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# Timing of Castration, with or without Analgesia, on Growth Performance, Hematology, and Behavior of Beef Calves

April C. Brown

*University of Arkansas, Fayetteville*

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TIMING OF CASTRATION, WITH OR WITHOUT ANALGESIA, ON GROWTH  
PERFORMANCE, HEMATOLOGY, AND BEHAVIOR OF BEEF CALVES

TIMING OF CASTRATION, WITH OR WITHOUT ANALGESIA, ON GROWTH  
PERFORMANCE, HEMATOLOGY, AND BEHAVIOR OF BEEF CALVES

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Animal Science

By

April Brown  
Missouri State University  
Bachelor of Science in Agriculture Business, 2011

December 2012  
University of Arkansas

## ABSTRACT

Angus-sired ( $n = 30$ ) and Hereford-sired ( $n = 32$ ) bull calves were assigned randomly to 1 of 4 treatments. Treatments were 1) surgical castration near birth, 2) oral administration of the analgesic meloxicam (1 mg/kg of body weight) followed by surgical castration near birth, 3) surgical castration at weaning, or 4) surgical castration at weaning with oral administration of the analgesic meloxicam (1 mg/kg of body weight). Data was recorded for behavior, hematology, and growth at birth and weaning. Calf standing and lying activity was monitored at both stages by recording x and y axis positions of a data logger accelerometer attached to the metatarsus of the right leg for 7 d. At birth, blood was collected on d 0 (birth), 1, 3, and 7 from a subset of calves, and post-weaning blood samples were collected on d 214 (weaning), 214 + 6 h, 215, 217, 221, and 228. Body weight was recorded on all calves on d 4, 33, 66, 116, 162, 199, and 214 (weaning). Body weight was recorded on all weaned calves on d 214 (weaning), 228, 246, and 270. Average daily gain did not differ between treatments ( $P \geq 0.88$ ) through weaning. The neutrophil to lymphocyte ratio at birth decreased with day ( $P < 0.0001$ ). Following castration at birth, there were no differences in treatments for any of the three observed positions. Overall, post-weaning ADG was greater ( $P = 0.02$ ) in steers compared to bulls castrated without meloxicam. At weaning the neutrophil to lymphocyte ratio displayed a d effect ( $P < 0.0001$ ), with an increase at 6 h post castration, and then decreasing. Post-weaning behavior following castration resulted in steers spending the least proportion of time standing, and bulls castrated with analgesia tended to spend less time, numerically, lying on sternum compared to steers.

This thesis is approved for recommendation  
to the Graduate Council.

Thesis Director:

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Dr. Jeremy Powell

Thesis Committee:

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Dr. Beth Kegley

---

Dr. Shane Gadberry

---

Dr. Yvonne Thaxton

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## **CHAPTER 1 – THESIS INTRODUCTION**

For centuries, cow-calf producers and feedlots have been castrating bull calves, with records dating as early as the sixteenth century (Capucille et al., 2002), and approximately 15 million bull calves are castrated each year in the United States (USDA, 2012). Even though bulls convert feed more efficiently and gain weight faster than steers (King et al., 1991), the aggressive behavior and less tender meat of the intact male were justifications for castrating (Bretschneider, 2005). While castration is necessary, it causes pain and stress that can temporarily reduce growth performance. Fisher et al. (1996) reported that castration reduces average daily gain (ADG) of beef calves, and Ballou et al. (2011) indicated that castration increased physiological stress parameters such as fibrinogen, haptoglobin, and Substance P. Castration can cause an alteration on normal behavior in beef calves (Sutherland et al., 2011) and an alteration on normal immunomodulatory effects (Chase et al., 1995). Castration has also been associated with an increased rate of bovine respiratory disease, resulting in greater treatment cost, labor cost, and reduced animal well-being (Daniels et al., 2000).

As public awareness and concern for animal well-being grows, research is necessary to determine the most humane and safe methods for performing even the most common animal management procedures. The American Veterinary Medical Association (AVMA, 2012) has voiced its concern with the important issue of pain management and encourages the use of pain relief during routine management practices. While the AVMA supports reducing or eliminating pain during dehorning and castration, it does not specifically address the use of analgesia or anesthesia. “The AVMA supports the use of procedures that reduce or eliminate the pain of dehorning and castrating of cattle. These procedures should be completed at the earliest

age practicable. Research in developing improved techniques for painless, humane castration and dehorning is encouraged.”

A survey conducted by Coetzee et al. (2010) reported that surgical castration with a scalpel was the most preferred method of castration by US bovine veterinarians. However, only 1 in 5 veterinarians reported using pain relief during castration. There is currently no non-steroidal anti-inflammatory drug (NSAID) approved by the Food and Drug Administration (FDA) for analgesia in cattle. Meloxicam, however, is an NSAID that is FDA approved and prescribed for pain relief in other species, such as companion animals. Some US veterinarians have prescribed the extra-label use of meloxicam to reduce inflammation in recently castrated beef cattle. Coetzee et al. (2011) reported meloxicam administered orally to newly received beef calves at 1 mg/kg body weight, 24 hours before castration reduced the incidence of bovine respiratory disease.

The objective of this study was to determine the effects of castration in beef calves at two stages of maturity, birth and weaning, with or without pain control (meloxicam), on growth performance, hematology, and behavior. Calves were evaluated for weight gain, blood parameters and behavior after castration, at birth and weaning. Outcomes from this research will enable producers and veterinarians to make informed decisions regarding proper and humane methods of castration for cattle, and it will contribute to our knowledge concerning animal health and wellbeing in order to better educate the general public on the humane treatment of beef cattle.

## **CHAPTER 2 – REVIEW OF LITERATURE**

### **A. INTRODUCTION**

Castration is a necessary husbandry practice in the beef cattle industry. It reduces aggressive behavior, unwanted pregnancies, and injury of animals. As the animal industry moves forward, the justification of many common animal husbandry procedures, such as castration, are beginning to be questioned by the public. Previous research has shown that castration can reduce weight gain of cattle (Fisher et al., 1996), alter physiological stress response (Ballou et al., 2011), and affect their stress level. While the level of stress can be measured in several ways, this literature review will focus on changes in behavior and physiological parameters that would suggest a stressful situation. The AVMA supports efforts to reduce or eliminate pain during dehorning and castration of cattle, which should be done at the earliest age possible. It is encouraged that research be done to improve techniques for castrating and dehorning to find methods that are less painful and more humane. In addition, it is recommended that practical alternatives to castration and dehorning be created and applied (Capucille et al., 2002). The purpose of this literature review is to report research results on the application of castration, pain mitigation, and the effects that castration, with or with analgesia, has observed with beef bulls at different stages of maturity.

### **B. METHODS OF CASTRATION**

While there are several methods to castrate cattle, research conducted by Capucille et al. (2002) found that in the United Kingdom, producers and farmers indicated that the emasculatome was the most popular method, followed closely by surgical (knife) and band (elastrator) castration (43%, 39% and 32%, respectively). Method varied based on the age at time of castration.

Even though the desired end result is the same, the methods used to obtain the result are quite different. An emasculator is an instrument designed for bloodless emasculation in all species but most adaptable to ruminants because of the extended scrotal neck in these species. The spermatic cord is crushed by force applied by a pair of double-action blunt blades. These blades have rounded edges to ensure that the skin is not cut or damaged. Coetzee et al. (2011) conducted a survey of preferred methods of US veterinarians; surgical castration (calves < 90 kg) was the most common method. Surgical castration uses a sharpened blade or scalpel to remove the lower half of the scrotum (Stafford et al., 2002). The goal is to create an opening large enough to allow drainage and prevent fluid buildup (Capucille et al., 2002). The testicles are gently pulled out, separately, until the spermatic cord breaks (Capucille et al. 2002; Stafford et al., 2002). This method can be used on any size of bull; however, as size increases, the recommended method with which to cut the scrotum and remove the testes also changes. The described method is recommended for bull calves under 500 pounds. Excessive hemorrhaging and ascending infection from the incision is a post-surgical complication associated with knife castration (Capucille et al., 2002). Knife castration results in immediate and complete removal of the testes with 100% confidence of sterilization.

The banding method leads to ischemic necrosis of the testes, this eventually causes testicular atrophy and sloughing of the scrotum (Capucille et al., 2002). The banding method requires a purpose-built band application device to place a wide, rubber, latex band on the neck of the scrotum proximal to the testes (Stafford et al., 2002). Occasionally, complications can result if the band is not tight enough leading to castration failure. If the band does not adequately constrict the blood vessels, it can lead to excessive pain and prolonged fertility (Capucille et al., 2002). Furthermore, a secondary complication associated with banding is tetanus. Although it



has not been reported in calves that are banded at birth, it has been commonly reported to occur in older calves. Veterinarians strongly recommend vaccinating for tetanus 7 to 10 d prior to banding. Capucille et al. (2002) indicated that the banding method has been advocated for bulls from birth to weaning and is frequently used to castrate larger bulls in feedlots.

King et al. (1991) reported that the method of castration (surgical or emasculator) had no effect on gain performance when compared to intact cohorts; however, castration of calves up to 78 d of age, regardless of method, caused minimal increase in plasma cortisol levels, but castrating calves at 167 d of age led to significant increases in plasma cortisol. According to Zweiacher et al. (1979), feeder calf health is not directly related to the time or method of castration, when comparing emasculator to banding.

### **C. PAIN ASSOCIATED WITH CASTRATION**

Pain is defined as “an aversive feeling or sensation associated with actual or potential tissue damage resulting in physiological, neuroendocrine, and behavioral changes that indicate a stress response” (Molony and Kent, 1997). The AVMA (2012) opinion is that castration is one of the most stressful experiences for livestock. While castration does inflict pain on animals and leads to a period of slow growth rate and poorer feed efficiency, there are benefits as well (AVMA, 2012). Castration improves meat quality, reduces aggressive behavior, prevents unwanted pregnancy, and increases the market value of that animal. Stafford (2007) reported that all physical methods of castration cause pain. Furthermore, animals exhibit pain responses during and after castration which may include struggling, kicking the hind legs, tail swishing, foot stamping, head turning, restlessness, stilted gait, reduced activity, abnormal standing posture, reduced interest in dams and each other, and reduced grazing and feed intake (AVMA, 2012). Understanding the significance of long term pain and its effects on the animal is an

important current research topic. Castration may illicit extended irritation, in addition to short term pain, leading to prolonged responses (Stafford, 2007). Assessments of chronic pain have primarily been based on reduced weight gain and growth rate, and findings suggest that pain may persist for several weeks following castration (AVMA, 2012).

Castration causes physiological stress and is shown through increased plasma cortisol concentrations, inflammatory reactions, pain associated behavior, suppression of immune function and a reduction in performance (Lorenz et al., 2011). According to Capucille et al. (2002) people assume that anything that can be stressful or painful to humans can also be to animals. With the growing concern for humane cattle management practices and the ever changing public perception of these practices, it is necessary to research methods with which to reduce pain which in turn, reduces stress and improves growth performance.

#### **D. NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AS ANALGESIA**

In the US, veterinarians and producers who perform routine castrations generally do not use any sort of pain mitigation. A survey conducted by Coetzee et al. (2010) indicated that only 1 in 5 veterinarians reported using an analgesic or local anesthetic during castration. Pain and central sensitization induced by surgery consists of 2 phases: an immediate phase due to the incision and a prolonged inflammatory phase that arises due to tissue damage (Kissin, 2000). The objective of administering analgesia, prior to castration, is to mitigate both the incisional and inflammatory phase of the pain response (Coetzee, 2011). Administration of some NSAIDs results in prolonged postoperative analgesia (AVMA, 2012). Pauly et al. (2012) evaluated the use of analgesia during castration on the behavior of 12 to 20 wk old calves and observed a positive association between analgesic treatment and the proportion of time calves spent lying down, indicating improved comfort and contentment.

Non-steroidal anti-inflammatory drugs produce anti-inflammatory effects and analgesic effects by reducing prostaglandin (PG) synthesis through inhibition of the enzyme cyclooxygenase (COX) in the peripheral tissues and central nervous system (Ochroch et al., 2003). There are 2 isoforms of COX, COX-1 and COX-2 which in the early 1990s led to the categorization of all NSAIDs according to their specificity to each of these isoforms (Draaijer, 2010).

There is currently no NSAID approved by the FDA for analgesia in cattle. Meloxicam is, however, an NSAID that is FDA approved and prescribed for pain relief in other species, such as companion animals. It has also been approved in the EU for adjunctive therapy of acute respiratory disease, diarrhea and acute mastitis in cattle when administered at 0.5 mg/kg of body weight by intramuscular or subcutaneous injection (Coetzee, 2011). Some US veterinarians prescribe an extra-label use of meloxicam to reduce pain and inflammation in recently castrated beef cattle. Coetzee et al. (2011) reported that when meloxicam was administered orally to newly received beef calves at 1 mg/kg of body weight, 24 h before castration, the incidence of bovine respiratory disease was reduced.

Meloxicam acts by inhibiting prostaglandin synthesis and inducible COX-2, leading to anti-inflammatory, analgesic, and antipyretic effects (Draaijer, 2010). Coetzee (2011) indicated that meloxicam had a mean peak plasma concentration of 3.10 µg/mL and a half-life of 27.5 h after oral administration. In European countries, meloxicam has a 15 d meat withdrawal time and a 5 d milk withdrawal time following administration (Coetzee, 2011). Coetzee and Kukanich (2012) reported that when meloxicam was administered orally to calves being dehorned, long term gain performance was improved compared to dehorned calves not receiving

meloxicam, and bull calves receiving meloxicam prior to castration were almost 50% less likely to develop bovine respiratory disease (BRD) compared with placebo treated controls.

Sutherland et al. (2011) reported 3 mo old calves receiving analgesia prior to castration and/or dehorning had a lower neutrophil:lymphocyte ratio (N:L), and did not exhibit a decrease in body weight compared to calves not receiving analgesia. A study conducted by Ballou et al. (2011) indicated that castrated calves exhibited an elevated total leukocyte count and N:L compared to non-castrated calves. Furthermore, the administration of anesthetic/analgesic, prior to castration, suppressed the leukocyte response, indicating that the administration of anesthetic/analgesic alleviates some adverse effects associated with castration.

Major arguments against the use of analgesia during castration are expense, lack of approved products, and increased labor for administration. While the use of analgesia to reduce or eliminate pain may benefit the animal, the cost of analgesic drugs and a lack of FDA approved products make pain control difficult for cattle producers, and the cost of employing a veterinarian to carry out castration may be prohibitive (Stafford et al., 2005). Stafford (2007) reported that large animal veterinarians are rare in many communities, thereby, minimizing the chance of analgesia for pain alleviation for castration to be used in the near future. In order for analgesia to be effectively incorporated into current cattle production systems, current management practices and legal hurdles would need to be changed (Adby, 2012).

#### **E. AGE AT CASTRATION**

A question asked by producers, veterinarians, and researchers is “what is the right age to castrate calves?” While steers typically bring more at time of sale, the question of proper age at castration is very pressing in research. Several studies have looked into the effects of castration, with or without other factors, such as dehorning, and their effects on the growth performance,

hematology, and behavior of calves castrated at weaning. However, there is little research evaluating the effects of castration performed at or near birth. According to Stafford (2007), the age at castration may be determined by the cattle management practices of the operation. Robertson et al. (1994) noted that younger calves experienced less pain; however, it may not be practical to castrate young calves on large beef operations where calves may not be gathered until they are 5 or 6 mo of age and then castrated, vaccinated, branded, and dewormed. However, this may be quite opposite on smaller operations where calves are easily accessible at an early age and can be castrated in the first days or weeks of life (Stafford, 2007).

Since both weaning and castration can cause stress, King et al. (1991) suggested that the 2 procedures should not be combined. Bretschneider (2005) reported that the stress response (plasma cortisol concentrations and haptoglobin) of calves castrated at  $\leq 6$  mo of age tended to be lower than that of cattle castrated at  $> 6$  mo of age, indicating that calves castrated at a younger age tended to suffer less stress. King et al. (1991) reported that calves castrated at 78 d of age or younger, compared to calves castrated at 167 d of age or older, experienced minimal physiological stress. They also reported that delaying castration until 2 wk before weaning resulted in no growth advantage for bulls left intact compared to steers.

When weight loss was measured, Bretschneider (2005) reported that castration near birth drastically reduced the castration-associated weight loss; however, when calves were castrated at weaning, weight loss was increased, causing a weight disadvantage when compared to those calves castrated at birth. Bagley et al. (1989), however, indicated calf gain performance and body condition scores were not affected by age at castration. The development of wounds in cattle castrated post-puberty with rubber bands indicates that this method is not ideal in post-pubertal

cattle (Bretschneider, 2005). Dr. Capucille, DVM (2002) recommended that calves be castrated before 2 mo of age.

When calves enter the feedlot or stocker operation, many times there are bulls and steers mixed together. The USDA (2012) estimates that 41% of beef operations in the US do not castrate bull calves before they are marketed, and of those that do castrate calves, 18.4% do not castrate calves until they are over 122 d old. Zweiacher et al. (1979) evaluated bull and steer calves that were weaned, purchased, and immediately shipped to a feedlot. They determined that calves purchased as steers gained faster than all bulls castrated on arrival or at 1 or 2 wk post-arrival to the feedlot. Many producers continue to not castrate their calves before weaning and marketing, even though according to Smith et al. (2000), steers marketed at weaning have a \$3.56/45.4 kg advantage compared to bulls. Troxel and Barham (2012) agreed that steers bring more than bulls. They collected data from 14 sale barns across the state of Arkansas and found that on average steers brought \$116.16/45.4 kg, while bulls only brought \$109.85/45.4 kg.

## **F. GROWTH PERFORMANCE**

Most studies find there is at least some initial decrease in ADG following castration. However, when it comes to overall growth, it has commonly been thought that bulls tend to gain weight faster than steers up until weaning. During puberty the testes primarily produce androgens, among which testosterone is the most potent. Androgens promote muscular development throughout the body, increasing nitrogen retention. This anabolic property of androgens, especially testosterone, influences the ADG of bulls to provide 19% advantage over steers (Bretschneider, 2005).

Coetzee et al. (2011) reported that castration performed at weaning reduced pen ADG over the first 14 d after surgery and from d 1 to 28. In a similar study bulls castrated at weaning,

with or without meloxicam, were compared to calves that were steers upon arrival. Steers were found to have a greater ADG d 1 to 14, yet differences in ADG were no longer apparent by d 28 (Coetzee et al., 2012). From d 15 to 28, ADG increased in castrated calves but not in steer calves. When weight gain and performance was measured from birth to 6 mo of age, Knight et al. (2000) reported calves castrated at birth had lower weight gain compared to intact bull calves that were not castrated until 6 mo or bulls that were left intact until 12 mo of age. When weight gain from 6 to 12 mo of age was evaluated, they found no significant difference in weight gain between all treatments, though they did record all calves to have low weight gain. Fisher et al. (1996), observed bulls castrated at 5.5 mo of age gained less from d 0 to 7 compared to intact bulls.

Some studies have reported post-weaning gain for bulls castrated at weaning are decreased when compared to calves castrated at birth. Lents et al. (2001) reported bulls castrated at weaning exhibited a decreased ADG during the 50 d after weaning compared to bulls castrated at birth. Worrell et al. (1987) reported that there was no difference in ADG from birth until weaning between calves castrated at 70, 230, or 410 kg. Klosterman et al. (1954) found bulls left intact were heavier at weaning, but their gains were decreased immediately following castration at weaning and subsequent weights were similar to the steers that had been castrated at a younger age. However, Peterson et al. (1989) reported that calves castrated 4 wk prior to weaning gained 12.73 kg less in the feedlot compared to those left intact. Calves that were castrated 10 wk prior to weaning exhibited similar weaning weights and similar weight gains in the feedlot compared to the non-castrated intact bulls.

Bagley et al. (1989) reported that castrating calves at birth did not reduce weaning weight compared with delaying castration until 4 mo of age. Imler et al. (2011) determined that calves

castrated at 36 d of age exhibited no difference in weaning weight when compared to calves that were castrated at 131 d, and there were no differences noted in ADG. Lents et al. (2001) found that when bulls were banded at 2 to 3 mo of age, they had a greater ADG than bulls that were left intact until weaning. Worrell et al. (1987) determined that bulls castrated at 70 kg had greater weight gains compared to bulls castrated at 320 kg. Bretschneider (2005) reviewed a number of studies looking at the effects of age at castration and noted that when castration is performed at birth, weight loss is scarce to zero, and weight loss is increased quadratically as the age of castration increased.

## **G. HEMATOLOGY**

Examining the immunomodulatory response of calves can be very beneficial for determining a response to stress or pain. Evaluating a complete blood count can give information regarding several parameters, such as red blood cells, white blood cells, and platelet counts. These parameters give key information regarding how the body is responding to environmental factors, such as an infection, inflammation, or injury.

Platelets play an important role in the healing process in animals. Platelets are responsible for clotting blood, especially at the site of an incision. The normal range for platelets, regardless of age in cattle, is 100 to 800 x 10<sup>3</sup>/μl (Smith, 2008). One study investigating platelet counts reported no differences between treatments when 5.5 mo old calves were left intact or castrated (Earley and Crowe, 2002).

Parameters that are commonly evaluated in castration studies include erythrocytes (red blood cells), packed cell volume (% PCV), also known as the hematocrit, and hemoglobin. According to Smith (2008), there is a range of normal values for each, depending on the age of the calf. At 48 h, the common red blood cell count is roughly 7.72 x 10<sup>6</sup>/μl. At 3 wk it has



increased to  $8.86 \times 10^6/\mu\text{l}$ , and by adulthood can range anywhere from 5 to  $10 \times 10^6/\mu\text{l}$ . For the hematocrit, at 48 h of age, the percentage is normally 32%, increasing to 35% by 3 wk of age, and ranging between 24 and 46% in adult cattle. Hemoglobin concentrations (g/dl) follow the same pattern, starting at 10.49 g/dl at 48 h, increasing to 11.32 g/dl at 3 wk and ranging from 8 to 15 g/dl in mature cattle. While a drop in the hematocrit is typically an indication of anemia, a slight decrease with age is expected. Smith (2008) explains that PCV must be interpreted in light of the animal's hydration status and level of excitement. When Earley and Crowe (2002) evaluated the hematological changes in bull calves castrated at 5.5 mo of age, with or without ketoprofen, a NSAID, to intact bull calves, they found no differences among treatments in red blood cell concentrations, hematocrit, or hemoglobin concentrations. Pang et al. (2006), however, found that when 5.5 mo old calves were castrated by banding, on d 7, banded calves had a greater number of red blood cells than non-castrated calves.

