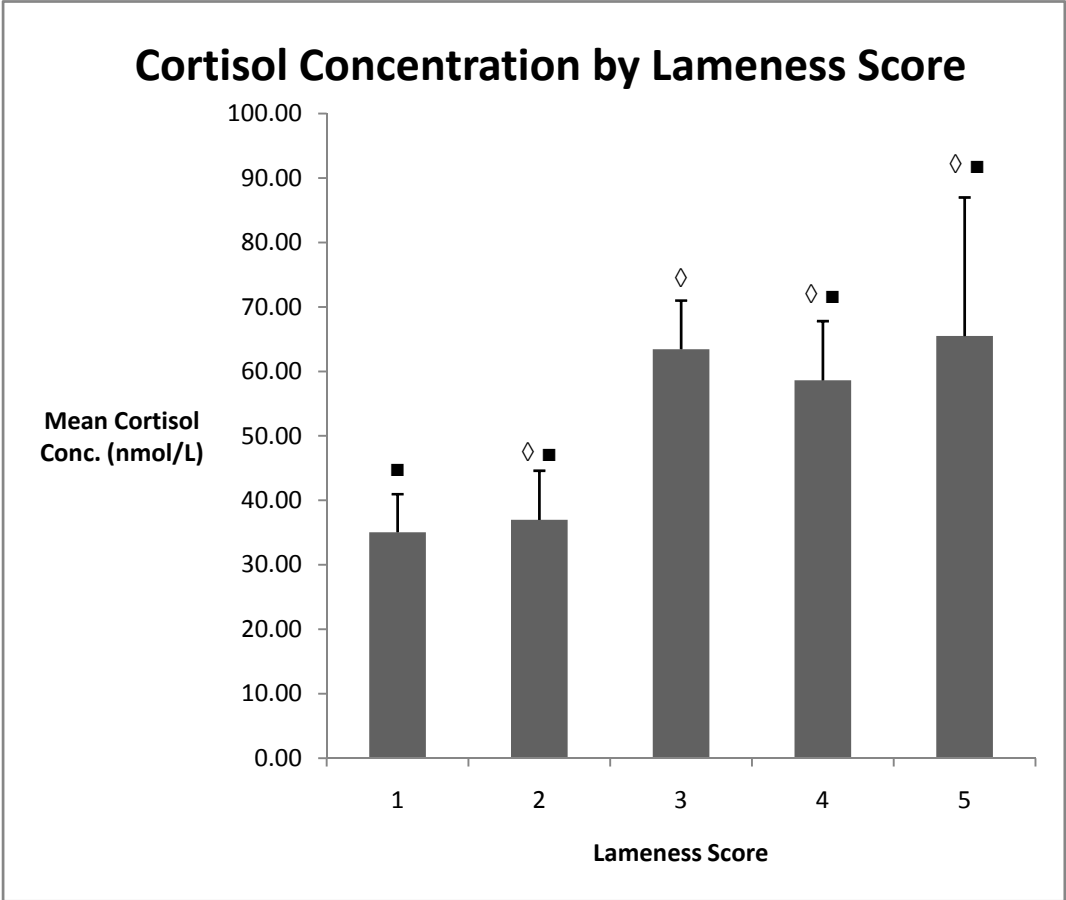


Figure 2.4 Mean surface area \pm SEM for all treatment groups. Data points with different symbols indicate $p < 0.05$.

