Pain assessment in cattle
Hans Coetzee
College of Veterinary Medicine, Iowa State University, Ames, IA

Introduction
The 21st century consumer is wealthier but also more detached from production agriculture than any in history. Therefore, animal welfare concerns are becoming an important issue to our clients and customers. We recognize that pain is an inevitable consequence of many routine animal husbandry procedures in farm animals. However, how can we assess if cattle are in pain and if our analgesic interventions are working? In this session we will examine novel approaches to measuring pain and analgesic drug efficacy in cattle. We will discuss a practical sub-anesthetic/analgesic drug combination you can use to take the edge off the fractious cases you encounter.

Nociception is an inevitable consequence of many routine management procedures in farm animals. Castration is considered one of the most stressful experiences for livestock by the American Veterinary Medical Association (AVMA) and is performed on approximately 15 million calves in the U.S. annually. Dehorning and castration are especially significant in terms of animal welfare because preemptive analgesia can be applied in advance of the painful stimulus, thereby preventing sensitization of the nervous system to subsequent stimuli that could amplify pain. The AVMA “supports the use of procedures that reduce or eliminate the pain of dehorning and castrating of cattle” and proposes that “available methods of minimizing pain and stress include application of local anesthesia and the administration of analgesics.” In spite of this, a recent survey of 184 bovine veterinarians conducted by our research group found that only one in five U.S. veterinarians use anesthesia or analgesics at the time of castration. One reason for this discrepancy is the lack of objective methodology to quantify the most effective pain mitigation strategies.

Keywords: Pain assessment, pain management, substance P, meloxicam, gabapentin

Castration
It is remarkable that although administration of local anesthesia prior to castration and dehorning is legislated in several European countries there are currently no analgesic drugs specifically approved for pain relief in livestock by the U.S. Food and Drug Administration (FDA). FDA Guidance Document 123 for the development of effectiveness data for non-steroidal anti-inflammatory drugs (NSAIDs) states that “validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in the target species.” The identification and validation of robust, repeatable pain measurements is therefore fundamental for the development and approval of effective analgesic drug regimens for use in livestock. Research to address our limited knowledge in this area is therefore essential to formulating science-based recommendations.

In practical terms, resistance to requiring injectable analgesia for routine castration and dehorning is largely based on time and logistical issues. However, a recent study evaluating novel methods of analgesia for tail docking in lambs demonstrated that castrating by banding in 1-2 day old lambs required an average of 28 seconds without analgesia and 68 seconds when an injectable local anesthetic was administered by jet-injector. While the U.S. has not followed other countries in legislating the use of local anesthesia during castration and dehorning, it is appropriate for the veterinary profession to pursue practical, rapid, and effective methods for the relief of pain related to these procedures. The authors are aware of practicing veterinarians that have adopted local anesthesia regimens for these procedures.

Candidate methods to assess pain associated with castration
The literature pertaining to behavioral response associated with castration has been summarized in an excellent review by Stafford and Mellor. The salient points pertaining to this discussion are summarized below.
Behavior. Assessment of individual animal behavioral changes in response to pain is highly subjective. Escape behaviors demonstrated at castration but not seen afterwards may reflect a pain response or a desire to escape confinement. Studies have reported that surgically castrated calves struggle and kick during the operation but calves castrated with rubber rings are quieter. Macauley and others reported that calves castrated surgically moved around much less than control calves or calves castrated using a Burdizzo emasculatome. Two days following castration, the surgical and Burdizzo emasculatome castrated calves were observed to be less active than control calves. Robertson and others found that rubber-ring, Burdizzo emasculatome and surgical castration caused significant behavioral responses indicative of pain during the first three hours after castration. Fisher and others reported that 14 month old bulls castrated surgically stamped their hind feet, swished their tails and grazed less in the afternoon following castration than control bulls and bulls castrated using bands. Behaviors indicative of a painful sensation such as turning the head towards the hindquarters, alternate lifting of the hindlegs, abnormal postures and slow movement of the tail has been reported weeks after rubber-ring castration. The relative level of pain associated with behaviors seen after castration has not been quantified. The characteristics of emotional states such as being fearful, anxious or happy; and other subjective states such as pain sensation and perception are such that they can never be precisely and accurately measured.