Another hematological parameter is leukocytes, or white blood cells, which are divided into 2 broad categories: granulocytes and mononuclear leukocytes (Smith, 2008). Neutrophils, eosinophils, and basophils make up the granulocytes, while mononuclear leukocytes consist of lymphocytes and monocytes. In calves at 48 h of age, the total white blood cell count is  $7.76 \times 10^3/\mu\text{l}$ , increasing to  $8.65 \times 10^3/\mu\text{l}$  at 3 wk, and ranging from 4 to  $12 \times 10^3/\mu\text{l}$  in adult cattle (Smith, 2008). Chase et al. (1995) found that 2 d after castrating bulls (21 mo of age) total white blood cell counts were greater in castrated calves compared to non-castrated bulls.

The largest white blood cell is a monocyte, which functions to remove dead and damaged tissue and takes microbicidal action against some bacteria, viruses, fungi, and protozoa (Smith, 2008). Monocytes regulate the immune responses of limbs, defend against tumors, and work to repair and remodel tissue. In calves at 48 h of age, the typical monocyte count is  $0.35 \times 10^3/\mu\text{l}$ ,

decreasing to  $0.23 \times 10^3/\mu\text{l}$  at three wk and ranging from  $0.025$  to  $0.84 \times 10^3/\mu\text{l}$  in adult cattle (Smith, 2008). Earley and Crowe (2002) noted no differences in the percentage of monocytes in calves left intact or castrated, with or without ketoprofen.

Another category of mononuclear leukocytes are lymphocytes, which can be divided into 2 broad groups: T cells and B cells (Smith, 2008). B lymphocytes transform into plasma cells and produce antibodies. T lymphocytes can be responsible for regulation of the immune response, delayed-type hypersensitivity (DTH), graft and tumor rejection, autoimmunity, and resistance to certain intracellular pathogens (Smith, 2008). The normal lymphocyte count in calves at 48 h following birth is  $2.85 \times 10^3/\mu\text{l}$  and  $5.05 \times 10^3/\mu\text{l}$  at 3 wk of age. In adult cattle the range of lymphocytes is  $2.5$  to  $7.5 \times 10^3/\mu\text{l}$  (Smith, 2008). Pang et al. (2006) noted that on d 35 following banding, 5.5 mo old calves had lower lymphocyte percentages compared to intact bulls.

Another set of leukocytes are the granulocytes. Neutrophils act to phagocytize and destroy foreign material, especially pathogenic bacteria (Smith, 2008). They migrate into the tissue within 2 h of infection, inflammation, or injury. In calves, 48 h old, the normal value for a neutrophil count is  $4.11 \times 10^3/\mu\text{l}$ , decreasing to  $2.92 \times 10^3/\mu\text{l}$  by 3 wk, and ranging from  $0.6$  to  $4 \times 10^3/\mu\text{l}$  in adult cattle. Eosinophils control parasitic infections and regulate allergic reactions (Smith, 2008). Their normal count in calves at 48 h of age is  $0.02 \times 10^3/\mu\text{l}$ . At 3 wk it is generally unchanged, remaining at  $0.02 \times 10^3/\mu\text{l}$ , and ranges from  $0$  to  $2.4 \times 10^3/\mu\text{l}$  in adult cattle. Basophils can elicit hypersensitivity reactions through secretion of vasoactive mediators, including histamine, leukotrienes and platelet-activating factor (Smith, 2008). In 48 h old calves, the basophil count is  $0.00002 \times 10^3/\mu\text{l}$ , and remains that in 3 wk old calves. The range for adult cattle is  $0$  to  $0.2 \times 10^3/\mu\text{l}$  (Smith, 2008).

Neutrophil to lymphocyte ratio is commonly evaluated on a complete blood count, and this ratio is expected to change with age. In calves 48 h of age, the N:L is 1.44, decreasing to 0.578 at 3 wk, and ranging between 0.3 and 0.6 in adult cattle (Smith, 2008). Sutherland et al. (2011) evaluated whole blood of 3 mo old calves castrated without an NSAID compared to castrated calves that received an NSAID and found that 6 h post-castration, the N:L was lower in castrated calves that received the NSAID compared with castrated calves that did not receive the NSAID. Ballou et al. (2011) found that in calves castrated at 3 mo old castration elevated total leukocyte count and the N:L.

## **H. BEHAVIOR**

Behavior analysis is often used to validate whether an animal is showing signs of physical pain by displaying behaviors or activities that deviate from normal. These include foot stamping, tail wagging, licking at lesions, lateral recumbency, and restlessness (Capucille et al., 2002). Calves also exhibit different standing and lying behavior when they are experiencing pain. Molony et al. (1995) described what is considered to be normal when evaluating standing and lying behavior, indicating that normal ventral lying is lying in ventral or sternal recumbency with all legs under the body with head positioned up or down. Abnormal ventral lying is lying in sternal recumbency with full or partial extension of one or both hind legs or lying on sternum with the scrotum held off the ground. Descriptions are continued with lateral lying being described as lying completely on side with 2 or more legs extended. Normal standing was illustrated as standing eating, playing or walking. The final posture explained was abnormal standing: standing or walking with an obviously abnormal gait or standing stationary for more than 10 s periods with only the head moving occasionally.

Molony et al. (1995) visually monitored 1 wk old calves over a 2 h period immediately following castration. They reported that the time spent standing abnormally was greater in all castrated calves than in control calves. They observed an increase in abnormal lying time for castrated calves, as well as more restlessness, foot stamping/kicking, stretching, easing quarters and a combined index of all the active behaviors in castrated calves. Ting et al. (2003) reported similar results when they visually observed 11 mo old calves post-castration, noting that the incidence of combined lying postures was less in castrated cattle compared to non-castrated controls within a 6-h post-castration period. They also noted that castrated calves had increased combined abnormal standing activities compared to intact cohorts.

Gonzalez et al. (2010) compared steers that had been castrated at 34 d of age to bulls that were castrated at 260 d of age for differences in physical activities using a digital video camera in each pen. They noted that castrated calves tended to spend less time lying down compared to steers (24.2 vs.  $31.4 \pm 3.00\%$ ). The length of steps measured for the back legs were also reduced in castrated calves compared to steers. ( $51.5$  vs.  $53.9 \pm 0.67$  cm/step, respectively).

While methods for observing behavior in cattle include video recording or physical observation, these can be either expensive or labor intensive and the presence of a human can cause altered behavior. With today's technology, a more efficient method of monitoring cattle is with a data logging accelerometer. According to Pauly et al. (2012), accelerometers have been shown to accurately record cattle behaviors (standing, lying, walking). They have been used in previous research to characterize behavioral differences in cattle following castration. White et al. (2008) utilized accelerometers to evaluate behavior following castration of calves ( $74 \pm 6.2$  kg) and noted that the accelerometer had a high degree of accuracy (98.3%). Furthermore, accelerometers monitored activity constantly and did not influence natural behavior patterns

(White et al., 2008). The accelerometers identified no difference in the proportion of hourly time spent standing prior to or after castration between the control group and the castration group. However, castrated calves spent more time standing (82.2%) after castration than they did before castration (37.9%).

Pauly and White (2011) used accelerometers to record differences in behavior in 2 studies. In the first study, they investigated the potential for different analgesics to mitigate pain after castration or dehorning (mean weight 154 kg  $\pm$  11.50 kg) and how the analgesic affected behavior. Regardless of treatment, all calves spent more time lying down and less time walking in the post-surgery compared to pre-surgery period (Pauly and White, 2011). The second study evaluated behavior changes for 6 d after castration and/or dehorning (mean weight 144 kg  $\pm$  40.07). Calves that were dehorned and castrated spent less time walking 1 d after the procedure compared to controls, but very few other behavioral differences were identified (Pauly and White, 2011).

Pauly et al. (2012) evaluated an analgesic effect on calf behavior after concurrent castration and dehorning. Calves between the ages of 16 and 20 wk old were assigned to a treatment: castration and dehorning with sodium silicate in drinking water (SS, added drinking water), castration and dehorning with a combination of ketamine and butorphanol (XKB, IM injection), castration and dehorning with a combination of the previous 2 treatments (XKBSS, IM injection), or left intact (CON). The analgesic treatment, time relative to surgery, and the interaction between treatment and time were all associated with the proportion of time calves spent lying down (Pauly et al., 2012). All calves spent more time lying down post-treatment compared to pre-treatment, and in the post-treatment period, XKBSS calves spent more time lying compared to XKB, while SS and CON calves were not different from either treatment.

When the time spent walking was compared between pre- and post-treatment, calves in all treatment groups, other than control calves, spent less time walking post-procedures. Control calves spent less time walking in the post-treatment period compared to the pre-treatment time frame (Pauly et al., 2012). These findings contradict previous research indicating cattle spend a greater percentage of time standing immediately following castration.

## **G. CONCLUSION**

Castration is a necessary procedure in beef operations. However, castration does cause pain and stress. With castration being necessary it is important to have a good understanding of how castration affects calf growth performance, physiological responses, and how their behavior changes. Many studies have looked at several aspects of castration and how it affects the calf and even the producer regarding cost and labor. However, further research is necessary to ensure that the most efficient and humane procedures are practiced.

As animal welfare become more prevalent in society, and more activists voice concerns about management practices, the importance of having research and results showing the most humane and efficient way of dealing with every day farm procedures is a must. Castration is painful and does cause stress, and being able to identify methods that make it less stressful and painful to the calf is the ultimate goal in conducting studies regarding castration and its effects.

From the method of castration, to the use of analgesia to reduce pain, there are different measures that can be taken to ensure a healthy calf and a smooth running operation. Research has shown that castration at or near birth gives better results in performance and reduced stress. However, the benefit of analgesia on calves castrated at different stages of maturity has not been researched in detail. Therefore, the objective of our study is to determine if the timing of

castration, near birth or at weaning, with or without analgesia, has an affect on the growth performance, hematology, or behavior of beef calves.

## CHAPTER 3 – EFFECTS OF CASTRATION WITH OR WITHOUT ANALGESIA ON GROWTH, HEMATOLOGY, AND BEHAVIOR IN NEONATAL BEEF CALVES

### A. ABSTRACT

Angus-sired ( $n = 30$ ) and Hereford-sired ( $n = 31$ ) bull calves were assigned randomly to 1 of 3 treatments. Treatments were 1) surgical castration near birth, 2) oral administration of the analgesic meloxicam (1 mg/kg of body weight) followed by surgical castration near birth, or 3) bulls remained intact. Calf standing and lying activity was monitored by recording x and y axis positions of a data logger accelerometer attached to the metatarsus of the right leg for 7 d. In addition, blood was collected on d 0, 1, 3, and 7 from a subset of calves. Body weight was recorded on all calves on d 4, 33, 66, 116, 162, 199, and 214. Average daily gain did not differ between treatments ( $P \geq 0.88$ ) through weaning, and no differences ( $P \geq 0.49$ ) in weights were recorded at any weigh date. White blood cell counts were not affected by treatment ( $P = 0.47$ ), but were affected by day ( $P = 0.002$ ) with d 1 and 3 being lower than d 0. A treatment x day interaction was noted for the percentage of neutrophils ( $P = 0.01$ ) and percentage of lymphocytes ( $P = 0.02$ ). The neutrophil to lymphocyte ratio decreased with day ( $P < 0.0001$ ). Data from accelerometers revealed 3 distinct behaviors being characterized as; lying flat on side, standing, and lying on sternum. Mean time spent lying flat on side and standing were not affected by treatment ( $P > 0.17$ ). There was, however, a tendency for treatment differences ( $P = 0.07$ ) in mean time lying on sternum, with calves castrated with analgesia spending the least amount of time and intact bulls spending the most amount of time lying on sternum. Overall, bull calves subjected to neonatal castration with or without meloxicam did not appear to exhibit negative diversions to the standing behavior, hematology, or growth performance through weaning when compared to intact bulls.



## **B. MATERIALS AND METHODS**

Angus-sired (n = 30) and Hereford-sired bull calves (n = 31) were born between September 1, 2011 and November 18, 2011. Treatments and procedures did not differ between sire groups. Treatment 1 (n = 14) included surgical castration near birth, without meloxicam; treatment 2 (n = 15) included surgical castration near birth with meloxicam; and treatment 3 (n = 32), calves were left intact until weaning. Throughout the study, all animals were housed and cared for in compliance with procedures approved by the University of Arkansas Animal Care and Use Committee.

On d 0, body weights were recorded and castration was performed if it applied. If the calf was assigned to receive meloxicam, it was administered orally immediately before castration. Meloxicam (Yung Shin Pharmaceutical Ind. Co. Ltd.; Tachan Taichung, Taiwan) was dosed based on weight at 1 mg/kg. It was crushed and mixed with 20 ml of water and drenched into the calf's mouth. The drench syringe was then rinsed with another 20 ml of water, and rinsed into the calf's mouth. Castration was performed with a scalpel, removing the bottom third of the scrotum. The testes were then pulled down from the inside of the scrotum, and the spermatic cord was severed with a scalpel. The wound was sprayed with a disinfectant and a fly control, and the calf was allowed to return to its dam. Thirteen Angus sired and 16 Hereford sired calves were selected randomly and with their dams were moved to a separate facility for further evaluation. All other calves were returned to their original pastures with their dams. Blood samples were taken from the relocated calves, via jugular venipuncture. Samples were collected into test tubes containing EDTA (Vacutainer, BD, Franklin Lakes, NJ). Each calf's rectal temperature was measured via digital thermometer, and each was fitted with an accelerometer (HoBeware Pendant G, Onset Computer Corp, Bourne, MA) to be in place for 7 d.

The accelerometer was attached lateral to the metatarsus of the right hind leg, using Velcro (Velcro USA, Inc, Manchester, NH) that had a heavy duty adhesive on the back of each side. The accelerometer was then secured further by placing Vetrap (3M, St Paul, MN) around the leg and over the accelerometer. The accelerometers were programmed to record at 20 s intervals, and date, time, X and Y axis positions were recorded for 7 d.

On d 1, 3 and 7, the relocated calves were again weighed and blood drawn, while the accelerometers continued to take readings, ending with 7 24 h periods. At the end of the 7 d period accelerometers were removed and calves were returned, with their dams, to their original pasture. A complete blood count was performed (Hemavet 950FS, Drew Scientific, Waterbury, CT) within 5 h of blood samples being collected on d 0, 1, 3 and 7.

Body weights (BW) were recorded from all calves on d 4 (d 1 representing average date of birth, September 26), 33, 66, 116, 162, 199, and 214. On d 66, calves were vaccinated with Pyramid 5 (modified live viral respiratory vaccine, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and Alpha 7 (clostridial vaccine, Boehringer Ingelheim Vetmedica, Inc.). Second injections of these vaccines, as well as Cydectin pour on dewormer (1 mL/1.1 kg of BW, Boehringer Ingelheim Vetmedica, Inc.), were administered on d 199. Weaning occurred on d 214.

The data stored on the accelerometers was downloaded using the HoBeware Lite Graphing and Analysis Software (Onset Computer Corp) and exported as a CSV file for analysis. An x-y graph was generated for each calf to examine evidence for clustering (Figure 1) using R plot function (R Project for Statistical Computing, [www.r-project.org](http://www.r-project.org)). Upon recognizing 3 potential clusters, a cluster analysis was performed by calf using the clara function, belonging to the cluster package for R (Maechler et al., 2012), setting the number of clusters to 3. Clusters

were coded 1 to 3 and graphical displays for each calf were generated using the R plot function and setting the data point colors to match their cluster category (Figure 2). The algorithm to cluster data does not necessarily categorize clusters similarly within the same x-y spatial plane for each calf. The clara package assigns a data point to a cluster dependent upon spatial location and number of points which varies by calf. Therefore, each calf's accelerometer data were graphed to ensure that all predicted clusters that fell within similar spatial planes on the x-y graph were coded similarly. If the cluster analysis labeled the data points as cluster 2 that fell within the same plane as cluster 1 for previous calves, then cluster 2 was recoded as cluster 1 for the new calf and cluster 1 would likewise be coded as 2 or 3 depending upon where in the spatial plane the cluster existed.

For calibration purposes, a data logger accelerometer was connected to a computer to monitor live x and y axis positions as the position of the accelerometer was altered to mimic calf leg position. The accelerometer position was modified until the x and y axis of the active accelerometer matched the x and y cluster medoid. The position where the accelerometer equaled the cluster medoid was used to establish position. The corresponding positions, based on logger position on the calf's leg, were lying flat on side (C1), standing (C2), or lying on sternum (C3).

The cluster positions were summarized within calf by day to establish the proportion of time spent each day in the lying on sternum, standing, or lying flat on side position. The proportion of activity exhibiting C1, C2, or C3 behavior was modeled for a binomial distribution. The effect of castration, meloxicam treatment, day, and all interactions on proportion of time exhibiting each of the 3 behaviors and the lying on sternum and lying flat on side combined behavior was estimated as a repeated measure using the GLIMMIX procedure of SAS v 9.2

(SAS Inst., Cary, NC). Kenward Rogers test was used as the degrees of freedom selection method. Contrast statements were used to compare calves receiving meloxicam against those that did not, and to compare all castrated to all non-castrated calves. When a treatment x day interaction was significant, treatment differences within day were compared using the slice option of the least squares means statement.

To examine the number of times a calf exhibited each of the 3 logged behaviors throughout the day and the duration of those events, an algorithm was constructed in R to create a variable that examined the current cluster value and record a new value each time the cluster changed. Within calf, a data frame was created from a table that summarized the frequencies of the new variable. For example, the new data frame would contain 1,1,1,3 corresponding to day = 1, cluster = 1, new variable = 1, frequency of new variable = 3. From this data frame, the number of times a calf exhibited cluster 1 (lying flat on side) behavior could be determined. Given that each record represented a 20 s duration, the frequency of the new variable indicated the amount of time exhibiting that behavior. The number of events for each behavior was determined within day for each calf and the average duration of each event was determined. The event and duration responses were modeled for a Poisson and normal distribution, respectively, and the event analysis included an over dispersion parameter. Both number of events and average duration of events were analyzed in GLIMMIX as a repeated measure.

Body weight, rectal temperatures, and hematological results were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Statistical significance was considered for a *P*-value of less than or equal to 0.05. Kenward Rogers test was used as the degrees of freedom selection method. Repeated measures were run for the blood data and rectal temperatures using day, and the subject of the statement was calf. Contrast statements were

made to compare all castrated to non-castrated, and to compare calves receiving meloxicam to those that did not. The random effect was sire and the subject of the statement was calf.

### C. RESULTS AND DISCUSSION

Birth weights ( $P = 0.76$ ), weights recorded on d 4 ( $P = 0.91$ ), d 33 ( $P = 0.65$ ), d 66 ( $P = 0.49$ ), d 116 ( $P = 0.63$ ), d 162 ( $P = 0.94$ ), d 199 ( $P = 0.96$ ), and weaning weight ( $P = 0.95$ ; Table 1) were similar between treatments. Average daily gain from birth to weaning was also similar between treatments ( $P = 0.88$ ). These results are also similar to Imler et al. (2011), who noted that calves castrated at 36 d of age showed no difference in weaning weight or ADG compared to calves castrated at 131 d of age, indicating that later castration did not benefit the weight gain of the animal. Worrell et al. (1987) also reported similar results when there was no difference in ADG from birth until weaning between calves castrated at 70, 230, or 410 kg. The expected results were for calves castrated at birth to have lower weaning weights compared to calves that were left intact.

White blood cell counts were not affected by treatment ( $P = 0.47$ ), but decreased on d 1 and d 3 ( $P = 0.002$ ; Figure 3) compared to d 0. Chase et al. (1995) reported a treatment x day interaction in white blood cell counts in bulls castrated at approximately 21 mo of age, white blood cell counts of castrated bulls tended to rise and fall over a wider range compared to non-castrated bulls for samples that were taken on d 0, 2, 5, 7, 9, 14, 21, 28, and 35.

Over the sampling period neutrophil percentage steadily decreased (day,  $P < 0.0001$ ), and there was a treatment x day interaction for percentage of neutrophils ( $P = 0.01$ ; Figure 4). On d 0, calves castrated without meloxicam had a greater percentage ( $P = 0.05$ ) of neutrophils compared to calves castrated with meloxicam. However, on d 7 calves that did not receive meloxicam tended to have a lower percentage of neutrophils ( $P = 0.06$ ) compared to calves that

received meloxicam. Over the sampling period lymphocyte percentages steadily increased (day,  $P < 0.0001$ ) and a treatment x day interaction was noted for the percentage of lymphocytes ( $P = 0.02$ ; Figure 5). On d 7, calves castrated without receiving meloxicam tended to have a greater percentage of lymphocytes ( $P = 0.06$ ) than calves castrated after receiving meloxicam.

The neutrophil to lymphocyte ratio decreased with day ( $P < 0.0001$ ; Figure 6), however, no differences were noted in treatment ( $P = 0.32$ ), and no treatment x day interaction was observed ( $P = 0.12$ ). Our results differed from those of Ballou et al. (2011), who noted that castration of 3 mo old bull calves elevated the neutrophil to lymphocyte ratio ( $P < 0.01$ ) 24 h after castration. These results were somewhat expected because as a calf ages to 2 d, the neutrophil count decreases, and by 3 wk the lymphocyte count is greater than that of the neutrophils (Smith, 2008). There were no differences recorded in the percentage of monocytes ( $P = 0.70$ ), the percentage of basophils ( $P = 0.93$ ), or the percentage of eosinophils ( $P = 0.94$ ; data not shown).

There was no treatment difference ( $P = 0.19$ ; Figure 7) or a treatment x day interaction ( $P = 0.88$ ) for red blood cell counts; however, there was a decrease noted in day ( $P < 0.0001$ ). The amount of hemoglobin in the blood followed the same pattern, with no difference in treatment ( $P = 0.42$ ), and no treatment x day interaction ( $P = 0.97$ ), but decreased hemoglobin with day ( $P < 0.0001$ ; data not shown). Overall, hematocrit was not affected by treatment ( $P = 0.29$ ; Figure 8). However, results indicated a day effect ( $P < 0.0001$ ) and a treatment x day interaction ( $P = 0.03$ ) for hematocrit. On d 1, hematocrit was greatest for bulls, intermediate for calves castrated with meloxicam, and least for calves castrated without meloxicam. Blood loss can commonly be a cause for a decrease in hematocrit, which would be expected in calves castrated.

Treatment differences were not noted for platelet count ( $P = 0.54$ ; Figure 9) or treatment x day ( $P = 0.49$ ), but an increase in day was noted ( $P < 0.0001$ ). Platelets are important for wound healing and clotting blood. After an incision, an increase in platelet count would be expected as the body prepares to heal and stop the bleeding.