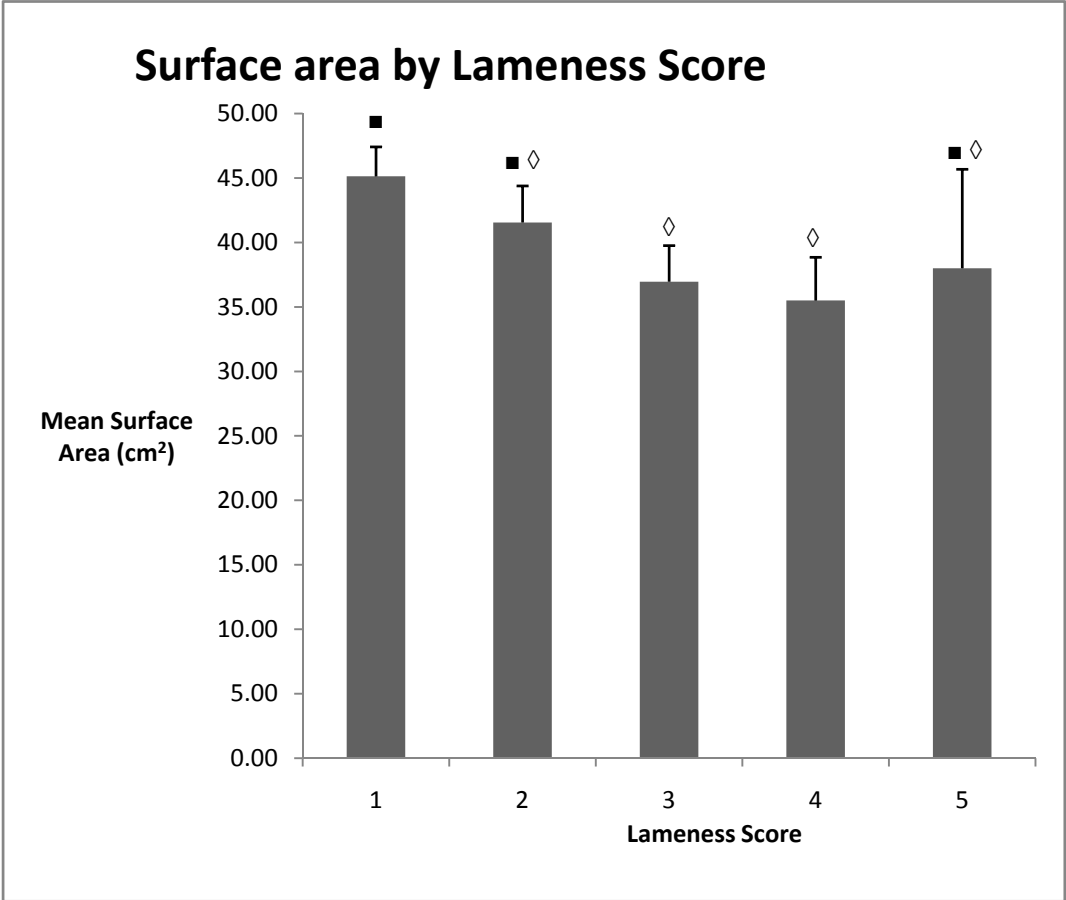


Figure 2.5 Mean contact pressure \pm SEM for all treatment groups. Data points with different symbols indicate $p < 0.05$.