Production parameters. Production parameters are often too imprecise to reflect the pain experienced by animals following castration. Furthermore, weight gain following castration may be negatively influenced by a decrease in testosterone following removal of the testes. However, assessment of production parameters is critical if animal well-being research is to have relevance to livestock producers. These assessments may take the form of a cost-benefit analysis or a measure of animal performance. In some studies, Burdizzo emasculatome or surgical castration had no effect on average daily gain (ADG) over a three month period following castration. The ADG of 7 week-old calves during the 5 weeks following castration using rubber rings, clamp or surgery have been reported to be lower than non-castrated calves but similar between the different castration methods. Rubber ring and surgical castration were reported to cause a decrease in ADG of 50% and 70% respectively in cattle aged 8 to 9 months. When 8, 9 and 14 month old cattle were castrated surgically or using latex bands, cattle castrated later had poorer growth rates than those castrated at weaning. Cattle castrated with latex bands also had lower growth rates than those castrated surgically during the following 4–8 weeks. In a study conducted by Oklahoma State University, 162 bull calves were used to determine the effects of latex banding of the scrotum or surgical castration on growth rate. Bulls that were banded at weaning gained less weight than bulls that were banded or surgically castrated at 2 to 3 months of age. In a second study, 368 bull calves were used in two separate experiments to examine the effect of method of castration on receiving health and performance. In the first experiment latex banding intact males shortly after arrival was found to decrease daily gain by 19% compared with purchasing steers, and by 14.9% compared with surgically castrating intact males shortly after arrival. In the second experiment purchased, castrated males gained 0.58 lbs more and consumed 1.26 lbs more feed per day than intact males surgically castrated shortly after arrival.

Cortisol response. Several studies have evaluated acute cortisol response as a method to determine the extent and duration of distress associated with castration in cattle. Studies reviewed by Stafford and Mellor indicate that surgical and latex band castrations, especially when performed in older cattle, appear to elicit higher plasma cortisol responses that remain above pre-treatment levels for longer periods. Peak cortisol concentration following surgical castration occurs within the first 30 minutes after castration and range from 45 nmol/L following rubber ring castration to 129 nmol/L following surgical castration. The duration of plasma cortisol response above pre-treatment levels typically ranges from 60 minutes following Burdizzo emasculatome castration to 180 minutes following surgical castration. Fisher and others reported that plasma cortisol response was significantly reduced during the first 90 minutes following surgical or Burdizzo emasculatome castration in calves when the
Lidocaine was administered prior to the procedure. Lidocaine is a commonly used local anesthetic that has a fast onset of action of 10-15 minutes, and an intermediate duration of action of 60–120 minutes. Based on these data, increases in plasma cortisol are believed to reflect pain experienced as a result of castration.

Cortisol has been widely used as a measurement of distress since its response magnitude, as indicated by peak height, response duration and/or integrated response usually accords with the predicted noxiouslyness of different procedures. At each end of the cortisol response range, however, interpretation is less straightforward. At the lower end, for example, studies have shown that tail docking with a ring and tail docking with a docking iron cause similar cortisol responses to control handling in older lambs. At the upper end of the range, there are several examples where cortisol responses do not increase proportionally to the severity of different treatments as might be expected. This may suggest a “ceiling effect” on plasma cortisol responses. Other studies have shown that plasma cortisol concentrations following surgical castrations vary greatly between animals. Based on these data, it has been hypothesized that low responses may be due to individuals having high pain thresholds. Variations may also come about due to differences in the way in which a particular castration method is performed by different operators. These data suggest that plasma cortisol levels may not always accurately reflect the extent of the pain response in animals.