Rectal temperature tended to be affected by treatment ( $P = 0.06$ ; Figure 10) and a day effect ( $P < 0.0001$ ) was found. Furthermore, all castrated calves were contrasted against intact bulls, castrated calves had a greater rectal temperature ( $P = 0.04$ ) compared to intact bulls. Overall, calves castrated with meloxicam had a greater temperature ( $P = 0.02$ ) compared to bulls left intact, while calves castrated without meloxicam fell in the middle. This indicated that the use of meloxicam did not help to reduce body temperature associated with castration. On d 0, calf rectal temperature was taken prior to administration of meloxicam. A difference in day was noted, with calves on d 1, 3 and 7 having a lower rectal temperature than calves on d 0. Multiple factors can affect the body temperature of calves; including, but not limited to, the stress of handling, separation from mother, pain or injury, and weather and environmental factors. Temperatures were taken prior to castration, thus the castration process cannot be attributed to the greater temperature on d 0. However, this would be the first time that the calf was handled and the calf was separated from its mother during processing. This initial stress could be a cause for an increase in initial temperature, as with stress it is common to see an increase in body temperature. As the calf becomes acclimated to being handled, a decrease in rectal temperature is expected. As the initial pain of castration subsided, a decreased temperature would be expected with time. Environmental factors could play a huge roll in the temperature data that was analyzed. For this portion of the study, calves were housed at different times of the year (between September and November) at the test facility. This allots for a large range of

differences in weather. Outdoor temperature has a large effect on the body temperature of the calf, and could have an effect on the increase or decrease of calf rectal temperature.

For the analysis of behavior, day represents a full 24 h period, with d 0 representing the first 24 h period, and so on. Overall, proportion of time expressed in lying flat on side (C1), standing (C2) and lying on sternum (C3) was 0.36, 0.23, and 0.41%, respectively. Proportion of daily activity expressed as C1 (lying flat on side) was not different between treatments ( $P = 0.23$ ; Figure 11). There was a treatment x day interaction ( $P = 0.05$ ). However, test of simple effects by day indicated no treatment differences on most days with the exception of d 5 where castration at birth with meloxicam differed from castration without meloxicam and non-castrated calves ( $P < 0.01$ ). Proportion of activity expressed as C2 (standing) did not differ among treatments ( $P = 0.50$ ) or over time ( $P = 0.13$ ; Figure 12). Molony et al. (1995) continuously evaluated 1 wk old calves visually for 180 min immediately following castration for behavioral changes, control calves spent less time standing compared to all castrated calves. Proportion of time expressed as C3 (lying on sternum) was 0.38, 0.40, and 0.42 ( $\pm 0.01$ ) for castrated with meloxicam, castrated without meloxicam, and non-castrated, respectively ( $P = 0.0017$ ). Proportion of time spent lying on sternum was decreased ( $P = 0.0005$ ; Figure 13) for castrated with meloxicam calves compared to non-castrated calves, and proportion of time spent lying on sternum tended to be less for castrated calves given meloxicam compared to those without meloxicam ( $P = 0.08$ ).

Accelerometer data was analyzed to determine how many times (events) calves exhibited C1, C2, and C3 behavior throughout the day. Calves castrated with meloxicam laid flat on side 44.26 times, while bulls laid down 33.64 and calves castrated without meloxicam laid down 33.14 times. There was no effect of treatment ( $P = 0.18$ ; Figure 14) or day effect ( $P = 0.14$ ) or a



treatment x day interaction ( $P = 0.14$ ). The event of standing was recorded to be 45.7, 44.6, and 41.9 ( $\pm 2.22$ ) times for calves castrated with meloxicam, castrated without meloxicam, and bulls left intact; respectively. While no treatment effects ( $P = 0.38$ ) were found, there was an effect of day ( $P = 0.03$ ) with an initial decrease noted on d 0 to 1 and d 1 to 3 and 5 (Figure 15). There was no treatment x day interaction ( $P = 0.22$ ). For the event C3, or lying on sternum, calves castrated with analgesia laid down 50.3 times, bulls 42.0, and calves castrated without analgesia 41.7 times, and no significant differences were found among treatments ( $P = 0.37$ ; Figure 16) or among days ( $P = 0.18$ ). There was also no treatment x day interaction ( $P = 0.14$ ).

The average amount of time (min) spent in each position was calculated. Mean duration among C1 events (lying flat on side) was 14.56, 19.34, and 18.86 ( $\pm 1.7$ ) min for castrated with meloxicam, castrated without meloxicam, and intact bulls, respectively; there was no effect of treatment ( $P = 0.17$ ; Figure 17), and no day effect ( $P = 0.58$ ) or treatment x day interaction ( $P = 0.70$ ). For C2 (standing) event duration, there was a tendency for a treatment x day interaction ( $P = 0.08$ ; Figure 18) but there was no a treatment or day effect ( $P = 0.79$ ,  $P = 0.59$ ; respectively). Calves castrated with analgesia had a mean duration of 6.82 min, calves castrated without analgesia recorded a mean duration of 8.00 min, and calves left intact: 8.33 min ( $\pm 0.0001$ ). There was a tendency for differences among treatments for mean duration ( $P = 0.07$ ; Figure 19) for C3 (lying on sternum) events. There was a day effect ( $P = 0.05$ ), but no treatment x day interaction ( $P = 0.67$ ). Lying on sternum duration for calves castrated with meloxicam, castrated without meloxicam, and bulls, was 12.18 ( $\pm 1.74$ ), 16.28 ( $\pm 1.61$ ), and 17.01 ( $\pm 1.10$ ) min, respectively.

While our evaluation methods can not show whether or not a calf lying was due to pain or because of contentment, or whether or not standing was a sign of pain, or if calves were

standing due to eating or moving, it is typically thought a lying position may be an indication of comfort and contentment. The procedure of castration causes pain at the site of the incision; however, the use of meloxicam is to reduce this pain, and thus increasing the amount of time spent lying (if lying is associated with contentment). The number of events exhibited in a day was never different between treatments for any of the three positions. Our behavior results, however, show that the proportion of time spent lying on sternum and the mean duration of time spent lying on sternum, were not increased when meloxicam was used with castration. The expected behavior of non-castrated calves was for them to exhibit a lower proportion of time standing compared to castrated calves; however, standing behavior was never different between treatments.

#### **D. CONCLUSION**

Overall, castration and meloxicam affected the lying behavior of calves when castration was performed near birth. Number of events recoded showed a treatment x day interaction or tendency for all positions, and the mean duration times of both lying position showed differences in treatments. Also, minor changes were noted in the percentages of neutrophils and lymphocytes during the first week, and an increased hematocrit was detected in bulls after the first day. Daily differences for blood data can be attributed to the calves being handled on d 1, 3, and 7 when blood samples and weights were recorded on calves. The added handling of calves on these days could cause an increased stress response of calves, causing differences in blood counts, as well as differences in temperature. As the week progressed and calves were handled more frequently, the signs of stress, blood parameters and body temperature, would be decreased. Also, not all calves were housed at the test facility at the same time, due to differing dates of birth. The difference in time causes there to be a difference in environment, and even

handling of cattle. These different situations can cause calves to have a different response to the environment around them, which could increase variability. However, calves were assigned to treatments following birth to balance for age therefore, treatment groups would be equally represented at similar points in time, and an overall examination of the behavior figures suggest that the days that did not involve cattle handling showed similar activities as days cattle were handled. The use of meloxicam when castrating did not benefit the calf, and instead caused an increase in body temperature compared to bulls left intact and those calves that did not receive meloxicam. Castrating near birth with or without meloxicam had no effect on calf weight or average daily gain up until weaning. These results suggest that there is no disadvantage to calves castrated at birth, and that the use of meloxicam did not improve calf productivity. The results suggest that though there are minor differences in blood parameters, as well as behavior, the impact was not great enough to recommend delaying castration, especially based on weight gain and growth performance.

## CHAPTER 4 – EFFECT OF CASTRATION WITH OR WITHOUT MELOXICAM ON GROWTH, HEMATOLOGY, AND BEHAVIOR OF BEEF CALVES CASTRATED AT WEANING

### A. ABSTRACT

Angus-sired ( $n = 29$ ) and Hereford-sired ( $n = 31$ ) calves were assigned randomly, upon birth, to 1 of 3 treatments. Treatments were 1) surgical castration near birth ( $n = 28$ , one calf died between time of birth and weaning), 2) surgical castration at weaning ( $n = 16$ ), or 3) surgical castration at weaning with oral administration of meloxicam (meloxicam at 1 mg/kg of body weight;  $n = 16$ ). Calf standing and lying activities were monitored by recording x and y axis positions of a data logger accelerometer attached to the metatarsus of the right leg for 7 d. In addition, blood was collected on d 0, 0 + 6 h, 1, 3, 7, and 14 post-weaning. Body weight was recorded on all weaned calves on d 0, 14, 28, and 56. Average daily gain was greater in steers compared to bulls castrated without meloxicam ( $P = 0.02$ ) from d 0 to 56. White blood cell counts were not affected by treatment ( $P = 0.17$ ), but had a day effect ( $P < 0.0001$ ). A treatment x day interaction was noted for the percentage of neutrophils ( $P < 0.001$ ) and percentage of lymphocytes ( $P < 0.001$ ). The neutrophil to lymphocyte ratio showed a treatment x day interaction ( $P = 0.0002$ ). Data from accelerometers revealed 3 distinct behaviors being characterized as; lying flat on side, standing, and lying on sternum. When proportions of time spent standing were compared among treatments, steers spent the least amount of time standing ( $P = 0.007$ ) when compared to bulls castrated with meloxicam, and bulls castrated with meloxicam had a greater proportion of time spent standing ( $P = 0.05$ ) compared to calves castrated without meloxicam. The duration of time spent lying on sternum was not affected by treatment ( $P = 0.22$ ). Overall, bulls subjected to castration at weaning with or without meloxicam did not appear to exhibit negative diversions to the hematology or lying behavior.

The ADG, however, was improved for steers, previously castrated at birth, compared to bulls castrated at weaning.

## **B. MATERIALS AND METHODS**

Angus-sired (n = 29) and Hereford-sired bull calves (n = 31) which were born between September 1, 2011 and November 18, 2011, were assigned randomly within sire group to a treatment upon birth. Treatment 1 (n = 28) included surgical castration near birth (this group included calves that did and did not receive meloxicam at birth); treatment 2 (n = 16), calves were castrated at weaning without meloxicam, and treatment 3 (n = 16), calves were castrated at weaning with meloxicam. Throughout the study, all animals were housed and cared for in compliance with procedures approved by the University of Arkansas Animal Care and Use Committee.

At 66 d of age, calves were vaccinated with Pyramid 5 (modified live viral respiratory vaccine, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and Alpha 7 (anti-clostridial vaccine, Boehringer Ingelheim Vetmedica, Inc.) and second injections of these vaccines, as well as Cydectin pour-on dewormer (1 mL/1.1 kg of body weight, Boehringer Ingelheim Vetmedica, Inc.), were administered on d 199.

Calves were weaned at 214 d of age (d 0). Body weights were recorded on all calves and castration was performed if applicable with or without meloxicam. Calves receiving meloxicam were dosed with meloxicam (Yung Shin Pharmaceutical Ind. Co. Ltd.; Tachan Taichung, Taiwan) immediately prior to castration at 1 mg/kg BW. Tablets were crushed and mixed with 20 ml of sterile water and dispensed into the calf's mouth as a drench solution. The syringe was then rinsed with another 20 ml of sterile water, and drenched into the calf's mouth. Castration was performed with a scalpel, removing the bottom third of the scrotum. The testes were then

pulled down from the inside of the scrotum, and the spermatic cord was severed using a scalpel. The area was sprayed with a disinfectant and a fly control.

Thirteen Angus-sired and 15 Hereford-sired calves were selected randomly and were assigned randomly to 1 of 4 0.45 hectare pens. All other calves were assigned randomly to 4 other 0.45 hectare pens. All 8 pens had *ad libitum* access to hay, grass, and water. Calves were fed up to 1.82 kg/d of a mixed grain supplement (Table 2) at 0800 each morning. Blood samples were taken from selected calves, via jugular venipuncture, and were collected into test tubes containing EDTA (Vacutainer, BD, Franklin Lakes, NJ). Calf lying and standing behaviors were determined by attaching a data logger accelerometer (HoBeware Pendant G, Onset Computer Corp, Bourne, MA) lateral to the metatarsus of the right hind leg, using Velcro (Velcro USA, Inc, Manchester, NH) that had a heavy duty adhesive on the back of each side. The accelerometer was further secured by placing Vetrap (3M, St Paul, MN) around the leg and over the accelerometer. The accelerometers were programmed to record at 20 s intervals, and date, time, X and Y axis positions were recorded for 7 d.

Accelerometers collected data from d 0 until they were removed on d 7, recording 7 24 h periods. On d 1, 3, 7, and 14, body weights were obtained from selected calves. A complete blood count was performed (Hemavet 950FS, Drew Scientific, Waterbury, CT) within 5 h from blood samples collected on d 0, 0 + 6 h, 1, 3, 7 and 14. Body weights were recorded on all calves d 0, 14, 28, and 56. On d 28 calves were moved to pasture where they had *ad libitum* access to grass and water and were fed corn gluten feed at a rate of 0.5% of their body weight at 0800 each morning. On d 64 calves were shipped to West Texas A&M in Canyon, TX for a summer grazing period. Calves had *ad libitum* access to water and sorghum x sudan hybrid grass and were supplemented a complete mineral. Calves were weighed on d 108 and 141.

The data stored on the accelerometers were downloaded using the HoBeware Lite Graphing and Analysis Software (Onset Computer Corp) and exported as a CSV file for analysis. An x-y graph was generated for each calf to examine evidence for clustering (Figure 1) using R plot function (R Project for Statistical Computing, [www.r-project.org](http://www.r-project.org)). Upon recognizing 3 potential clusters, a cluster analysis was performed by calf using the clara function, belonging to the cluster package for R (Maechler et al., 2012), setting the number of clusters to 3. Clusters were coded 1 to 3 and graphical displays for each calf were generated using the R plot function and setting the data point colors to match their cluster category (Figure 2). The algorithm to cluster data does not necessarily categorize clusters similarly within the same x-y spatial plane for each calf. The clara package assigns a data point to a cluster dependent upon spatial location and number of points which varies by calf. Therefore, each calf's accelerometer data were graphed to ensure that all predicted clusters that fell within similar spatial planes on the x-y graph were coded similarly. If the cluster analysis labeled the data points as cluster 2 that fell within the same plane as cluster 1 for previous calves, then cluster 2 was recoded as cluster 1 for the new calf and cluster 1 would likewise be coded as 2 or 3 depending upon where in the spatial plane the cluster existed.

For calibration purposes, a data logger accelerometer was connected to a computer to monitor live x and y axis positions as the position of the accelerometer was altered by calf position. The accelerometer position was modified until the x and y axis of the active accelerometer matched the x and y cluster medoid. The position where the accelerometer equaled the cluster medoid was used to establish position. The corresponding positions, based on logger position on the calf's leg, were lying flat on side (C1), standing (C2), or lying on sternum (C3).

The cluster positions were summarized within calf by day to establish the proportion of time spent each day in the lying on sternum, standing, or lying flat on side position. The proportion of activity exhibiting C1, C2, or C3 behavior was modeled for a binomial distribution. The effect of castration, meloxicam treatment, day, and all interactions on proportion of time exhibiting each of the 3 behaviors and the lying on sternum and lying flat on side combined behavior was estimated as a repeated measure using the GLIMMIX procedure of SAS v 9.2 (SAS Inst., Cary, NC). Kenward Rogers test was used as the degrees of freedom selection method. Contrast statements were used to compare calves receiving meloxicam against those that did not, and to compare all castrated to all non-castrated calves. When a treatment x day interaction was significant, treatment differences within day were compared using the slice option of the least squares means statement.

To examine the number of times a calf exhibited each of the 3 logged behaviors throughout the day and the duration of those events, an algorithm was constructed in R to create a variable that examined the current cluster value and record a new value each time the cluster changed. Within calf, a data frame was created from a table that summarized the frequencies of the new variable. For example, the new data frame would contain 1,1,1,3 corresponding to day = 1, cluster = 1, new variable = 1, frequency of new variable = 3. From this data frame, the number of times a calf exhibited cluster 1 (lying flat on side) behavior could be determined. Given that each record represented a 20 s duration, the frequency of the new variable indicated the amount of time exhibiting that behavior. The number of events for each behavior was determined within day for each calf and the average duration of each event was determined. The event and duration responses were modeled for a Poisson and normal distribution, respectively,



and the event analysis included an over dispersion parameter. Both number of events and average duration of events were analyzed in GLIMMIX as a repeated measure.

Results for blood counts, rectal temperature, and body weights were analyzed using the PROC MIXED procedure of SAS. Statistical significance was considered for a *P*-value of less than or equal to 0.05. Kenward Rogers test was used as the degrees of freedom selection method. Repeated measures were run for blood data and rectal temperature using day, and the subject of the statement was calf. The covariance model structure used was SP(POW). A contrast statement was run for steers vs. all castrated bulls, as well as bulls castrated with meloxicam vs. calves castrated without meloxicam. The random effect was sire and the subject of the statement was calf. Correlations between proportions of time standing or lying and ADG for the 7 d post-castration period were analyzed using PROC CORR.

## **C. RESULTS AND DISCUSSION**

Weaning weights ( $P = 0.52$ ), weights recorded on d 14 ( $P = 0.84$ ), d 28 ( $P = 0.81$ ), and d 56 ( $P = 0.86$ ; Table 3) were similar among treatments. Average daily gain from weaning to d 56 was greater in steers ( $P = 0.02$ ) compared to bulls castrated without meloxicam, while bulls castrated with meloxicam fell intermediately and were not significantly different from the other treatments. Steers had a greater ADG when contrasted with all bulls castrated ( $P = 0.04$ ). The ADG from d 0 to 14 was greater in steers ( $P = 0.002$ ) compared to bulls castrated without meloxicam, and again, bulls castrated with meloxicam fell intermediately. Klosterman et al. (1954) found differing results when comparing weaning weights. They found that bulls left intact were heavier at weaning, but their gains decreased immediately following castration at weaning and their subsequent weights were similar to the early castrated steers. Coetzee et al. (2011) reported that castration performed at weaning reduced ADG over the first 14 d after

surgery and from d 1 to 28. When bulls castrated at weaning, with or without meloxicam, were compared to steers, Coetzee et al. (2012) found that the ADG of steers was greater than castrated bulls from d 1 to 14, but these effects were not apparent by d 28. When weights during the grazing period were analyzed, no differences were found on d 108 ( $P = 0.81$ ; Table 4) or d 141 ( $P = 0.84$ ). No difference ( $P = 0.61$ ; Table 4) was noted in the ADG during the grazing period, indicating the cattle castrated at weaning did not have any compensatory gain following the decreased gain immediately post-castration.

Total white blood cell count was not affected by treatment ( $P = 0.17$ ; Figure 20), however, there tended to be a treatment x day interaction ( $P = 0.09$ ) with an increase at 6 h post-castration in cattle castrated without meloxicam. There was an effect of day ( $P < 0.0001$ ) greater white blood cell concentrations were found at d 0 + 6 h than at other sampling times. Chase et al. (1995) found similar results when they evaluated whole blood of 21 mo old bulls that had been castrated, and noted a treatment x day interaction for the total white blood cell counts. They found that 2 d following castration bulls left intact exhibited lower total white blood cell counts compared to castrated males.

Treatment ( $P < 0.01$ ; Figure 21) and day ( $P < 0.0001$ ) effects, and a treatment x day ( $P = 0.0002$ ) interaction was noted for the neutrophil to lymphocyte ratio (N:L). All castrated bulls displayed a greater ratio ( $P = 0.0019$ ) when contrasted to steers. There was an increase in the N:L at 6 h post castration, but then the N:L decreased through d 14. Six h after castration on d 0, steers had lower mean N:L compared to both bulls castrated without meloxicam ( $P < 0.0001$ ) and bulls castrated with meloxicam ( $P < 0.0001$ ). The same was true on d 1, with steers again exhibiting a lower N:L compared to bulls castrated without meloxicam ( $P = 0.0018$ ) and bulls castrated with an meloxicam ( $P = 0.0058$ ). By d 3, steers continued to exhibit a lower N:L

compared to calves castrated with meloxicam ( $P = 0.009$ ). Ballou et al. (2011) noted similar results in calves castrated at 3 mo of age exhibiting elevated N:L compared to bulls left intact. Davis and Maerz (2008) suggest that with an increase in stress, it is very common to see an increase in N:L. An increase in neutrophil count is expected after an injury or painful procedure. This increase is the body's natural defense against infection and disease. As the neutrophil count increases, the lymphocyte count decreases, causing an increased N:L. Sutherland et al. (2011) evaluated whole blood of 3 mo old calves castrated without an NSAID compared to castrated calves that received an NSAID and found that 6 h post-castration, the N:L was lower in castrated calves that received the NSAID compared with castrated calves that did not receive the NSAID. Though not significant, data from the present study showed that numerically calves castrated with meloxicam displayed a greater N:L.

The percentage of neutrophils differed by treatment ( $P = 0.03$ ; Figure 22), and there was an initial increase, then decrease in day ( $P < 0.0001$ ). A treatment x day interaction ( $P = 0.0008$ ) was noted. Steers were contrasted against all bulls castrated, and steers exhibited a lower mean neutrophil percentage ( $P = 0.0075$ ) across all days compared to bulls. At 6 h post-castration, steers exhibited a lower neutrophil percentage compared to bulls castrated without meloxicam ( $P = 0.0002$ ) and bulls castrated with meloxicam ( $P = 0.0002$ ). On d 1, following castration, steers were again exhibiting a lower percentage compared to bulls castrated without meloxicam ( $P = 0.0012$ ) and bulls castrated with meloxicam ( $P = 0.02$ ). Bulls castrated without meloxicam ( $P = 0.04$ ) and bulls castrated with meloxicam ( $P = 0.005$ ) had a greater percentage of neutrophils on d 3 compared to steers.

Differences were found in the percentage of lymphocytes due to treatment ( $P = 0.03$ ; Figure 23) and an initial decrease, then increase was noted for day ( $P < 0.0001$ ). A treatment x

day interaction ( $P = 0.0024$ ) was noted. Overall, steers had a greater percentage of lymphocytes ( $P = 0.0093$ ) when contrasted against all bulls castrated. Steers had a greater percentage of lymphocytes compared to bulls castrated without meloxicam ( $P = 0.01$ ). Six hours after castration, steers had a greater lymphocyte percentage compared to both bulls castrated without meloxicam ( $P = 0.0003$ ) and bulls castrated with meloxicam ( $P = 0.0002$ ). The same pattern was noted on d 1, with lymphocyte percentages in steers being greater compared to bulls castrated without meloxicam ( $P = 0.0094$ ) and bulls castrated with meloxicam ( $P = 0.02$ ). By d 3, lymphocyte percentage in steers was greater compared to bulls castrated with meloxicam ( $P = 0.0043$ ). Pang et al. (2006) found that on d 35, 5.5-mo-old calves that had been banded had lower lymphocyte percentages than control calves.

Percentages of monocytes (data not shown) were not affected by treatment ( $P = 0.13$ ) and no treatment x day ( $P = 0.23$ ) interaction was found. However, a day effect ( $P < 0.0001$ ) was noted. An initial decrease at 6 h was found and then an increase until d 14. Our results are similar to that of Earley and Crowe (2002), who noted no differences in monocyte percentages in 5.5 mo old calves left intact or castrated, with or without ketoprofen. The current study noted no differences in the percentage of basophils ( $P = 0.92$ ; data not shown) or percentage of eosinophils ( $P = 0.93$ ; data not shown).