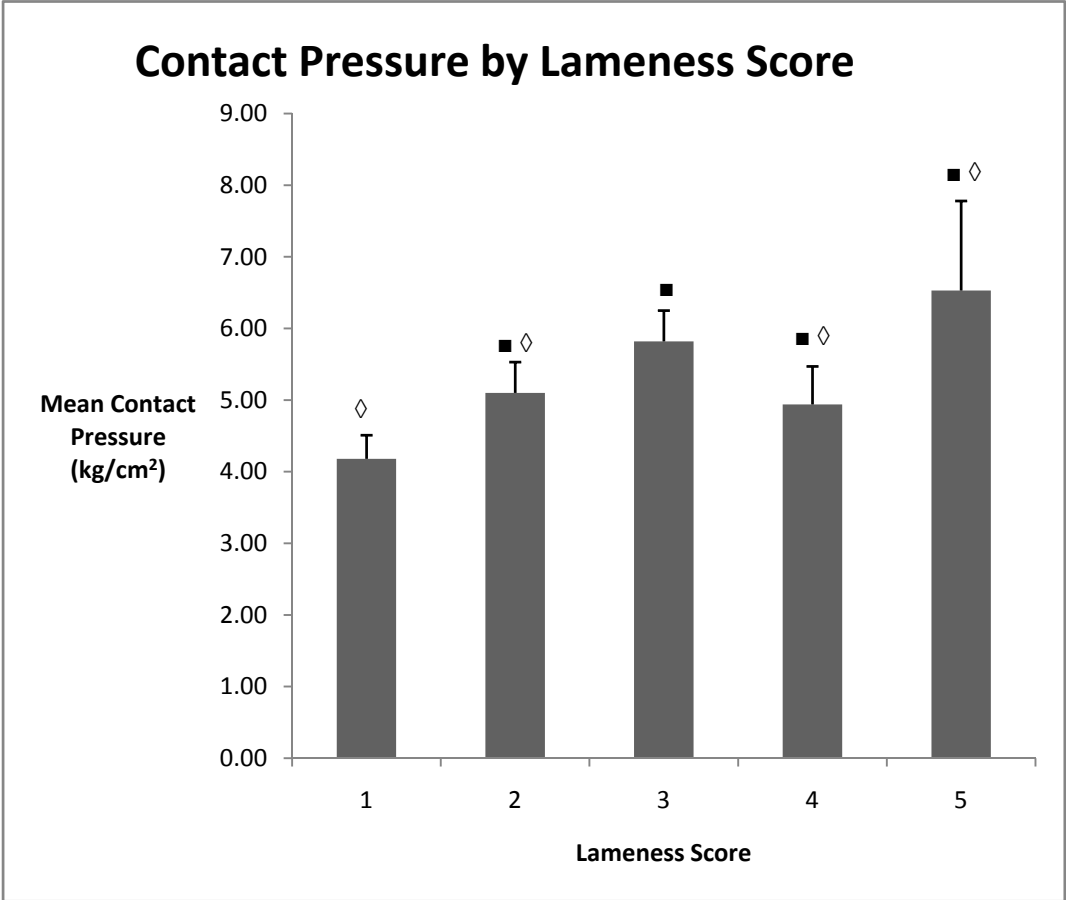


Figure 2.6 Semilogarithmic plot of plasma salicylate concentrations following intravenous administration of sodium salicylate at 50 mg/kg immediately after lameness induction.

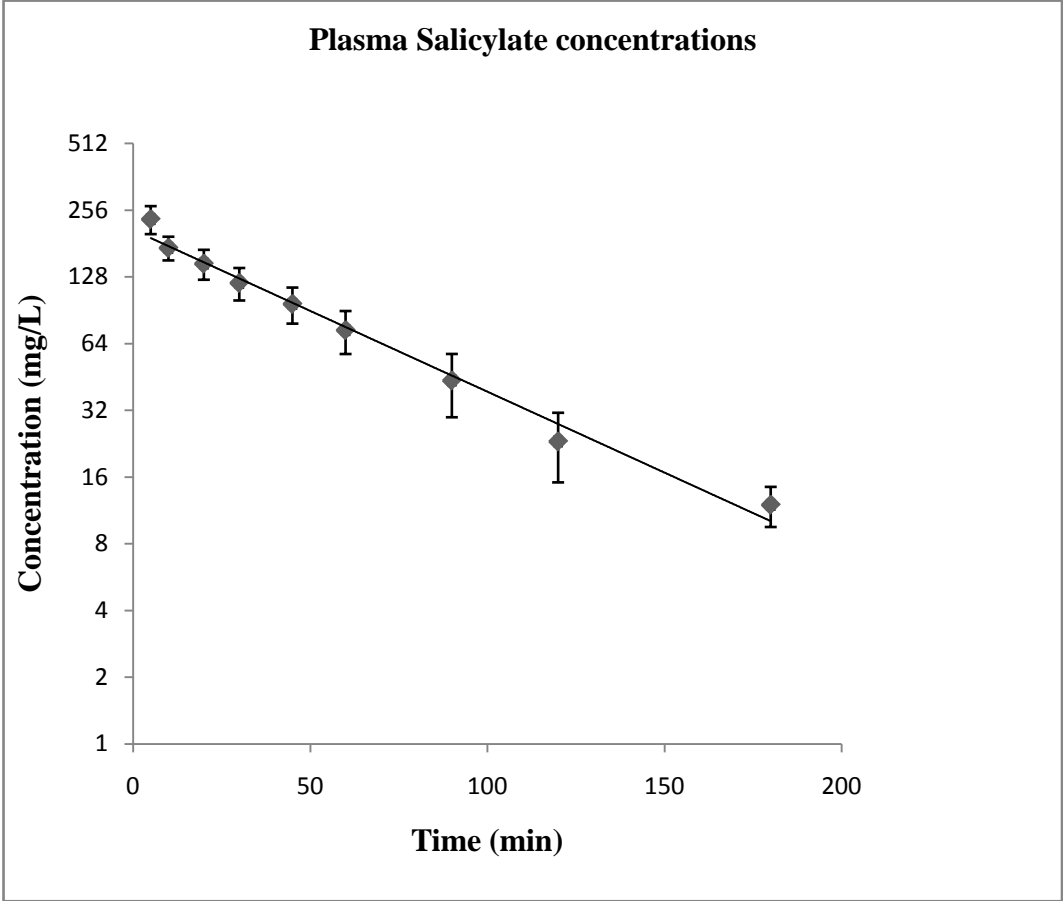


Table 2.1 Sprecher lameness scoring system.