Substance P. Substance P (SP) is an 11-amino acid prototypic neuropeptide that regulates the excitability of dorsal horn nocireceptive neurons and is present in areas of the neuroaxis involved in the integration of pain, stress, and anxiety. Studies have shown that plasma SP levels are up to 27-fold greater in human patients with soft tissue injury than healthy controls. Our research group recently conducted a study to evaluate plasma SP and cortisol response following castration. Calves were acclimated for 5 days prior to random assignment based on scrotal circumference to a castration or uncastrated control group. Blood samples were collected at -24, -12, and 0 hours pre-castration and 3, 10, 20, 30, 45, 60, 90, 120, 150, 180, and 240 minutes post-castration. Vocalization and attitude scores were determined at the time of castration or simulated castration. Plasma SP and cortisol were determined using competitive and chemiluminescent enzyme immunoassay, respectively. Data were analyzed by repeated measures analysis using a mixed effects model allowing for unequal variances. No significant difference in plasma cortisol response between castrated and uncastrated calves was observed over time (p=0.644). In contrast, mean plasma SP concentrations were significantly higher in castrated calves compared to uncastrated controls over the course of the study (p=0.042). Cortisol responses over time in calves with vocalization scores of 0 were not significantly different from calves with vocalization scores of 3 (p=0.17). However, calves with vocalization scores of 3 had significantly higher SP levels when compared to calves with scores of 0 (p=0.033). These findings contradict previous reports that show an increase in plasma cortisol relative to pain post-castration. Significant increases in plasma SP concentration post-castration suggest that this measurement may be associated with nociception; however, further investigation is necessary.

Accelerometers. Accelerometers have been used in other species to detect lameness and remotely monitor level of animal activity. Our research group has utilized video observations to determine the accuracy of accelerometers to measure behavior changes in cattle and to determine differences in beef bull behavior post-castration. Three Holstein calves and 12 healthy beef bulls had two-dimensional accelerometers placed on three animals and data were logged simultaneous to video recording of animal behavior. The subsequent data set was used to generate and validate a predictive model to classify animal posture (standing or lying) and type of activity (standing in place, walking, eating, getting up, lying awake, or lying sleeping). The algorithms developed were used to conduct a prospective trial to determine differences in bull behavior in the first 24 hours post-castration (N=6) compared to both control animals (non-castrated) (N=6) and pre-castration readings from the same bulls. Based on the analysis of the 2-D accelerometer signal, posture can be classified with a high degree of accuracy (98.3%) and the specific activity can be estimated with a reasonably low misclassification rate.
(23.5%). Employing the system to compare behavior post-castration revealed that castrated calves spent a larger (P < 0.05) amount of time standing (79.3%) compared to either pre-castration readings (51.2%) or control calves after castration (64.3%). Animals also spent a lower percentage of the time eating in the post-castration phase. The 2-D accelerometers provided accurate classification of animal posture and reasonable classification of animal activity. Collected data allowed quantification of behavioral differences between animals after a surgical procedure and provides a valuable tool to compare research with behavioral endpoints.

**Radar speed cameras.** Burrows and Dillon and Fell et al. used radar speed cameras to measure the speed of cattle exiting a squeeze chute. Cattle with faster exit speeds had lower weight gains, more sickness, and more dark cutting meat. The major problem with chute exit speed as a means to determine pain is that it does not work well for highly acclimated, very tame animals such as a dairy cattle. There are also certain breeds such as *Bos indicus* breeds that are more prone to demonstrate rapid chute exit speeds when compared with *Bos taurus* breeds.

**Pain management in cattle**

It has been suggested that a surgical stimulus such as castration in calves is so brief that little difference can be observed or measured between animals having or not having local anesthetic applied. However, alleviating pain associated with surgical castration by administration of local anesthesia increased weight gain in cattle for 35 days following castration. This suggests that alleviating acute pain at the time of castration may have economic benefit. Ketoprofen, a NSAID analgesic not approved for use in cattle in the U.S., has been shown to reduce acute plasma cortisol response in cattle following administration at the time of castration. Giving both a local anesthetic and intravenous ketoprofen before surgery-cut castration was found to virtually abolish the post-surgery cortisol response. Ketoprofen given alone was also found to reduce the plasma cortisol response to Burdizzo emasculatome castration more effectively than a local anesthetic or an epidural. Similar studies examining NSAIDs that are approved for use in food-producing animals in the U.S. have not been conducted. Furthermore, all these studies examining the efficacy of analgesic drugs in farm animals fail to report associated plasma drug concentrations essential for designing efficacious analgesic regimens. Some of the parameters described above may be useful to allow us to determine the efficacy of analgesics in food animals.