Red blood cells had no effect of treatment ( $P = 0.21$ ; Figure 24) or day ( $P = 0.26$ ). There was, however, a treatment x day interaction ( $P = 0.0065$ ). All castrated bulls, regardless of meloxicam treatment, had a lower ( $P = 0.05$ ) red blood cell (RBC) count when contrasted with steers. This decrease in RBC for castrated bulls could be due to hemorrhaging at the site of castration. Steers tended to have a greater ( $P = 0.08$ ) RBC count compared to bulls castrated without meloxicam. On d 0, 6 h after castration, steers had a greater ( $P = 0.05$ ) red blood cell

count compared to bulls castrated with meloxicam. On d 3, RBC count in steers was greater compared to bulls castrated without meloxicam ( $P = 0.008$ ) and greater compared to bulls castrated with meloxicam ( $P = 0.03$ ). By d 7, RBC count only tended ( $P = 0.06$ ) to be greater in steers compared to bulls castrated without meloxicam. Pang et al. (2006) reported when 5.5 mo old calves were banded, that RBC count were greater in castrated than control, non-castrated, bulls on d 7. Though his results do differ from ours, the 2 different methods employ different mechanisms to complete the castration process, which could account for the different results when comparing experiments.

Hemoglobin concentration (data not shown) was not affected by treatment ( $P = 0.29$ ), but a day effect was noted ( $P < 0.0001$ ) and there tended to be a treatment x day interaction ( $P = 0.08$ ). On d 3, steers had a greater hemoglobin concentration ( $P = 0.01$ ) compared to bulls castrated without meloxicam, and steers tended to have a greater ( $P = 0.07$ ) hemoglobin concentration on d 7 compared to bulls castrated without meloxicam. Earley and Crowe (2002) evaluated the hematological changes in bull calves castrated at 5.5 mo of age. Intact bulls compared to bull calves castrated with or without ketoprofen showed no difference in hemoglobin concentration or hematocrit. Results from the current study showed however, there was no day effect ( $P = 0.26$ ; Figure 25) or affect of treatment ( $P = 0.22$ ) but there was a treatment x day interaction ( $P = 0.0049$ ) for the hematocrit. Overall, all castrated bulls tended ( $P = 0.08$ ) to have a lower hematocrit when contrasted to steers; 29.71 and 31.81, respectively. On d 0, 6 h after castration, steers tended ( $P = 0.10$ ) to have a greater hematocrit compared to bulls castrated without meloxicam, and exhibited a greater ( $P = 0.05$ ) hematocrit compared to bulls castrated with meloxicam. On d 3, hematocrit in steers was greater compared to bulls castrated without meloxicam ( $P = 0.006$ ) and bulls castrated with meloxicam ( $P = 0.05$ ). By d 7,

hematocrit in steers only tended ( $P = 0.08$ ) to be greater compared to bulls castrated without meloxicam. A decrease in RBC will cause a decrease in hematocrit, which could be the reason for lower hematocrit in bulls that were castrated, due to a loss of blood.

Early and Crowe (2002) found no differences between treatments in platelet count for 5.5 mo old bulls that were castrated or left intact. Data from the current study indicated a day effect ( $P < 0.0001$ ; Figure 26) and a treatment x day interaction ( $P < 0.0001$ ). All bulls that were castrated tended ( $P = 0.07$ ) to have a greater platelet count when contrasted with steers. Six h following castration, steers tended to have a greater platelet count compared to bulls castrated without meloxicam ( $P = 0.09$ ) and bulls castrated with meloxicam ( $P = 0.06$ ). On d 3, bulls castrated without meloxicam had a lower platelet count ( $P = 0.05$ ) than bulls castrated with meloxicam. On d 7, steers had a lower ( $P < 0.0001$ ) count compared to both bulls castrated with and without meloxicam. By d 14 the results were the same, steers had a lower platelet count compared to bulls castrated without meloxicam ( $P = 0.0017$ ) and bulls castrated with meloxicam ( $P = 0.0001$ ). Platelets are important in clotting blood; however, a large amount of blood loss will commonly cause a decrease in platelet counts. Barbucci et al. (2002) conducted research to determine how platelets reacted to stress. They found stress increased the degree of platelet adhesion, increased platelet spreading, and caused a dramatic increase in the number of platelets.

Rectal temperatures were not affected by treatment ( $P = 0.89$ ; Figure 27), however, there was an effect of day ( $P < 0.0001$ ). Overall temperature for d 0 was 40.05° C, dropping to 39.21° C on d 1, and continuing to decrease from d 0 to d 3 and 7. Rectal temperatures were taken prior to castration; therefore, castration would not have caused the increase in temperature. These calves had just been weaned from their dams, and transported a few miles to the university stocker unit. The stress of handling, separation from their dam due to weaning, as well as

transportation could have caused an increase in rectal temperature. Body temperature decreased from d 0 to 1, but then plateaued for the rest of the testing period.

Overall proportion of time expressed in lying flat on side (C1), standing (C2) and lying on sternum (C3) was 0.20, 0.58, and 0.21, respectively. The position denoted as C1 (lying flat on side) was analyzed among treatments for differences; there was no treatment effect ( $P = 0.76$ ; Figure 28), but there was day effect ( $P < 0.0001$ ). The proportion of time standing differed with treatment ( $P = 0.02$ ; Figure 29), as well as day ( $P < 0.0001$ ), but no treatment x day interaction ( $P = 0.43$ ) was observed. Steers ( $P = 0.0073$ ) and bulls castrated without meloxicam ( $P = 0.05$ ) had lower proportions of time standing compared to bulls castrated with meloxicam. A decrease in proportion of time spent standing was noted for day up until d 3 where it began to plateau. This plateau could be due to the stress of weaning beginning to subside. Cluster 3, or time spent lying on sternum, demonstrated the same pattern; a difference among treatments ( $P = 0.03$ ; Figure 30) and an increase in day ( $P < 0.0001$ ), but no treatment x day interaction ( $P = 0.61$ ). Steers spent a greater ( $P = 0.01$ ) proportion of time lying on sternum compared to bulls castrated with meloxicam. Gonzalez et al. (2010) compared steers that had been castrated at 34 d of age to bulls that were castrated at 260 d of age for differences in physical activities using a digital video camera in each pen. They noted similar results in that castrated calves tended to spend less time lying down compared to steers (24.2 vs.  $31.4 \pm 3.00\%$ ).

Each treatment was evaluated to determine how many times, on average, calves were in each of the 3 positions, or the number of times an event occurred. On average, steers were found to be lying flat on side 20.18 times per day, while bulls castrated with meloxicam laid down 18.27 and bulls castrated without meloxicam laid down 16.83. There were no differences due to treatment ( $P = 0.47$ ; Figure 31) and there was no treatment x day interaction ( $P = 0.72$ ) for C1

events. There was however, an initial increase from d 0 to d 1, then a decrease on d 2 ( $P < 0.0001$ ). The same results were noted for number of events standing (Figure 32). Numerically, steers exhibiting this behavior 74.67 times a day, with bulls castrated at without meloxicam standing 71.84 times and bulls castrated with meloxicam 71.84 times. Cluster 3 (lying on sternum; Figure 33) also showed similar numerical results. Bulls castrated with meloxicam were found to be lying down 54.47 times a day, followed by steers lying down 56.6 times and then bulls castrated without meloxicam 60.24 times. A day effect ( $P < 0.0001$ ) was noted for the number of events for both standing and lying on sternum with an increase in both on d 1, and events decreasing drastically to d 2 and 3, but beginning to flatten out after that.

These events were then evaluated for the average amount of time spent exhibiting each event. Steers spent on average 20.92 min lying flat on side, while bulls castrated with meloxicam spent 21.98 min, and bulls castrated without meloxicam spent 28.44 min, lying flat on side. There were no differences among treatments ( $P = 0.36$ ; Figure 34) and no treatment x day interaction ( $P = 0.68$ ), however, there was a difference in day ( $P = 0.0018$ ), however these differences began to plateau at d 2. The same responses were noted for the behavior classified as lying on sternum (Figure 35); treatment had no effect ( $P = 0.22$ ) and there was no treatment x day interaction ( $P = 0.23$ ), but there was a difference in day ( $P < 0.0001$ ), beginning to plateau around d 3. Differences were found due to treatment ( $P = 0.04$ ; Figure 36) and day ( $P < 0.0001$ ) when standing durations were analyzed, but no treatment x day ( $P = 0.57$ ) interaction was noted. Steers ( $P = 0.01$ ) and bulls castrated without meloxicam ( $P = 0.04$ ) were found to, on average, spend less time standing compared to bulls castrated with meloxicam. The amount of time spent standing increased from d 1 through d 3.



The differences recorded due to day could be attributed to the fact that the calves were gathered and run through the chute 6 h after castration, and then again on d 1, 3, and 7 for further evaluations. The plateau around d 2 or 3 could be attributed to a decrease in stress associated with weaning. On average, they would be penned for up to 3 h, which would cause an increase in standing proportions, events, and times on those work days. Standing and lying behavior is also affected by the time of day and the environmental conditions. During hot periods of the day, cattle are typically found under shade, and grazing during cooler times such as night (Kendall et al., 2006). This experiment was not designed to determine if the effects of weaning played into the stress levels of a calf, but whether or not the added stress of castration at weaning had negative or positive affects on calves.

There was no correlation found between proportion of time standing and ADG ( $P = 0.97$ ) or between proportion of time lying and ADG ( $P = 0.15$ ). This suggests that even though steers spent, on average, the greatest amount of time lying (in both positions), and the least amount standing, that their ADG was not affected by their behavior. Increased lying behavior is typically associated with comfort and contentment; however, increased lying means a decrease in grazing time.

#### **D. CONCLUSION**

Waiting until weaning to castrate bull calves is often thought to improve gain performance for the calf. We conclude from our results that leaving bulls intact did not improve pre-weaning gain performance compared to steers castrated near birth. However, bulls castrated at weaning did exhibit lower ADG from d 0 to 14 and d 0 to 56 compared to steers, and administering meloxicam improved ADG for 14 d following castration at weaning. While blood parameter results did show differences, most were found at 6 h post-castration, and within a few

d showed no effects. Most often, the bull calves administered meloxicam were found to have values that were intermediate compared to steers and bull calves that did not receive meloxicam. However, there were some instances when the meloxicam did not appear to be help. Bulls castrated at weaning without meloxicam exhibited intermediate relaxed and content behavior out of all of the treatments. Rectal temperatures were not affected by castration, and meloxicam neither helped nor hurt the calves in anyway.

In some instances, meloxicam showed to be beneficial to the calf, and in others it did not show signs of relief. Meloxicam has a half life of 27.54 h when administered orally. In our study, meloxicam was dosed immediately prior to castration. Administering the drug several hours prior to processing may lead to different results, and possibly show a greater advantage to the use of meloxicam. The added amount of handling on d 1, 3, 7 and 14, however, could also have an affect on the performance of the calves, affecting both behavior and physiological aspects of the calf.

## CHAPTER 5 – THESIS CONCLUSION

Castration is a common procedure performed on cattle operations around the world. It is necessary to help reduce the aggressive behavior, as well as improve meat quality, and to help prevent unwanted pregnancies. Although it is a necessary production practice, castration does cause pain and stress from the process, regardless of method. This response can cause a temporary alteration in performance; including growth reduction, biochemical, and abnormal behavior.

As there is an increase in the public desire for more humane treatment of animals, addressing common farm practices that are known to be stressful and painful to cattle is necessary. The lack of public education regarding these practices, and this increased desire for humane treatment of animals has stimulated research for evaluating measures that could be utilized to ensure everyday farm animal management practices are carried out efficiently and humanely. The use of equipment, and following procedures that reduces pain and stress, as well as speed along the recovery and healing process, is encouraged by organizations such as the AVMA. The goal of research is to have an answer for the public concern of how beef cattle are typically managed in today's production schemes.

The use of meloxicam, alongside anesthesia or alone, has been tested for its ability to reduce the immediate phase of pain associated with castration, as well as the long-term, inflammatory phase of pain. While several have been shown in research to have positive effects on the calf, there is not an analgesic currently approved in the US for use in cattle. Despite this, several studies have investigated meloxicam, an NSAID, which has been approved for use in companion animals, and for cattle in other countries. The goal of administering the NSAID is to

help reduce the initial pain associated with castration, as well as help combat the inflammatory phase, reducing recovery time.

The objective herein was to determine if castration at 2 different stages of maturity, birth and weaning, with or without pain control, would have an effect on growth performance, hematology, and behavior. We hypothesized that calves castrated at birth would exhibit poorer gain performance until weaning compared to bulls left intact and calves receiving meloxicam would perform better than those not receiving the meloxicam. Our results, however, showed that the use of meloxicam does not benefit the calf if castrated at birth. There was also no benefit to leaving calves intact until weaning for growth performance, and blood parameters and behavior showed little differences between calves castrated at birth compared to those that were not. Due to the greater market value of steers, our results suggest castrating calves near birth would benefit the producer. The data demonstrated that when bulls are castrated at weaning that the use of meloxicam does increase short-term ADG. This increase not only benefits the calf, but also brings an increased amount in profit for the farmer, since the increased income outweighs the cost of meloxicam, which is roughly \$0.21/15 mg tablet. The blood data did not provide significant evidence to meloxicam restoring biochemical profiles to non-stressed control calf equivalents. Rectal temperatures were not influenced by the use of analgesia, and castration did not affect rectal temperature. Our results indicate that castration should be performed as young as possible to minimize stress on the animal. If castration is performed at a later age, the use of meloxicam is encouraged and recommended to benefit calf performance and potentially increase the income of the producer.

## CHAPTER 6 – LITERATURE CITED

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## Tables

**Table 1. Effects of castration at birth, with or without meloxicam, on body weight and average daily gain (kg).**

	<b>Castration at birth without meloxicam</b>	<b>Castration at birth with meloxicam</b>	<b>Bulls</b>	<b>P-Value</b>
<b>BW</b>				
Birth weight	33 ± 1.6	34 ± 1.5	33 ± 1.1	0.76
d 4*	52 ± 4.5	50 ± 4.5	50 ± 3.2	0.91
d 33	71 ± 4.5	75 ± 4.5	70 ± 3.1	0.65
d 66	99 ± 6.7	95 ± 6.6	89 ± 4.6	0.49
d 116	135 ± 7.2	130 ± 6.9	127 ± 4.7	0.63
d 162	162 ± 8.6	160 ± 8.3	158 ± 5.7	0.94
d 199	190 ± 10	188 ± 9.7	191 ± 6.6	0.96
d 214 (weaning)	216 ± 11	215 ± 10.4	219 ± 7.1	0.95
<b>ADG</b>				
d 4 to 33	0.81 ± 0.09	1.06 ± 0.09	1.01 ± 0.06	0.13
d 33 to 66	0.97 ± 0.07	0.79 ± 0.07	0.85 ± 0.05	0.19
d 66 to 116	0.81 ± 0.08	0.78 ± 0.08	0.83 ± 0.04	0.73
d 116 to 162	0.57 ± 0.06	0.62 ± 0.05	0.67 ± .04	0.36
d 162 to 199	0.82 ± 0.07	0.83 ± 0.07	0.96 ± 0.05	0.18
d 199 to 214	0.96 ± 0.09	0.94 ± 0.08	0.96 ± 0.06	0.97
d 0 to 214	0.83 ± 0.04	0.81 ± 0.03	0.84 ± 0.03	0.88

\*Using the average birth date as d 1

**Table 2: Ingredient composition of mixed grain supplement fed to calves after weaning.**

<b>Ingredients</b>	<b>%</b>
Cracked corn	68.36
Dried distillers' grain	26
Salt, white	1
Limestone	2
Vitamin A, D, E premix <sup>a</sup>	0.1
Vitamin E premix <sup>b</sup>	0.05
Corn/rumensin premix <sup>c</sup>	0.4
NB-8675 ruminant trace mineral premix	0.085
Molasses	2

<sup>a</sup>ADE premix contains 4,000,000 IU/ 0.45 kg Vitamin A, 800,000 IU/ 0.45 kg Vitamin D, and 500 IU/0.45 kg Vitamin E

<sup>b</sup>Vitamin E contains 20,000 IU/ 0.45 kg.

<sup>c</sup>This intermediate mix provided 10 g monensin/0.45 kg

**Table 3. Effects of castration at weaning, with or without meloxicam, on body weight and average daily gain (kg).**

	Steers	Castrated at weaning without meloxicam	Castration at weaning with meloxicam	SE	P-value
<b>BW</b>					
d 0*	215.91	228.24	216.67	8.47	0.52
d 14	244.26	250.00	242.49	8.75	0.84
d 28	260.39	266.00	257.15	8.83	0.81
d 56	273.32	278.38	270.70	9.15	0.86
<b>ADG</b>					
d 0 to 14	2.02 <sup>a</sup>	1.55 <sup>b</sup>	1.85 <sup>a</sup>	0.09	0.002
d 14 to 28	1.15	1.14	1.05	0.09	0.67
d 28 to 56	0.46	0.44	0.48	0.06	0.92
d 0 to 56	1.02 <sup>a</sup>	0.89 <sup>b</sup>	0.96 <sup>ab</sup>	0.04	0.02

\*D 0 represents day of weaning

<sup>a,b</sup> Superscripts that are different in the same row indicate a tendency towards a significant difference ( $P < 0.06$ )

**Table 4. Effects of castration at weaning, with or without meloxicam, on body weight and average daily gain (kg) during grazing period.**

	<b>Steers</b>	<b>Castrated at weaning without meloxicam</b>	<b>Castrated at weaning with meloxicam</b>	<b>SE</b>	<b>P-value</b>
<b>BW</b>					
d 108	316.49	323.47	314.36	9.73	0.81
d 141	352.62	355.14	345.79	10.47	0.84
<b>ADG</b>					
d 108 to 141	0.93	0.903	0.88	0.03	0.61

## Figures

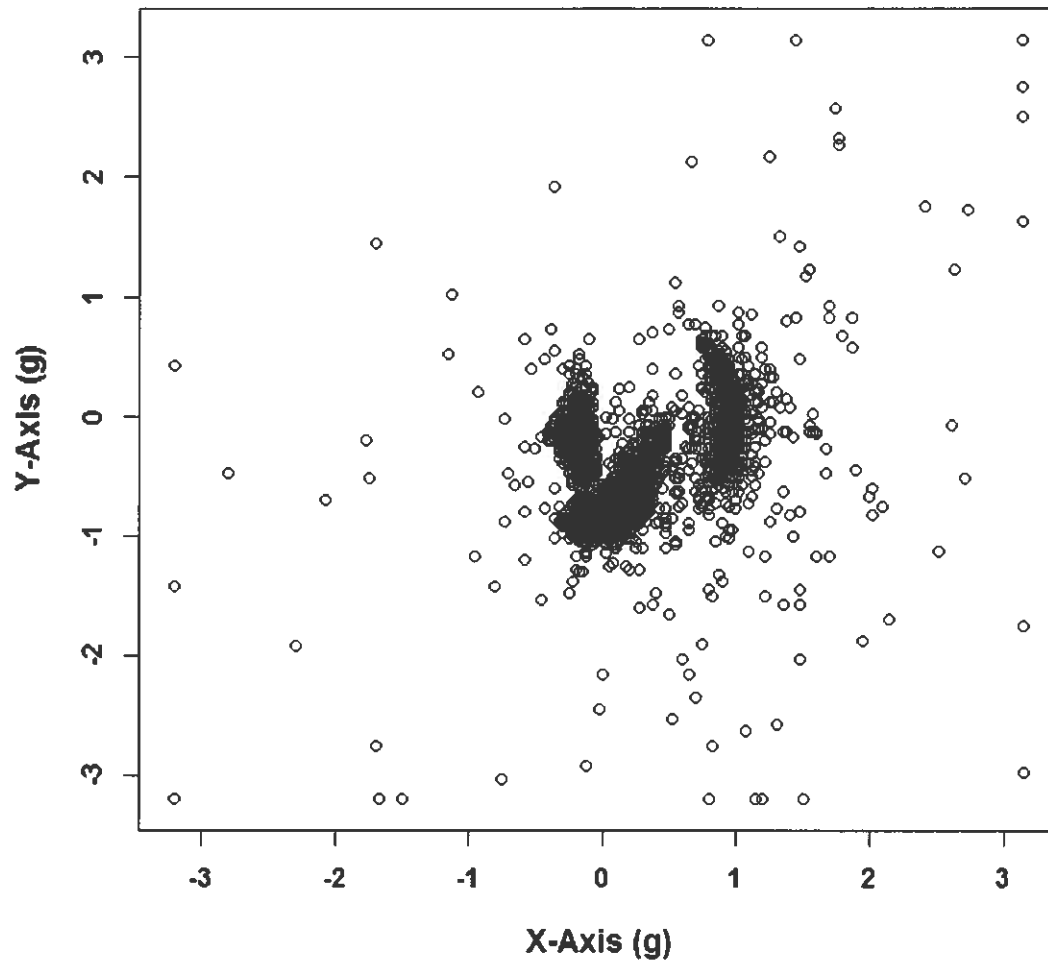
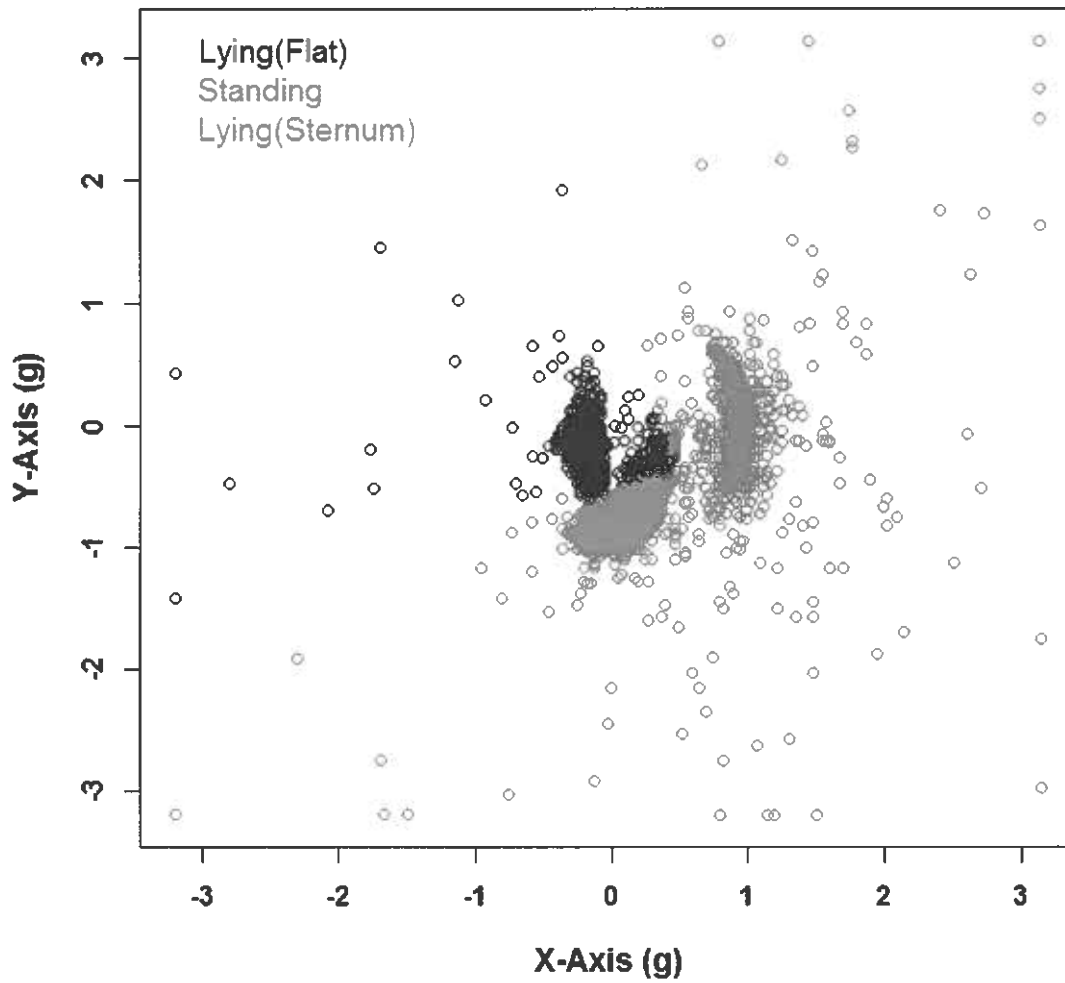
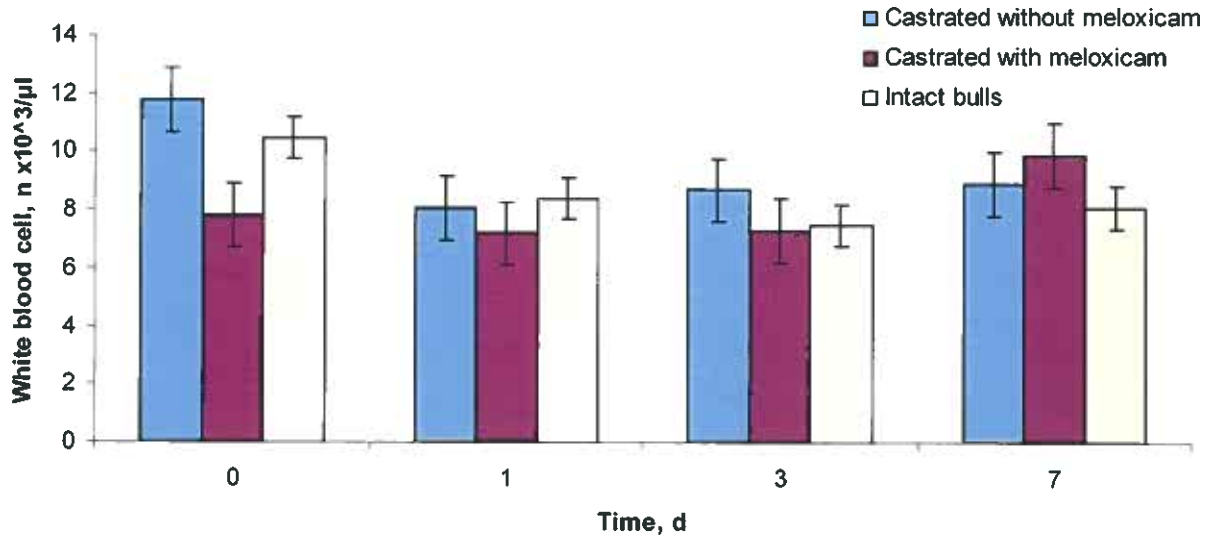