Lameness Score	Clinical Description
1	Normal-Stands and walks normally, with all feet placed with purpose
2	Mildly lame-Stands with flat back, but arches when walks, gait is slightly abnormal
3	Moderately lame-Stands and walks with an arched back, and short strides with one or more legs
4	Lame-Arched back standing and walking, with one or more limbs favored but at least partially weight bearing
5	Severely lame-Arched back, refuses to bear weight on one limb, may refuse or have great difficulty moving from lying position

Table 2.2 Effect of period on cortisol, surface area, contact pressure, stance phase duration, heart rate, and electrodermal activity comparing repeat measures analysis to area under the time-effect curves. Differing superscripts indicate $p < 0.05$.

Parameter	Method of analysis	Period 1		Period 2		Period 3	
		Control LSM \pm SEM	Salicylate LSM \pm SEM	Control LSM \pm SEM	Salicylate LSM \pm SEM	Control LSM \pm SEM	Salicylate LSM \pm SEM
Cortisol, nmol/L	Repeated measures	46.41 \pm 3.	40.81 \pm 2.	62.00 \pm 3.	73.60 \pm 2.	36.51 \pm 3.	43.71 \pm 3.
	AUEC	439.99 \pm 101.04	314.41 \pm 93.55	767.65 \pm 101.04	839.42 \pm 93.55	322.83 \pm 101.04	335.52 \pm 93.55
Surface area,	Repeated measures	43.65 \pm 0.77	46.70 \pm 0.70	39.27 \pm 0.76	40.16 \pm 0.70	37.87 \pm 0.76	40.98 \pm 0.70
	AUEC	478.52 \pm 31.57	525.60 \pm 29.23	440.42 \pm 31.57	455.89 \pm 29.23	452.23 \pm 31.57	491.43 \pm 29.23
Contact pressure, kg/	Repeated measures	3.52 \pm 0.14	3.60 \pm 0.13	5.13 \pm 0.14	5.14 \pm 0.13	5.24 \pm 0.14	5.03 \pm 0.13
	AUEC	41.45 \pm 2.77	42.52 \pm 2.56	61.51 \pm 2.77	60.79 \pm 2.56	62.32 \pm 2.77	58.91 \pm 2.56
Stance phase duration, s	Repeated measures	0.79 \pm 0.	0.79 \pm 0.	0.82 \pm 0.	0.80 \pm 0.	0.77 \pm 0.	0.84 \pm 0.
	AUEC	8.51 \pm 0.49	8.80 \pm 0.45	9.07 \pm 0.49	8.99 \pm 0.45	9.09 \pm 0.49	9.46 \pm 0.45
Heart rate, bpm	Repeated measures	82.61 \pm 1.66	84.21 \pm 1.55	86.88 \pm 1.70	89.79 \pm 1.56	97.59 \pm 1.73	104.38 \pm 1.55
	AUEC	1000.97 \pm 77.64	1069.08 \pm 71.88	1019.85 \pm 77.64	1226.86 \pm 71.88	1204.52 \pm 77.64	1340.07 \pm 71.88
Electrodermal activity	Repeated measures	8.43 \pm 0.06	8.55 \pm 0.06	8.34 \pm 0.06	8.38 \pm 0.06	8.49 \pm 0.06	8.33 \pm 0.06
	AUEC	102.96 \pm 1.15	104.15 \pm 1.06	100.62 \pm 1.15	101.15 \pm 1.06	101.17 \pm 1.15	100.45 \pm 1.06

Table 2.3 Number of calves within each lameness score by treatment group for the entire study.