Our research group recently conducted a study to examine the effect of oral aspirin and intravenous sodium salicylate on acute plasma cortisol response following surgical castration. Twenty bulls, randomly assigned to the following groups: 1) uncastrated, untreated controls, 2) castrated, untreated controls, and 3) 50mg/kg sodium salicylate IV pre-castration and 4) 50mg/kg aspirin (acetylsalicylic acid) per os pre-castration, were blood sampled at 3, 10, 20, 30, 40, 50 minutes and 1, 1.5, 2, 4, 6, 8, 10 and 12 hours post-castration. Samples were analyzed by competitive chemiluminescent immunoassay and fluorescence polarization immunoassay for cortisol and salicylate respectively. Data were analyzed using noncompartmental analysis, a simple cosine model, ANOVA and t-tests. Intravenous salicylate Vdss was 0.18 L/kg, Clb was 3.36 mL/min/kg and T½λ was 0.63 hours. Plasma salicylate concentrations above 25 µg/mL coincided with significant attenuation in peak cortisol concentrations (p=0.029). Peak salicylate concentrations following oral aspirin administration was less than 10 µg/mL and failed to attenuate cortisol response. Once salicylate concentrations decreased below 5 µg/mL, cortisol response in the castrated groups were significantly higher than uncastrated controls (p=0.018). To our knowledge this is the first study relating plasma analgesic drug concentrations directly to mitigation of plasma cortisol response post-castration. These findings have important implications for designing effective analgesic regimens to alleviate the stress response associated with painful routine animal husbandry procedures.

A protocol for use of IM butorphanol/xylazine/ketamine (BXK) was presented by Dr. Matt Miesner, with credit given to Dr. Eric Abrahamsen, at the 2007 Kansas State University June Conference. The regimen consists of butorphanol (0.01–0.025 mg/kg) + xylazine (0.02–0.05 mg/kg) + ketamine (0.04–0.1 mg/kg). Dr. Miesner noted that for a 450 kg animal, 5 mg butorphanol, 10 mg xylazine, and 20 mg ketamine would constitute the low end of the dosing range. Note that
calculation should involve 2x xylazine as compared to butorphanol and 2x ketamine as compared with xylazine. They have noted up to an hour of cooperation using this protocol but more fractious patients may require increased doses. Dr. Miesner suggests giving no more than 10 mg butorphanol or 20 mg of xylazine the initial dose (this would be for the 450 kg animal on up).

Stun “recipe” for large groups of cattle:

- 5 ml ketamine (100mg/mL solution)
- 10 ml SMALL ANIMAL! xylazine (20 mg/mL)
- 10 ml of butorphanol (10 mg/mL)
- Makes a 25 ml stock solution to be used at one time due to stability concerns
- Docile (dairy) cattle- 1 mL/ 400 Kg (880 lbs)
- Fractious (beef) cattle- 1 mL/ 200 kg (440 lbs)
- Onset: IV faster than IM faster than SC
- Duration of effect: SC > IM > IV
- Risk of recumbency: IV> IM > SC (High dose > Low dose)

Meloxicam

Meloxicam is a NSAID of the oxicam class that is approved in the European Union for adjunctive therapy of acute respiratory disease; diarrhea and acute mastitis when administered at 0.5 mg/kg IV or SC. Meloxicam is considered to bind preferentially to cyclooxygenase-2 (COX-2) inhibiting prostaglandin synthesis although definitive evidence of COX-selectivity in calves is deficient in the published literature. Heinrich et al.5 demonstrated that 0.5 mg/ kg meloxicam IM combined with a cornual nerve block reduced serum cortisol response for 6 hours in 6-12 wk old calves compared with calves receiving only local anesthesia prior to cautery dehorning. Furthermore, calves receiving meloxicam had lower heart rates and respiratory rates than placebo treated control calves over 24 hours post-dehorning. Stewart et al.6 found that meloxicam administered IV at 0.5 mg/kg mitigated the onset of pain responses associated with hot-iron dehorning in 33 ± 3 day old calves compared with administration of a cornual nerve block alone as measured by heart rate variability and eye temperature. These findings indicate that administration of meloxicam at 0.5 mg/kg IV or IM decreases physiological responses that may be linked to pain and distress associated with cautery dehorning in preweaning calves.