Figure 1. Example x-y plot of accelerometer data points collected for calf 1130.

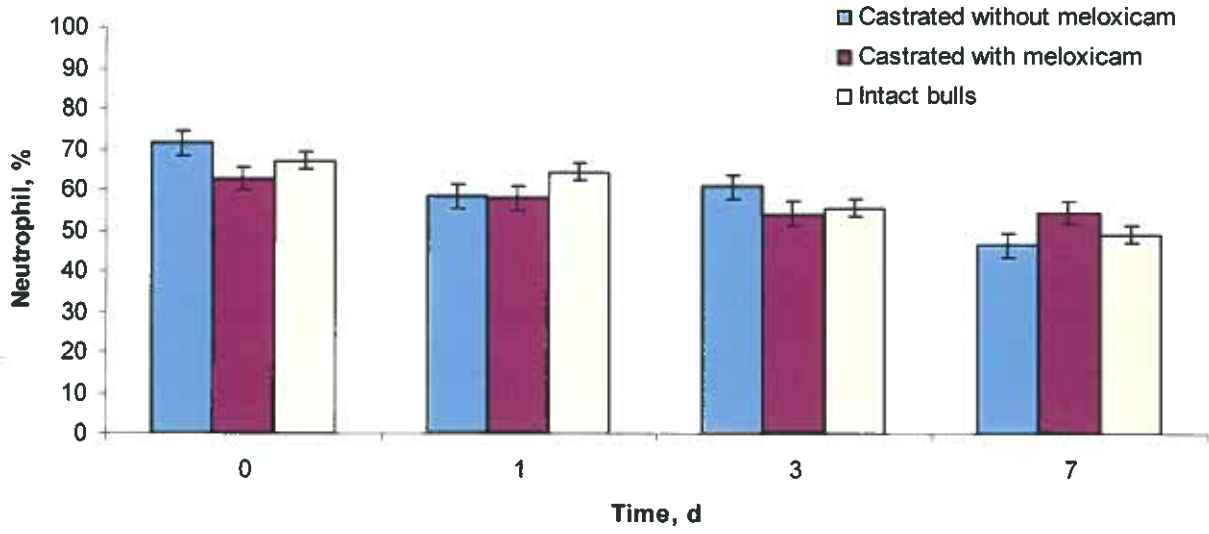


**Figure 2. Example x-y plot of accelerometer data points collected for calf 1130 colored for each of 3 predicted clusters (cluster 1 = blue, cluster 2 = orange, and cluster 3 = green).**

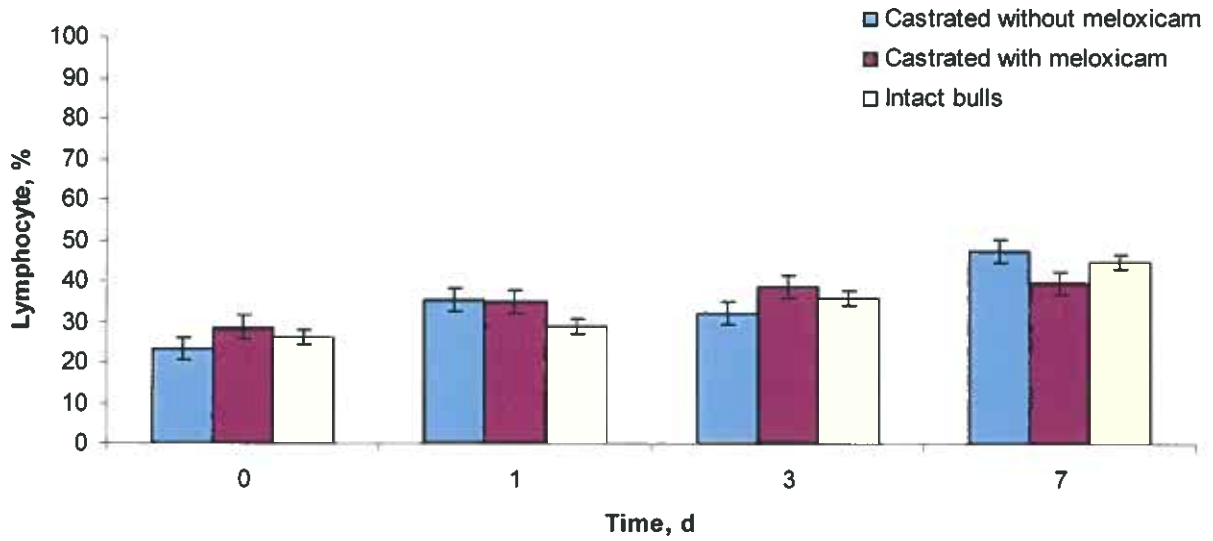


**Figure 3. Effect of castration at birth, with or without meloxicam, on white blood cell count. Effect of treatment ( $P = 0.47$ ), day ( $P = 0.002$ ), treatment x day ( $P = 0.13$ ).**

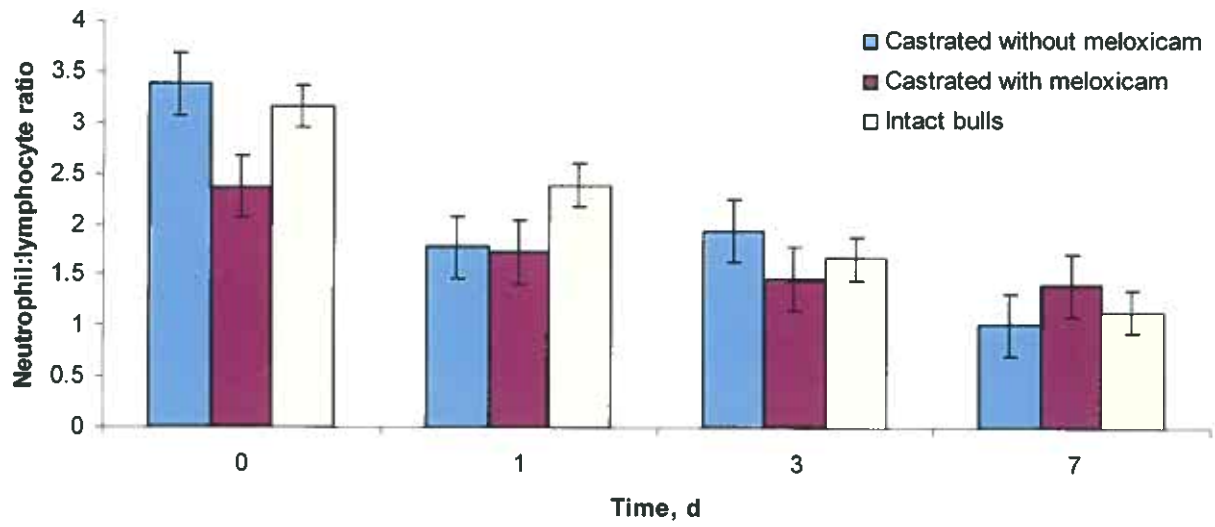




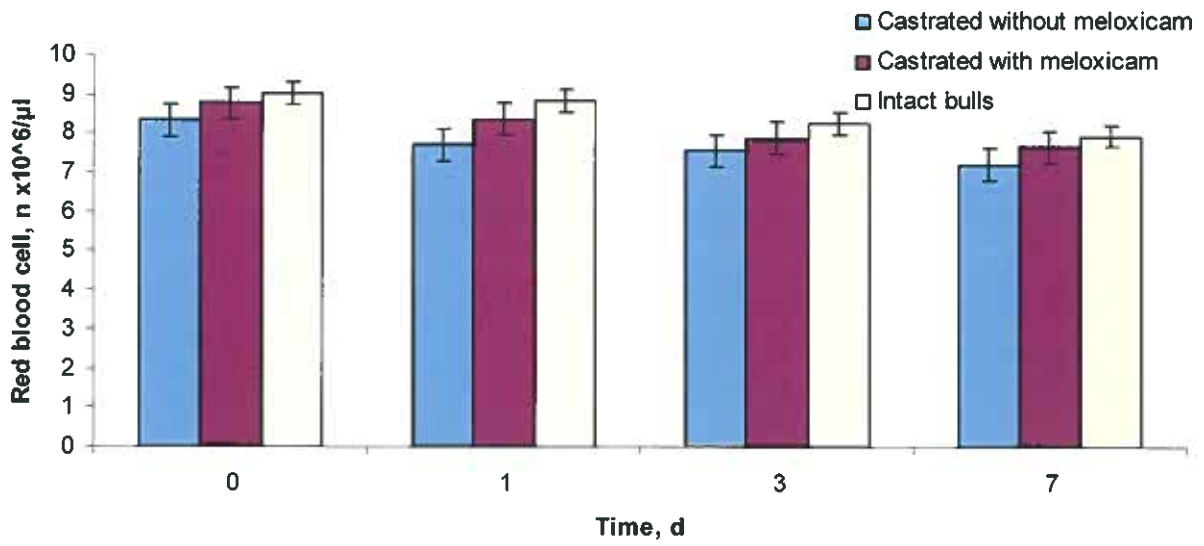
**Figure 4. Effect of castration at birth, with or without meloxicam, on percentage of neutrophils. Effect of treatment ( $P = 0.76$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.01$ ).**



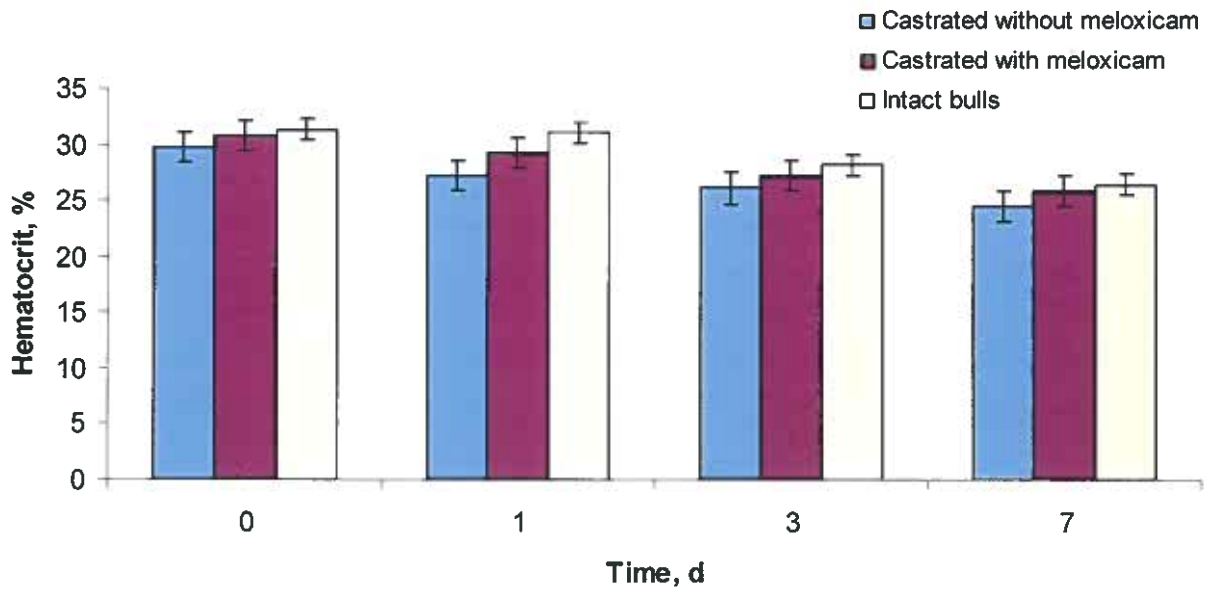
**Figure 5. Effect of castration at birth, with or without meloxicam, on the percentage of lymphocytes. Effect of treatment ( $P = 0.79$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.02$ ).**



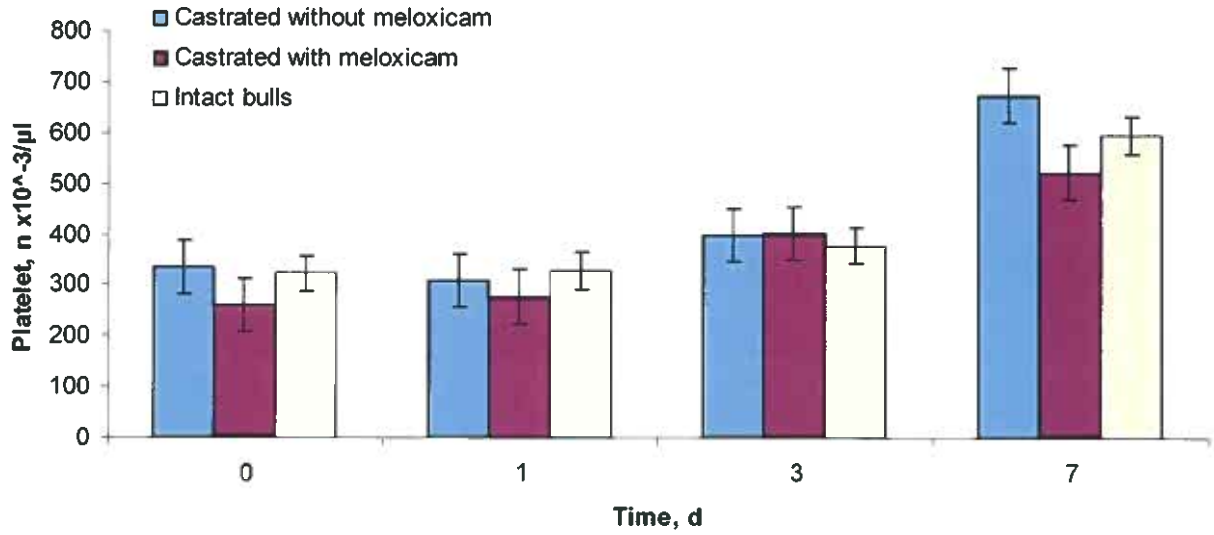
**Figure 6. Effect of castration at birth, with or without meloxicam, on neutrophil to lymphocyte ratio. Effect of treatment ( $P = 0.32$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.12$ ).**



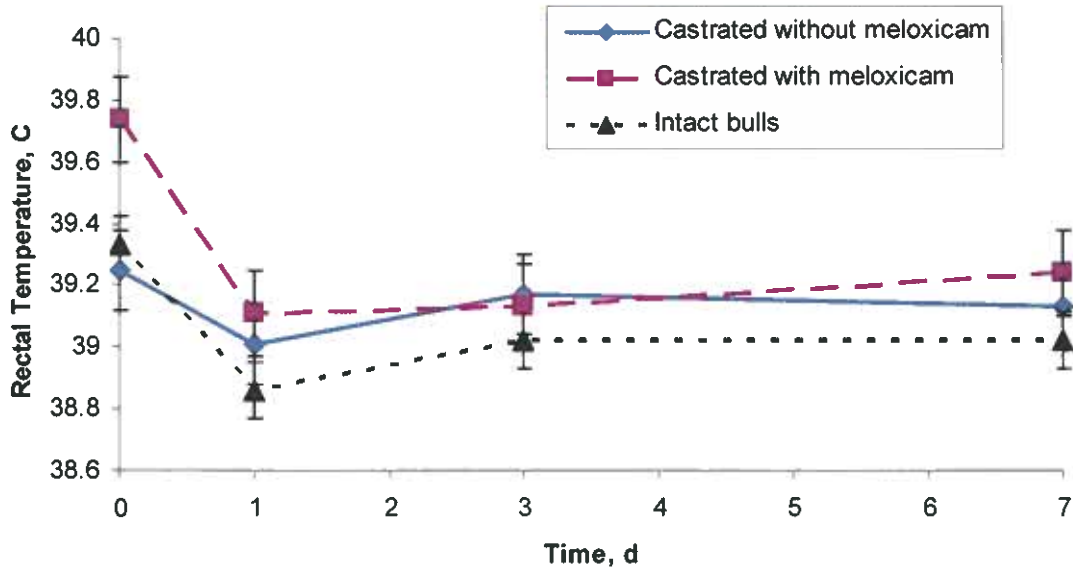
**Figure 7. Effect of castration at birth, with or without meloxicam, on red blood cell count. Effect of treatment ( $P = 0.19$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.88$ ).**



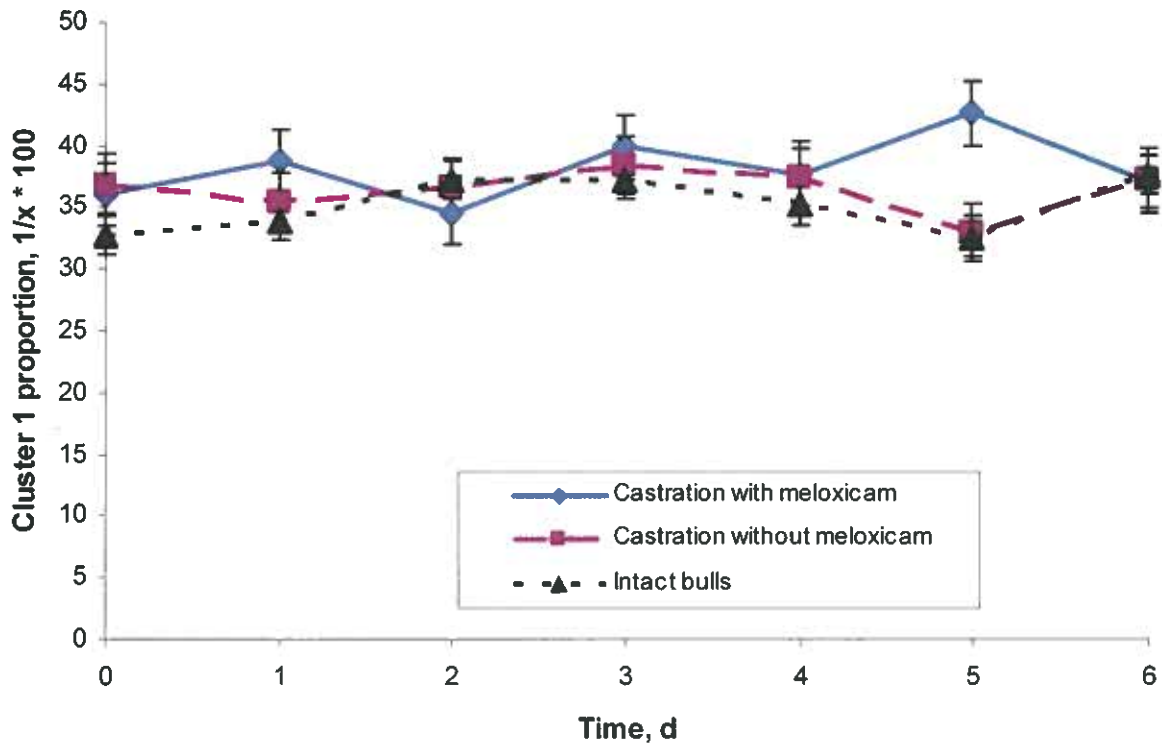
**Figure 8. Effect of castration at birth, with or without meloxicam, on hematocrit. Effect of treatment ( $P = 0.29$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.03$ ).**



**Figure 9. Effect of castration at birth, with or without meloxicam, on platelet count. Effect of treatment ( $P = 0.54$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.49$ ).**