Lameness Score	Period 1				Period 2				Period 3			
	Control		Salicylate		Control		Salicylate		Control		Salicylate	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	6	6	8	8	6	0	8	0	0	0	0	0
2	1	1	0	0	1	0	0	0	2	5	4	4
3	0	0	0	0	0	5	0	5	3	1	2	2
4	0	0	0	0	0	2	0	2	2	1	1	2
5	0	0	0	0	0	0	0	0	0	0	1	0

Table 2.4 Mean \pm SE pharmacokinetic parameters describing disposition of sodium salicylate after IV administration of 50 mg/kg to steers.

Pharmacokinetic parameter	Mean \pm SE
AUC, min*mg/L	12099.0 \pm 509.7
T _{1/2el} , min	36.9 \pm 1.2
K ₁₀ , 1/min	0.02 \pm 0.0007
C ₀ , mg/L	226.9 \pm 4.9
CL _B , mL/min*kg	4.3 \pm 0.2
V, L/kg	0.2 \pm 0.005
MRT, min	53.2 \pm 1.7

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Sincerely,

Susan Pollock
Managing Editor
Journal of Dairy Science

CHAPTER 3 - Implications for further research

From the research performed as detailed in Chapter 2, several conclusions for further research can be made regarding our lameness model, analgesic drugs to be used, study design, and measurements to be taken.

First, we proved that our induced lameness model of 15 and 20 mg of Amphotericin B injected in the distal interphalangeal joint was effective in producing lameness and this lameness was found to be controlled and transient in dairy calves. This can be seen in the statistical analysis by looking at the period effects. When looking at contact surface area, significantly more contact surface area for the affected foot occurred in Period 1 as compared to Period 2 and Period 3. This is intuitive because the calves were lame in Period 2 and Period 3 and were unwilling to put as much contact surface area down on the affected hooves. Likewise, during lameness (Period 2 and Period 3), the calves placed more contact pressure down on less contact surface area of their affected foot, resulting in significantly less contact pressure in Period 1 compared to Period 2 and Period 3. However, in contrast to these measurements, cortisol concentration was found to be elevated in Period 2 compared to Period 1 and Period 3. This suggests that cortisol concentration is a more acute indication of pain, since the calves were clinically lame as well as apparently painful as indicated by the other measurements. In contrast, heart rate appears to be a more sensitive measure of increased pain due to chronicity of a condition. Our results indicate that Period 3 heart rate was significantly higher compared to Period 2 heart rate, which was significantly higher compared to Period 1 heart rate.

Our dosage of 50 mg/kg sodium salicylate intravenously once daily was not effective in providing analgesia for the above mentioned induced lameness model. Areas of interest for further research include using an oral formulation, either as a bolus or sustained in the drinking

water, and using a higher dosage. A benefit of oral dosage of aspirin in the ruminant is prolonged concentrations of the drug due flip flop kinetics in the rumen; the absorption rate is much slower than the elimination rate. Aspirin has been found to be up to 90% protein bound at low concentrations; however, at high concentrations, protein binding has been found to be as low as 70%. Due to these above mentioned reasons, a study comparing IV to oral aspirin at higher dosages would be beneficial. The lameness model would also be useful for looking at the analgesic efficacy of other drugs.

In our study, we looked at one subjective visual measurement and several objective physiological measurements of pain in lame cattle. One tool that was not relevant in our research was electrodermal activity. While the other instruments detected differences, this tool was ineffective in finding a difference. The pressure mat data appeared to be very relevant in finding differences in data because it directly measures output on the affected foot. Cortisol concentrations appear to be a measurement of acute pain, as detailed above. However, it would be of interest to see how cortisol responds at higher drug concentrations. The heart rate data suggests that chronic pain indeed is more painful than the acute pain. Subjective, blinded lameness scores appear to be a good measure of pain. Our results found a significant difference in heart rate comparing clinically normal (LS 1), LS 2, and LS 4 cattle; a significant decrease in cortisol concentration in clinically normal cattle (LS 1) compared to LS 3 cattle; a significant increase in surface area in clinically normal cattle (LS 1) compared to LS 3 cattle and LS 4 cattle; and a significant decrease in contract pressure in clinically normal cattle (LS 1) compared to LS 3 cattle.

Beneficial suggestions for further research include using the lameness model as described, using an oral form of aspirin at a higher dosage or choosing another analgesic drug,

removing the electrodermal activity measurement, and consider eliminating the cortisol concentrations.