The purpose of this study was to investigate the pharmacokinetics and oral bioavailability of meloxicam in ruminant calves.7 Six Holstein calves (145–170 kg) received either meloxicam IV at 0.5 mg/kg or oral meloxicam at 1 mg/kg in a randomized cross-over design with a 10-day washout period. Plasma samples collected up to 96 hours post-administration were analyzed by LC-MS followed by noncompartmental pharmacokinetic analysis. A mean peak plasma concentration (Cmax) of 3.10 ug/mL (range: 2.64–3.79 ug/mL) was recorded at 11.64 hours (range: 10–12 hours) with a half-life (T ½ λz) of 27.54 hours (range: 19.97–43.29 hours) after oral meloxicam administration. The bioavailability (F) of oral meloxicam corrected for dose was 1.00 (range: 0.64–1.66). These findings indicate that oral meloxicam administration could be an effective and convenient means of providing long-lasting analgesia to ruminant calves.
In the U.S., meloxicam administered to cattle by any route constitutes extra-label drug use (ELDU). 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. Therefore, use of oral meloxicam to alleviate suffering associated with dehorning and castration in calves in the U.S. would be required by law to comply with these regulations.

Currently the only NSAID approved for use in cattle in the U.S. is flunixin meglumine. The plasma elimination half-life of flunixin is reported to be 3–8 hours therefore requiring once daily administration. Although this drug class is recognized as having analgesic properties, flunixin is only indicated for control of fever associated with respiratory disease or mastitis, and fever and inflammation associated with endotoxemia, rather than for control of pain. Studies demonstrating the analgesic effects of flunixin at the approved dose of 2.2 mg/kg are deficient in the published literature. Use of flunixin meglumine is further complicated by the requirement for intravenous administration which is more stressful on the animal and involves more skill and training on the part of the operator. Several reports have suggested that the IM administration of flunixin may result in significant myonecrosis and tissue residues. In the absence of data demonstrating that flunixin reduces signs of pain and distress associated with dehorning and castration in calves, it could be argued that use of oral meloxicam for this purpose can be justified under AMDUCA. Meloxicam (20 mg/ml) is approved for use in cattle in several European countries with a 15 day meat and five day milk withdrawal time following administration of 0.5 mg/kg IM or SC. An oral meloxicam suspension (1.5 mg/mL) and injectable formulation (5 mg/mL) are approved in the U.S. for the control of pain and inflammation associated with osteoarthritis in dogs. Furthermore, an injectable formulation (5 mg/ml) is approved for the control of post-operative pain and inflammation in cats. Several generic tablet formulations containing meloxicam (7.5 and 15 mg) have recently been approved for relief of signs and symptoms of osteoarthritis in human medicine. The cost of administering IV meloxicam to calves in the present study was approximately U.S. $58.00/100 kg bodyweight and the cost of administering oral meloxicam was U.S. $0.30/100 kg bodyweight.
Gabapentin

Gabapentin is a γ-aminobutyric acid (GABA) analogue indicated for treatment of neuropathic pain. This study determined the pharmacokinetics of oral gabapentin alone or in combination with meloxicam in ruminant calves. Gabapentin capsules at 10 mg/kg PO or gabapentin powder (from capsules) and meloxicam tablets at 15 mg/kg and 0.5 mg/kg PO, respectively was administered to six beef calves. Plasma drug concentrations were determined over 48 h post-administration by liquid chromatography/mass spectrometry followed by non-compartmental pharmacokinetic analysis. The mean (±SD) Cmax, Tmax and elimination half-life (t½λz) for gabapentin (10 mg/kg) alone was 2.97±0.40 μg/mL, 9.33±2.73 h and 11.02±3.68 h, respectively. The mean (±SD) Cmax, tmax and t½λz for gabapentin (15 mg/kg) co-administered with meloxicam was 3.57±1.04 μg/mL, 7.33±1.63 h and 8.12±2.11 h, respectively. The mean (±SD) Cmax, Tmax and t½λz for meloxicam was 2.11 ± 0.19 μg/mL, 11.67 ± 3.44 h and 20.47 ± 9.22 h, respectively. Plasma gabapentin concentrations >2 μg/mL were maintained for up to 15 h and meloxicam concentrations >0.2 μg/mL for up to 48 h. The pharmacokinetic profile of oral gabapentin and meloxicam supports clinical evaluation of these compounds for management of neuropathic pain in cattle.

Acknowledgement

Supported by the USDA- CSREES Animal Protection (Animal Well-being) NRI Grant (No. 2008-35204-19238).

References