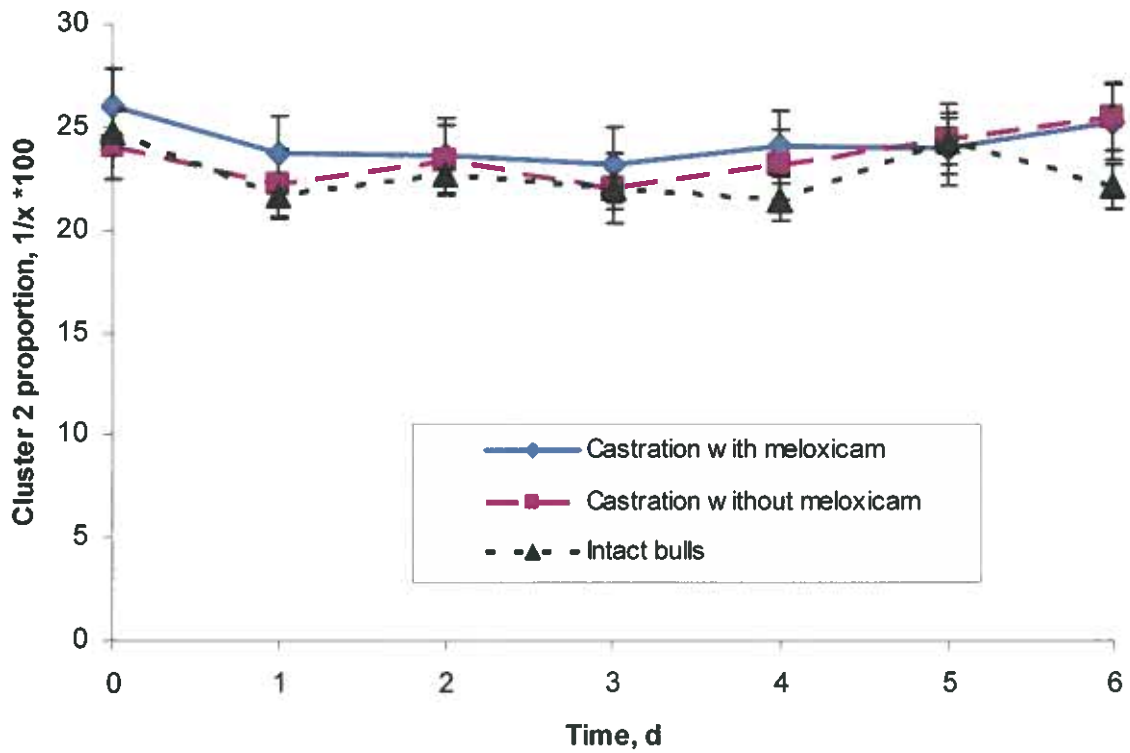


**Figure 10. Effect of castration at birth, with or without meloxicam, on rectal temperature of beef calves. Effect of treatment ( $P = 0.06$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.53$ ).**

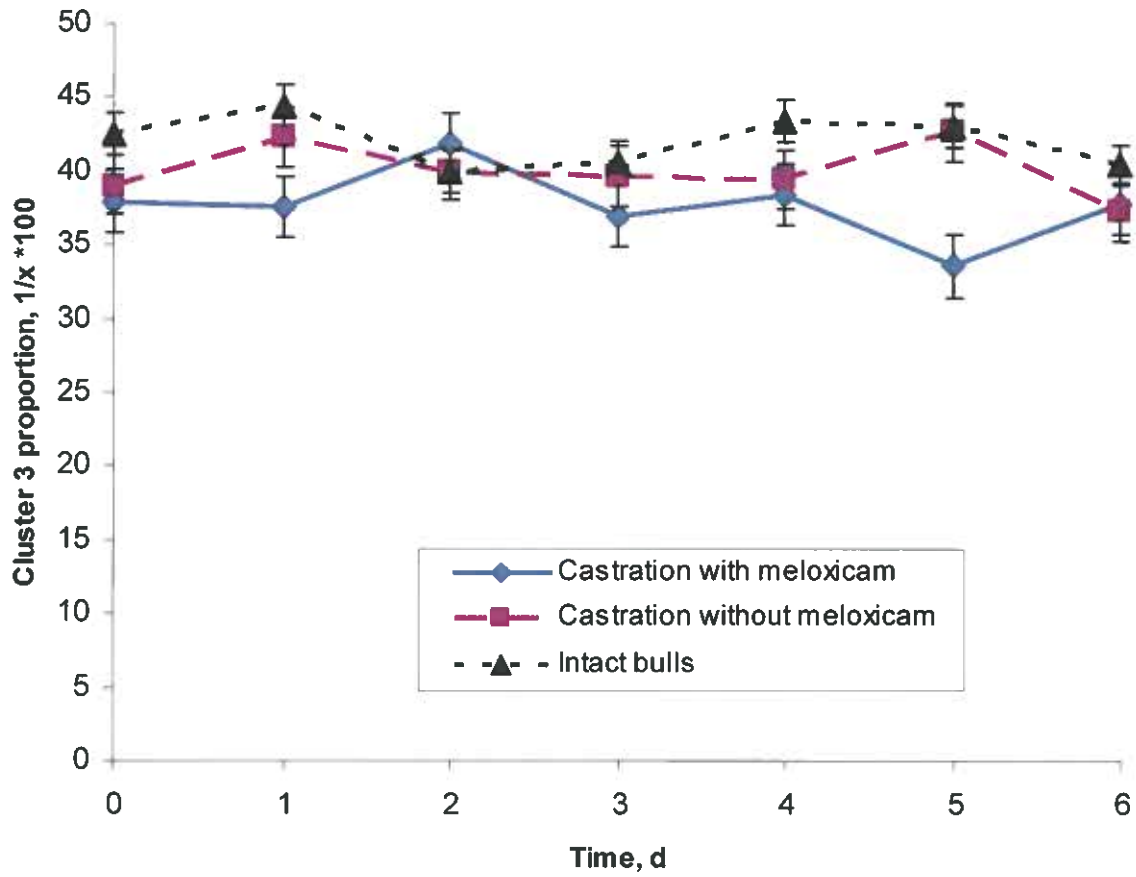


**Figure 11. Effect of castration at birth, with or without meloxicam, on cluster 1, proportion of time spent lying flat on side. Effect of treatment ( $P = 0.23$ ), day ( $P = 0.44$ ), treatment x day ( $P = 0.05$ ).**

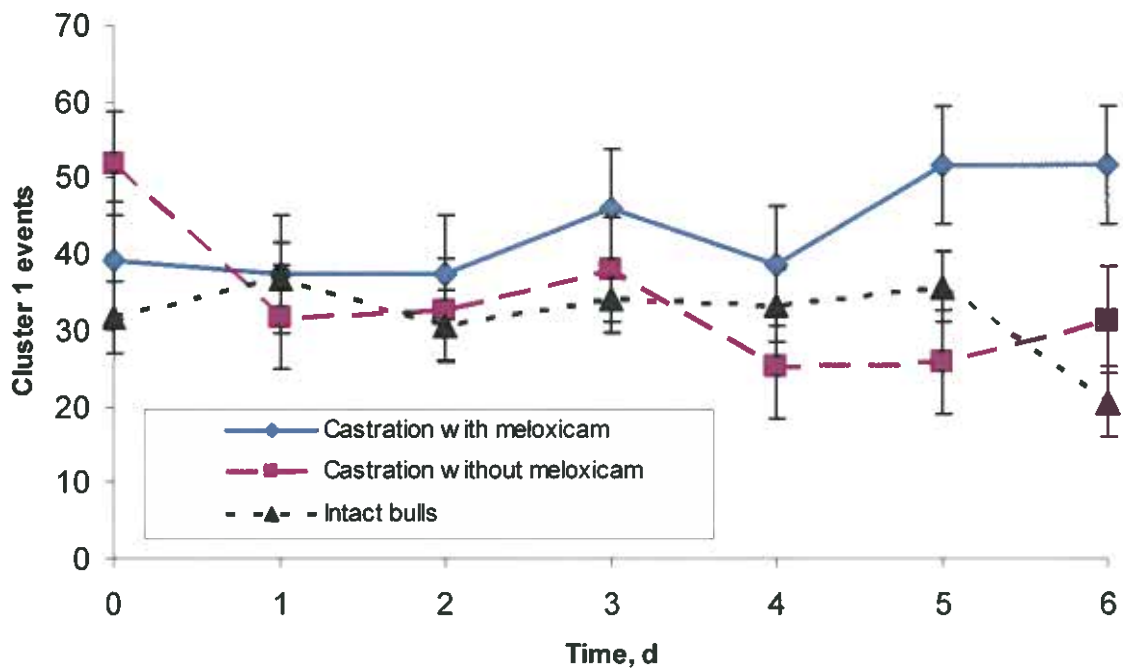




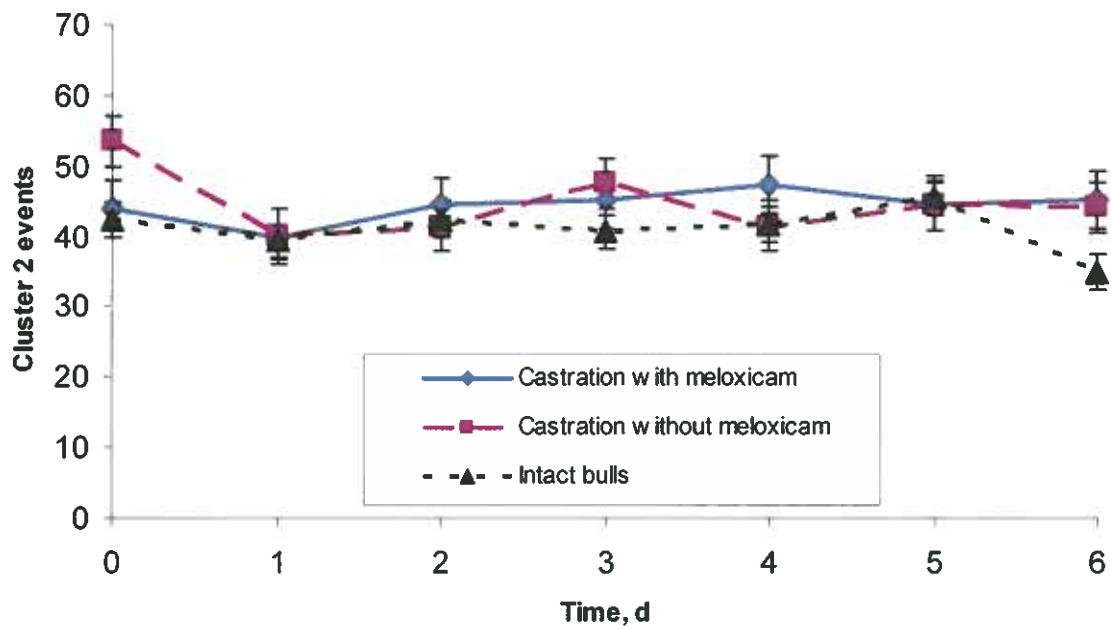
**Figure 12. Effect of castration at birth, with or without meloxicam, on cluster 2, proportion of time spent standing. Effect of treatment ( $P = 0.50$ ), day ( $P = 0.13$ ), treatment x day ( $P = 0.87$ ).**



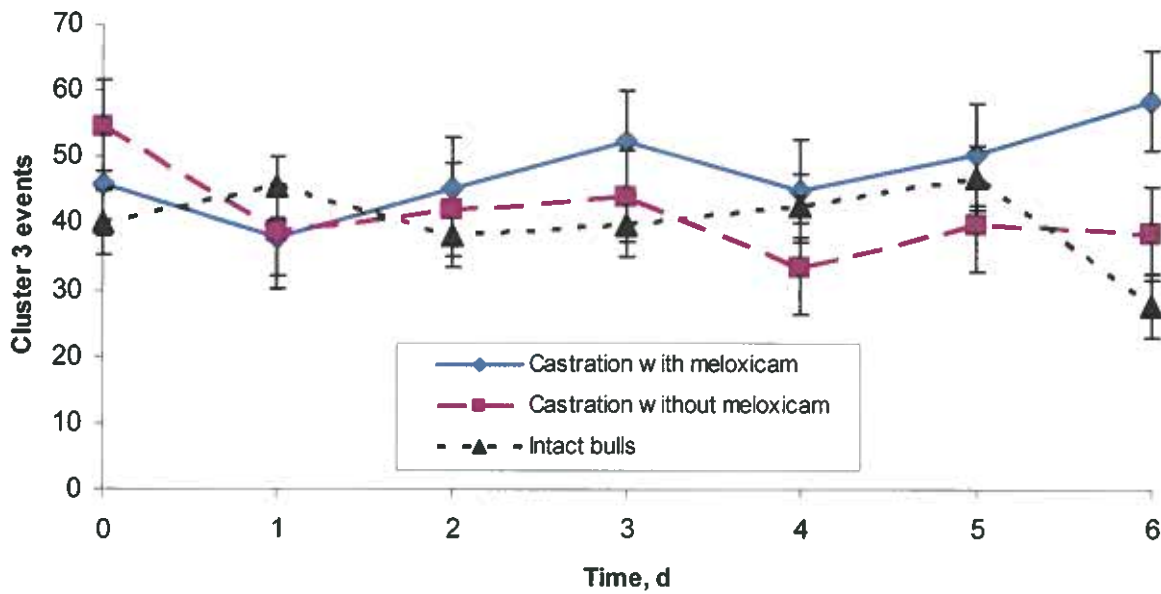
**Figure 13. Effect of castration at birth, with or without meloxicam, on cluster 3, proportion of time spent lying on sternum. Effect of treatment ( $P = 0.0017$ ), day ( $P = 0.54$ ), treatment x day ( $P = 0.12$ ).**



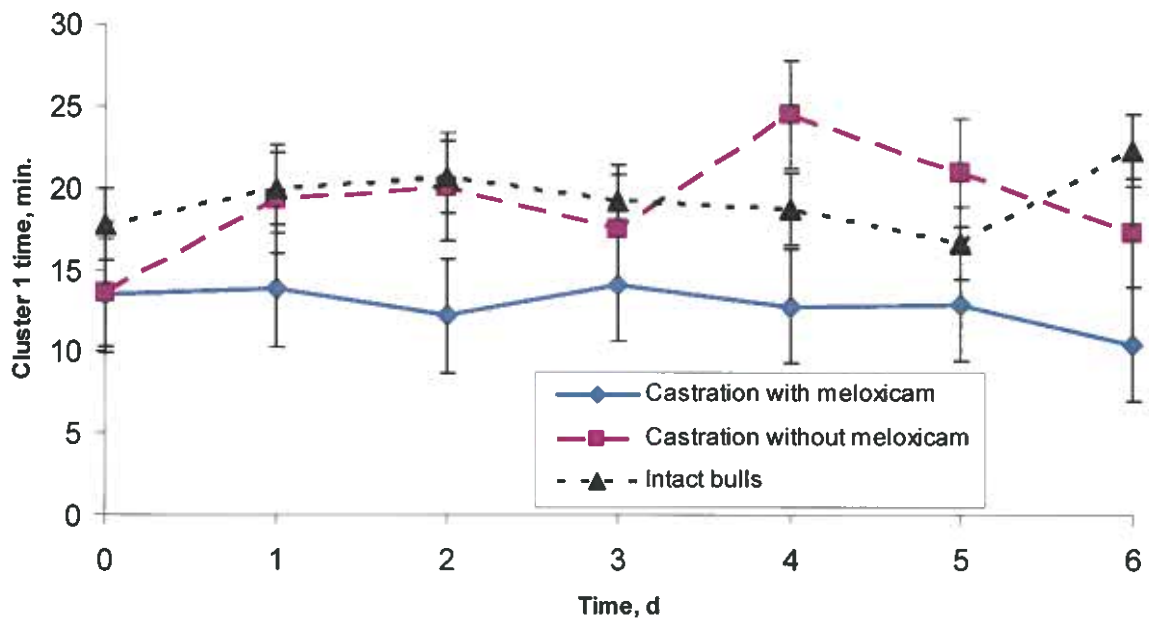
**Figure 14. Effect of castration birth, with or without meloxicam, on cluster 1, number of events occurring: lying flat on side. Effect of treatment ( $P = 0.18$ ), day ( $P = 0.14$ ), treatment x day ( $P = 0.14$ ).**



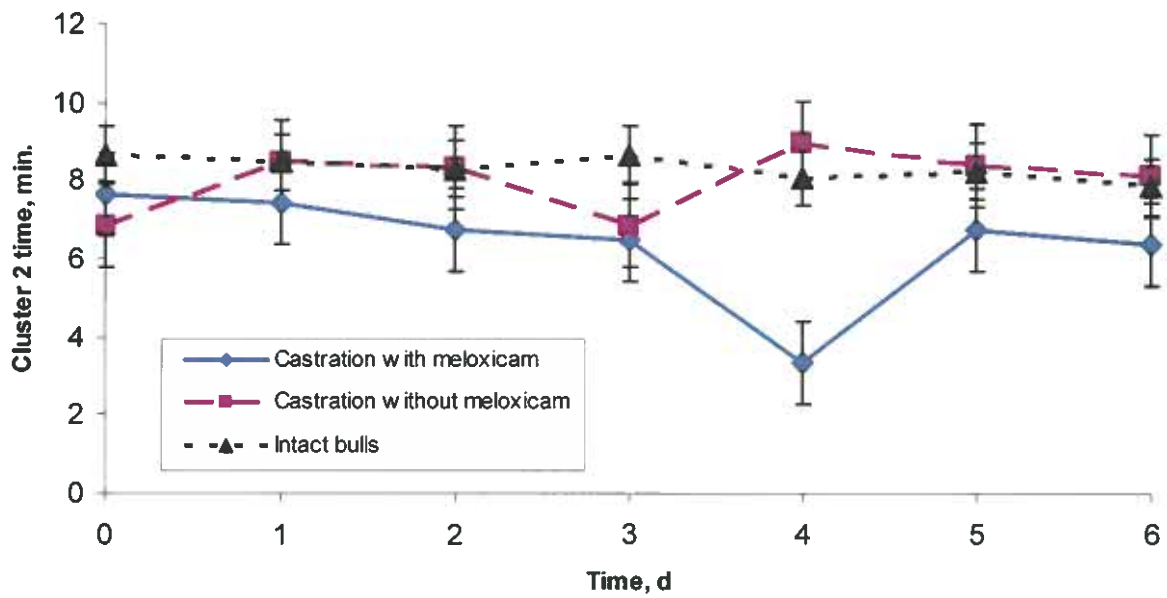
**Figure 15.** Effect of castration at birth, with or without meloxicam, on cluster 2, number of events occurring: standing. Effect of treatment ( $P = 0.38$ ), day ( $P = 0.03$ ), treatment x day ( $P = 0.22$ ).



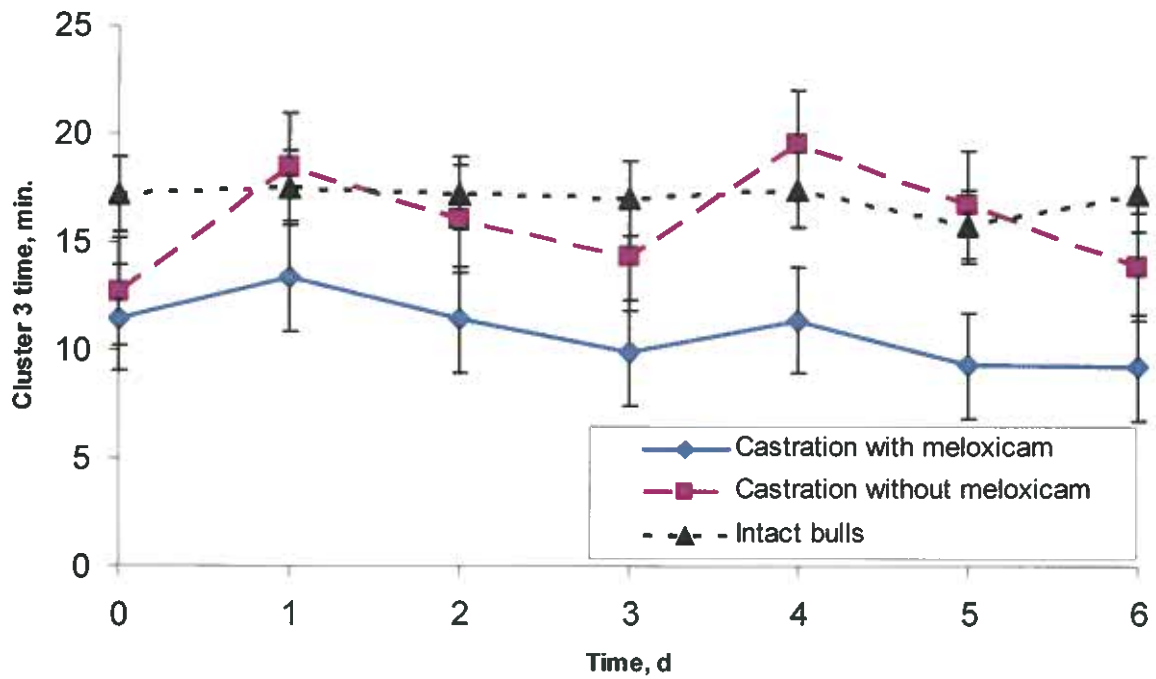
**Figure 16. Effect of castration at birth, with or without meloxicam, on cluster 3, number of events occurring: lying on sternum. Effect of treatment ( $P = 0.37$ ), day ( $P = 0.18$ ), treatment x day ( $P = 0.14$ ).**



**Figure 17.** Effect of castration at birth, with or without meloxicam, on cluster 1, mean duration of time (min) spent lying flat on side. Effect of treatment ( $P = 0.17$ ), day ( $P = 0.58$ ), treatment x day ( $P = 0.70$ ).

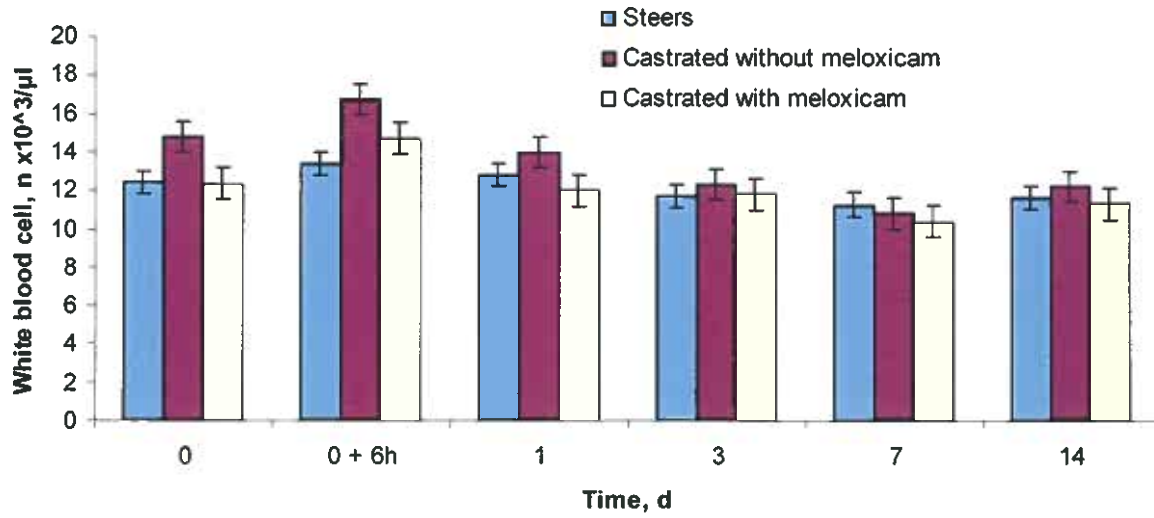


**Figure 18. Effect of castration at birth, with or without meloxicam, on cluster 2, mean duration of time (min) spent standing. Effect of treatment ( $P = 0.79$ ), day ( $P = 0.59$ ), treatment x day ( $P = 0.08$ ).**

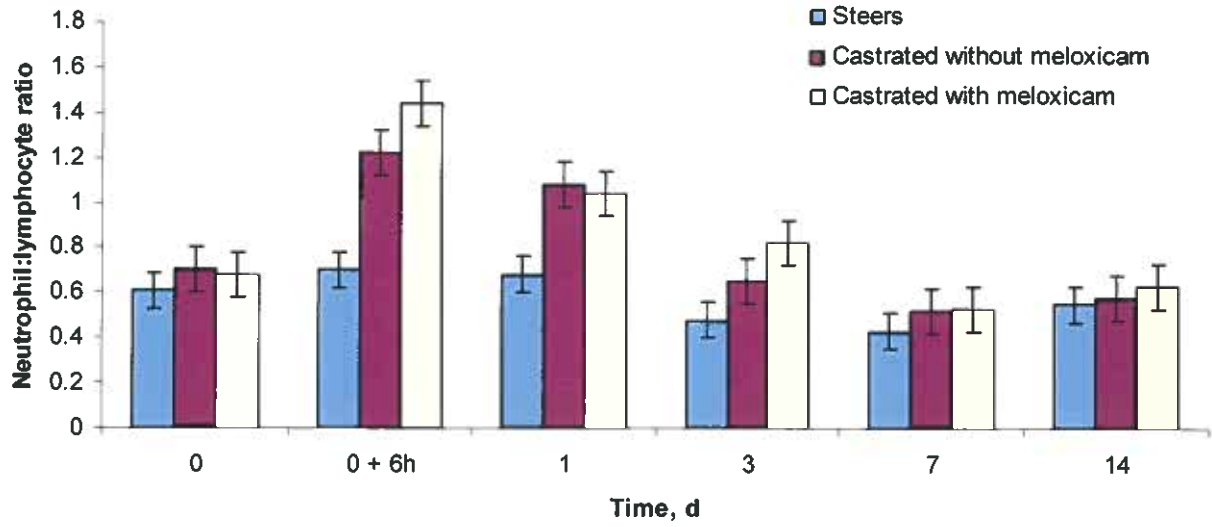


**Figure 19. Effect of castration at birth, with or without meloxicam, on cluster 3, mean duration of time (min) spent lying on sternum. Effect of treatment ( $P = 0.07$ ), day ( $P = 0.05$ ), treatment x day ( $P = 0.67$ ).**

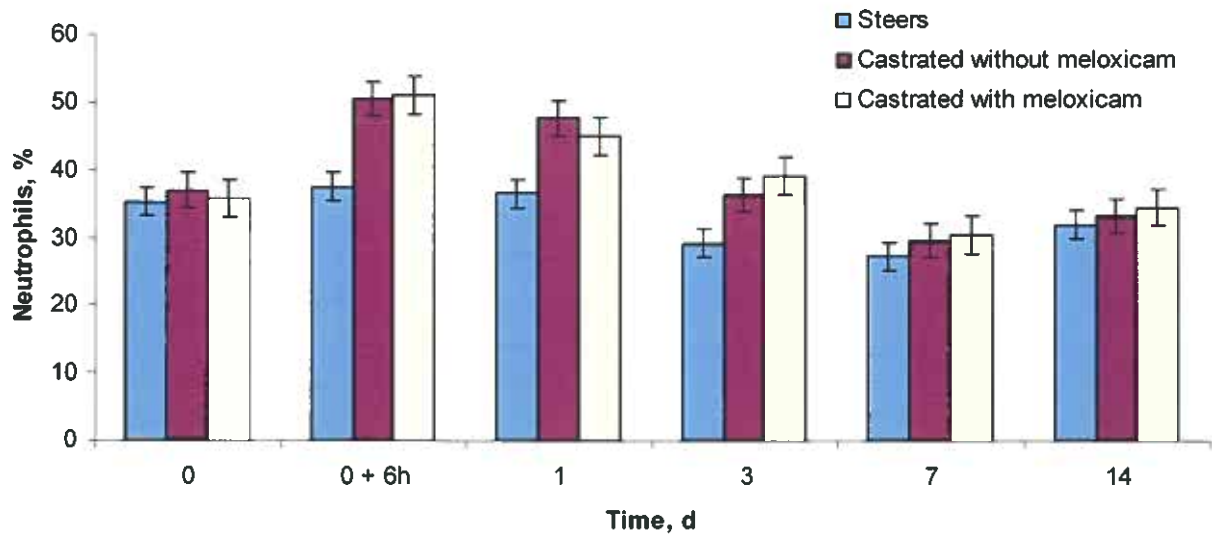




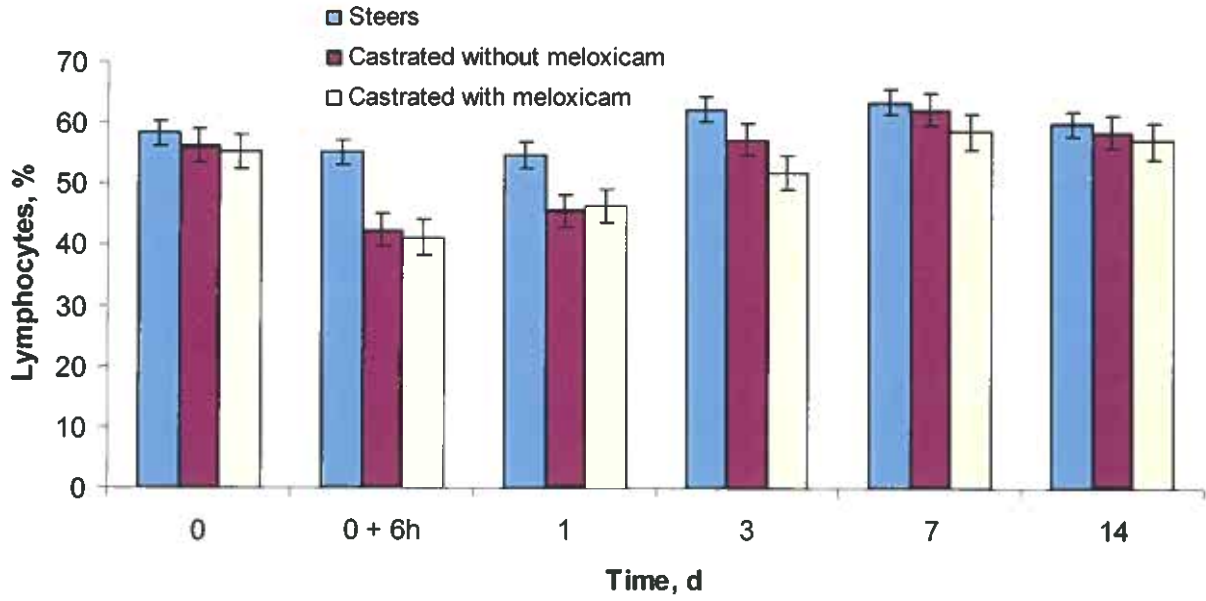
**Figure 20. Effect of castration at weaning, with or without meloxicam, on white blood cell count. Effect of treatment ( $P = 0.17$ ), day ( $P < 0.001$ ), treatment x day ( $P = 0.09$ ).**



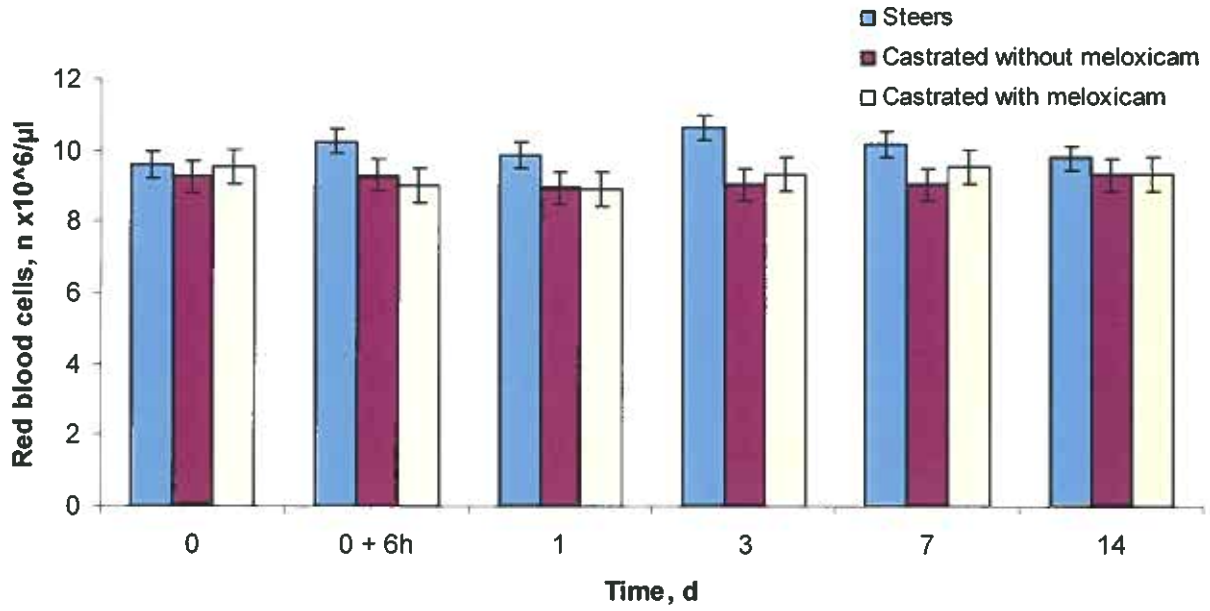
**Figure 21. Effect of castration at weaning, with or without meloxicam, on neutrophil to lymphocyte ratio. Effect of treatment ( $P = 0.0066$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.0002$ ).**



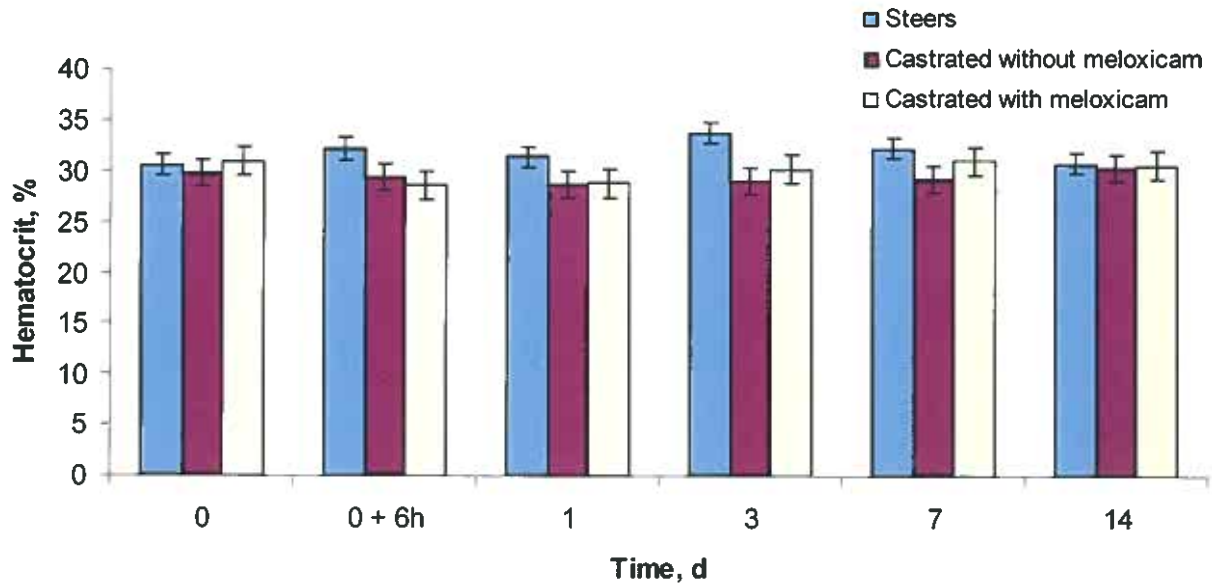
**Figure 22. Effect of castration at weaning, with or without meloxicam, on percentage of neutrophils. Effect of treatment ( $P = 0.03$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.0008$ ).**



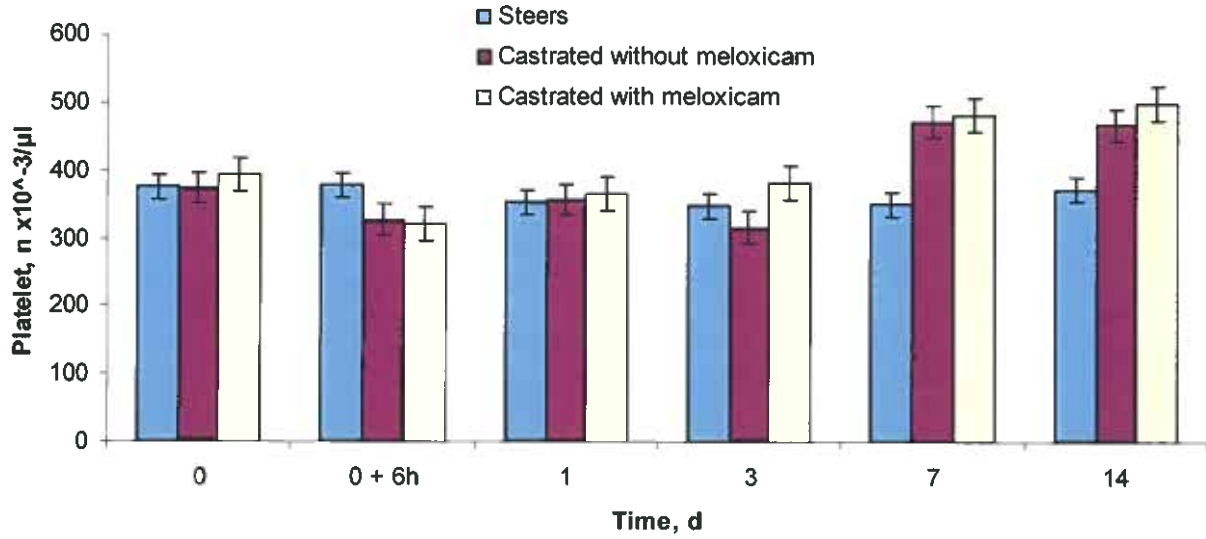
**Figure 23. Effect of castration at weaning, with or without meloxicam, on the percentage of lymphocytes. Effect of treatment ( $P = 0.03$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.0024$ ).**



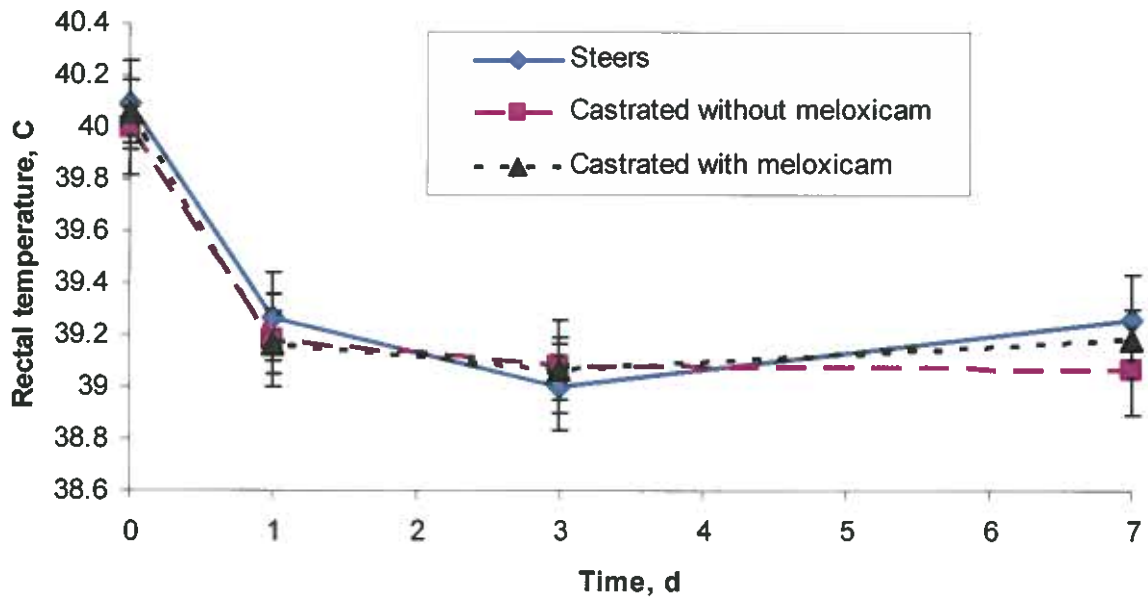
**Figure 24. Effect of castration at weaning, with or without meloxicam, on the red blood cell count. Effect of treatment ( $P = 0.21$ ), day ( $P = 0.26$ ), treatment x day ( $P = 0.0065$ ).**



**Figure 25. Effect of castration at weaning, with or without meloxicam, on hematocrit. Effect of treatment ( $P = 0.26$ ), day ( $P = 0.22$ ), treatment x day ( $P = 0.0049$ ).**

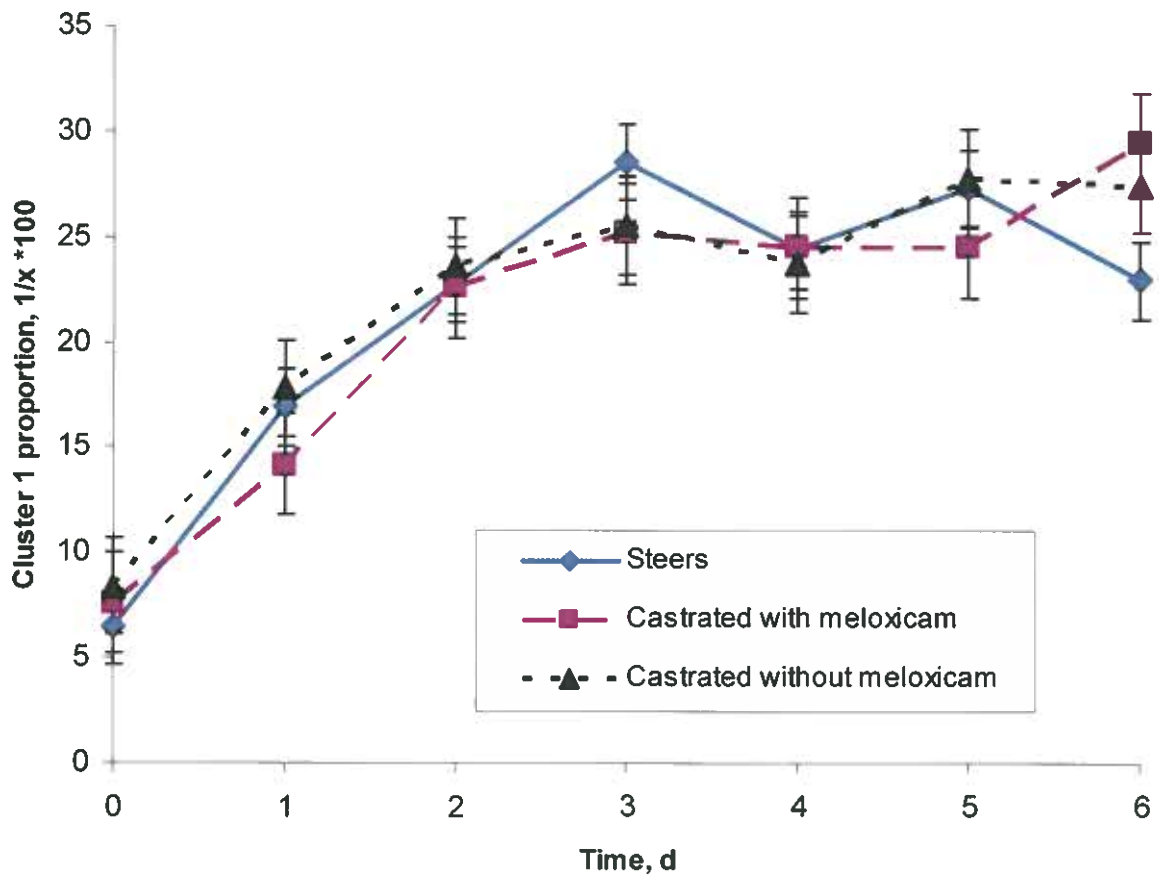


**Figure 26. Effect of castration at weaning, with or without meloxicam, on platelet count. Effect of treatment ( $P = 0.13$ ), day ( $P < 0.0001$ ), treatment x day ( $P < 0.0001$ ).**

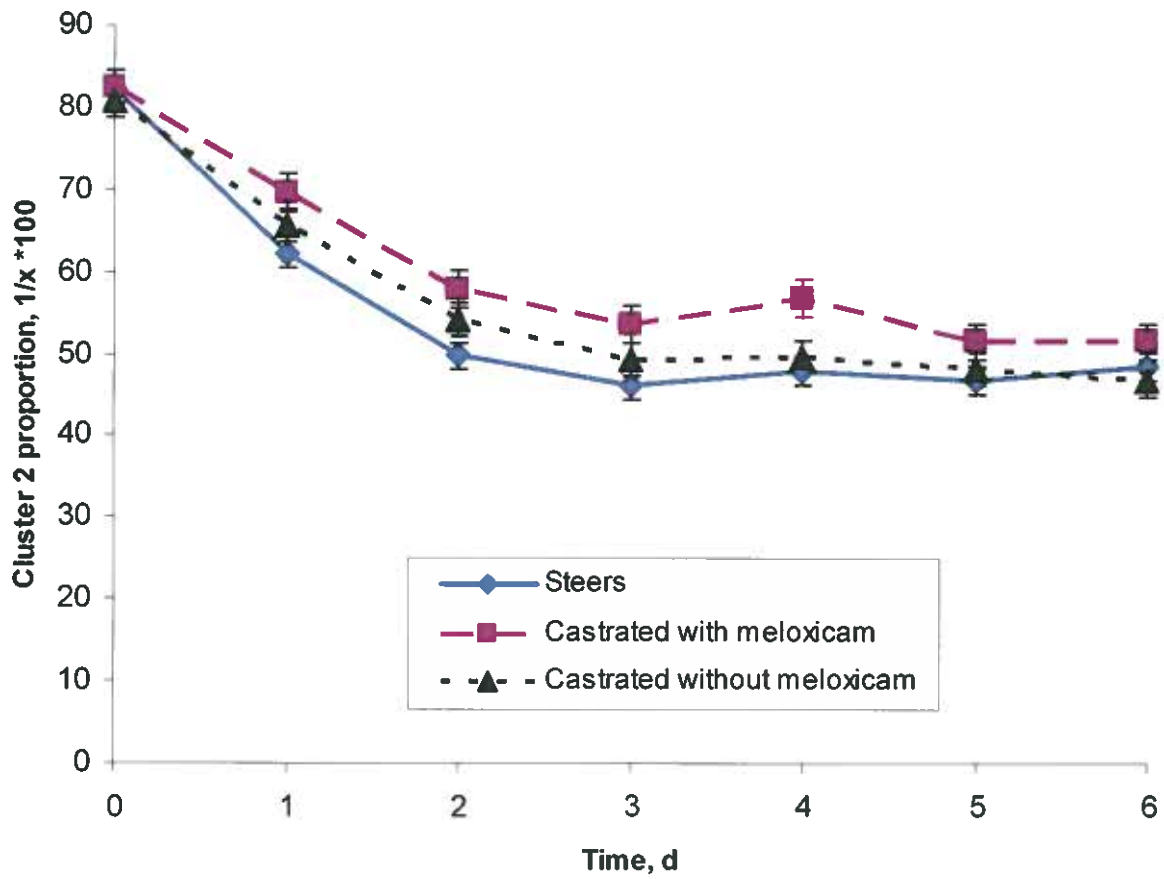


**Figure 27. Effect of castration at weaning, with or without meloxicam, on rectal temperature. Effect of treatment ( $P = 0.89$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.98$ ).**

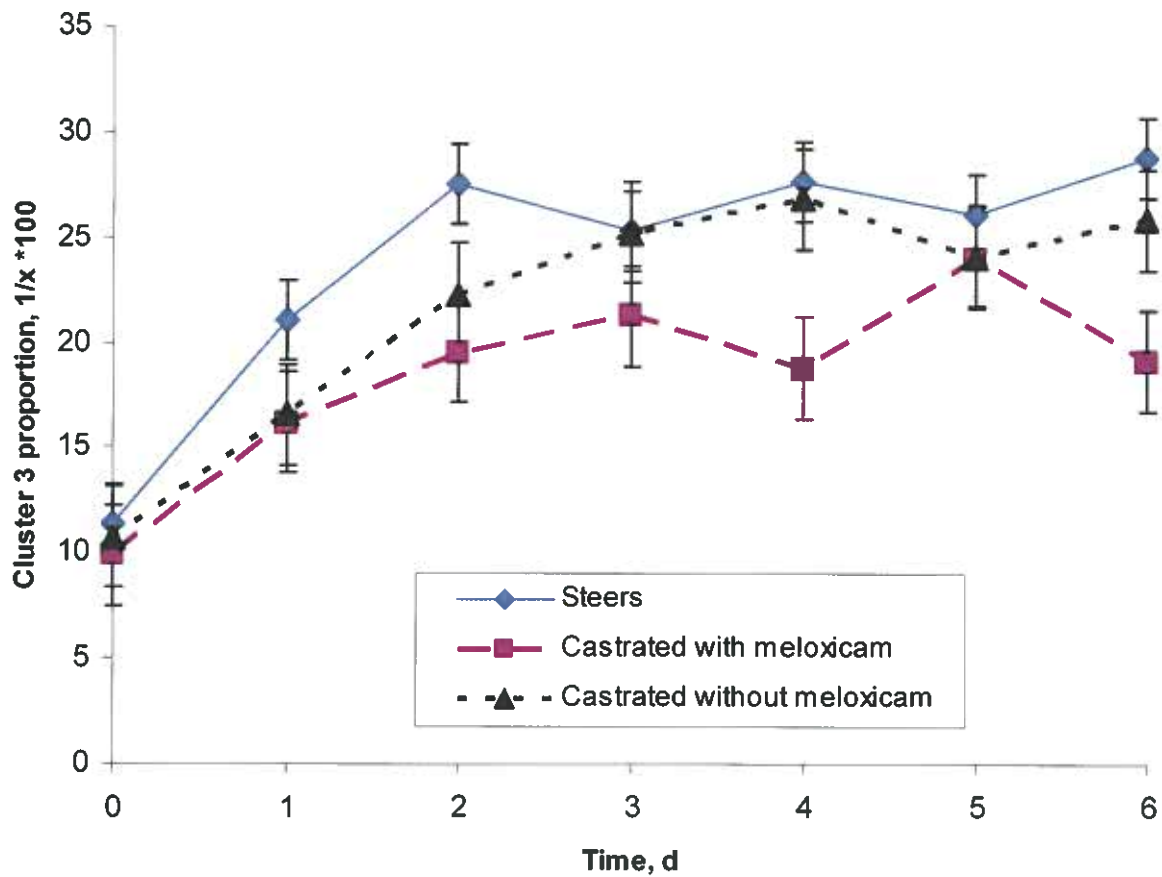




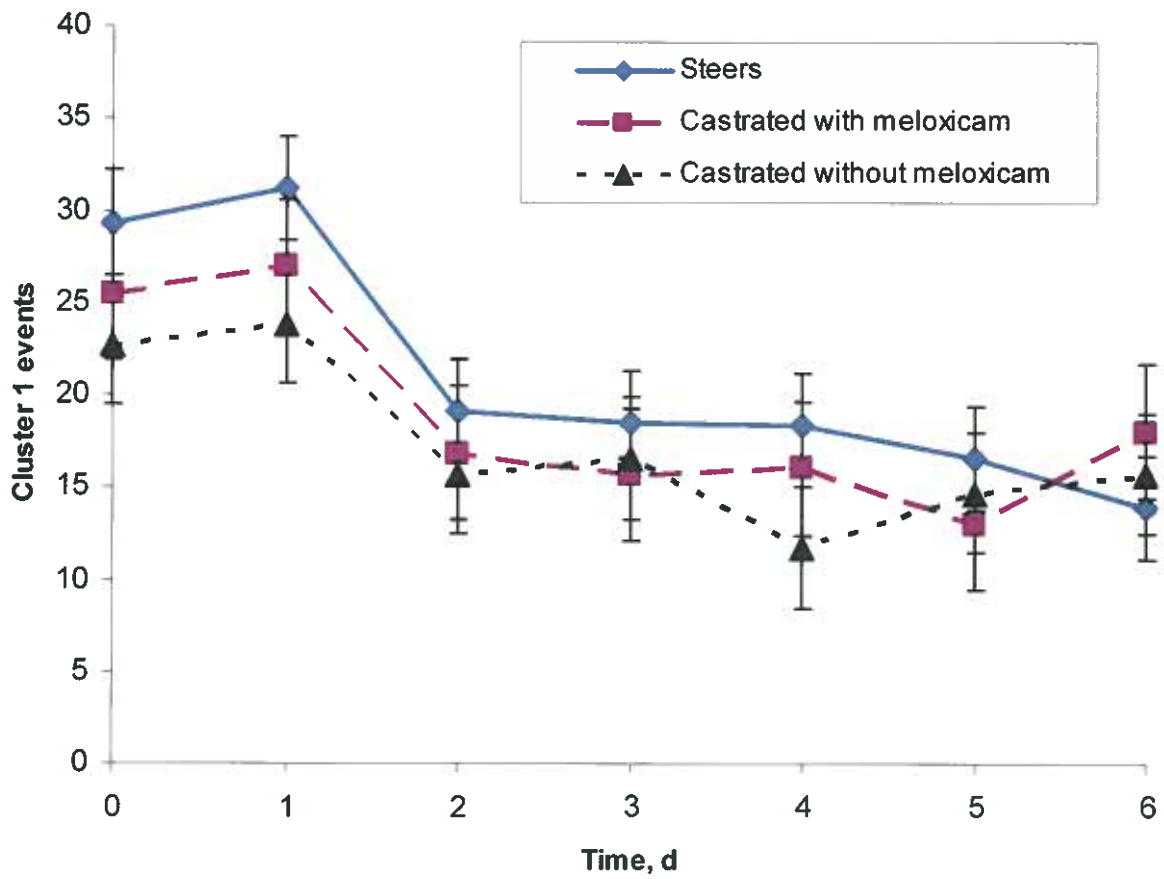
**Figure 28. Effect of castration at weaning, with or without meloxicam, on cluster 1, proportion of time spent lying flat on side. Effect of treatment ( $P = 0.76$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.41$ ).**



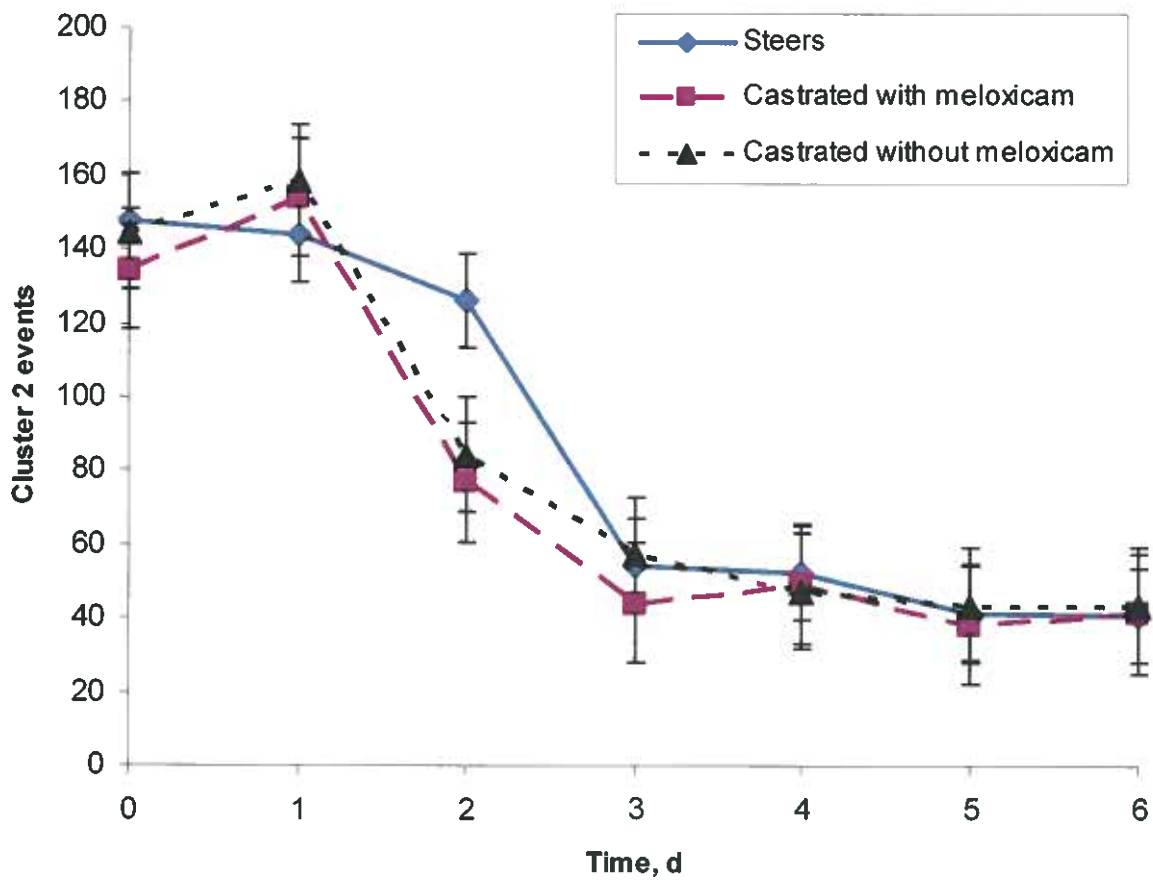
**Figure 29. Effect of castration at weaning, with or without meloxicam, on cluster 2, proportion of time spent standing. Effect of treatment ( $P = 0.02$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.43$ ).**



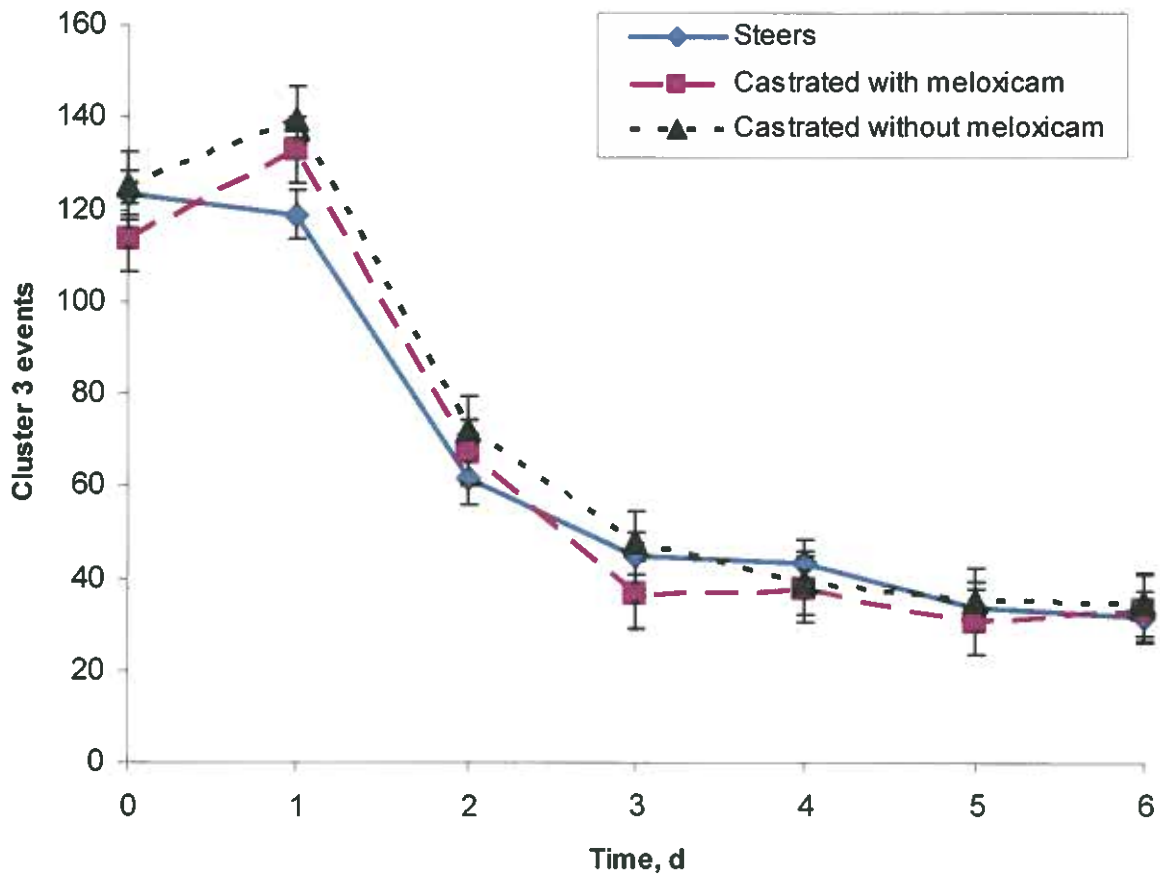
**Figure 30. Effect of castration at weaning, with or without meloxicam, on cluster 3, proportion of time spent lying on sternum. Effect of treatment ( $P = 0.03$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.61$ ).**



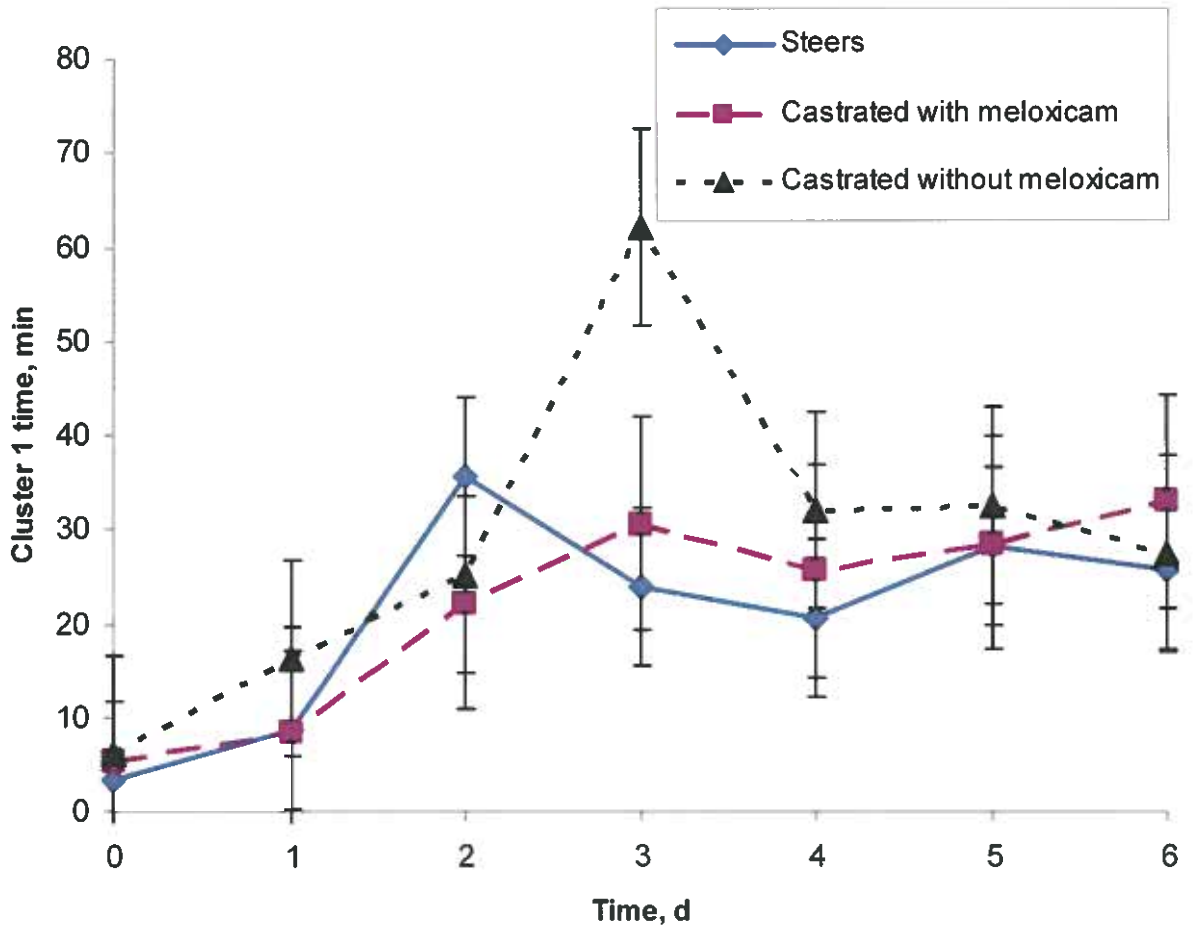
**Figure 31. Effect of castration at weaning, with or without meloxicam, on cluster 1, number of events occurring: lying flat on side. Effect of treatment ( $P = 0.47$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.72$ ).**



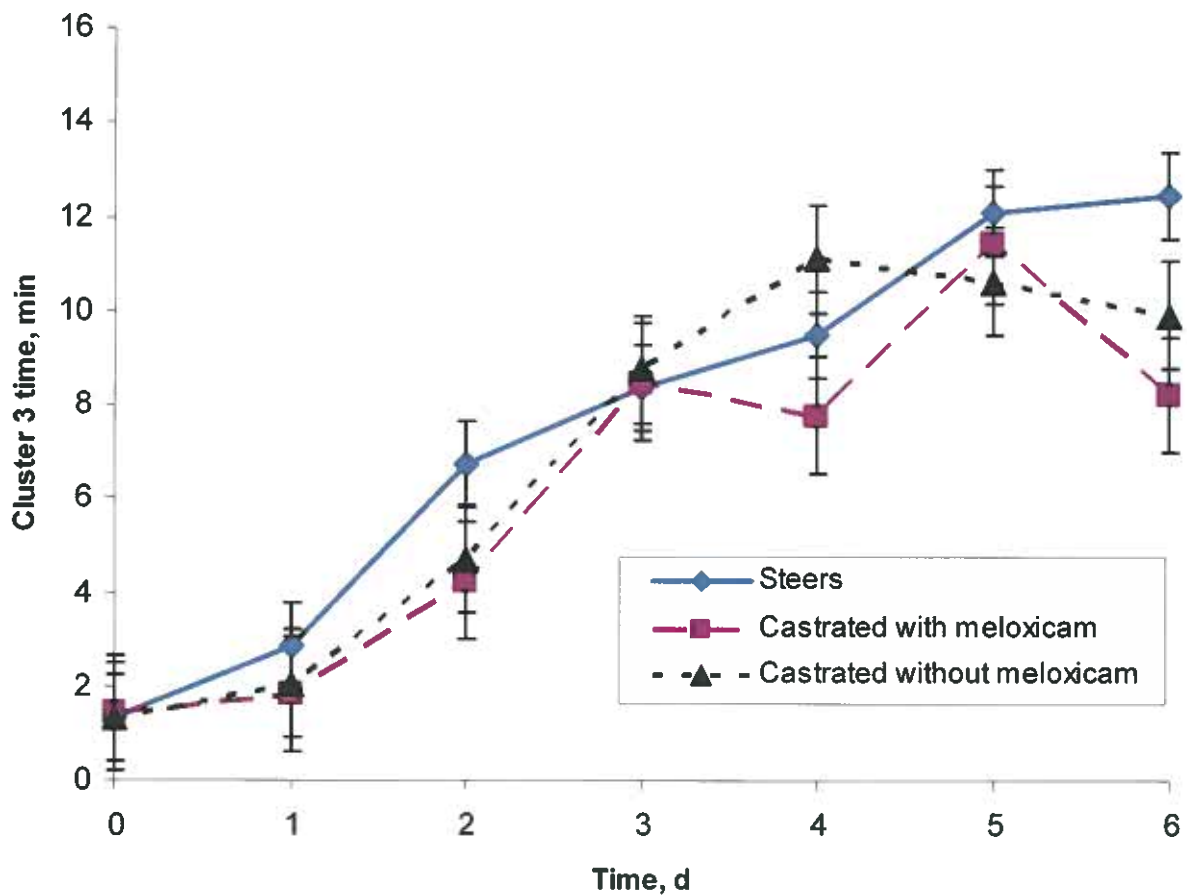
**Figure 32. Effect of castration at weaning, with or without meloxicam, on cluster 2, number of events occurring: standing. Effect of treatment ( $P = 0.59$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.97$ ).**



**Figure 33. Effect of castration at weaning, with or without meloxicam, on cluster 3, number of events occurring: lying on sternum. Effect of treatment ( $P = 0.60$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.79$ ).**

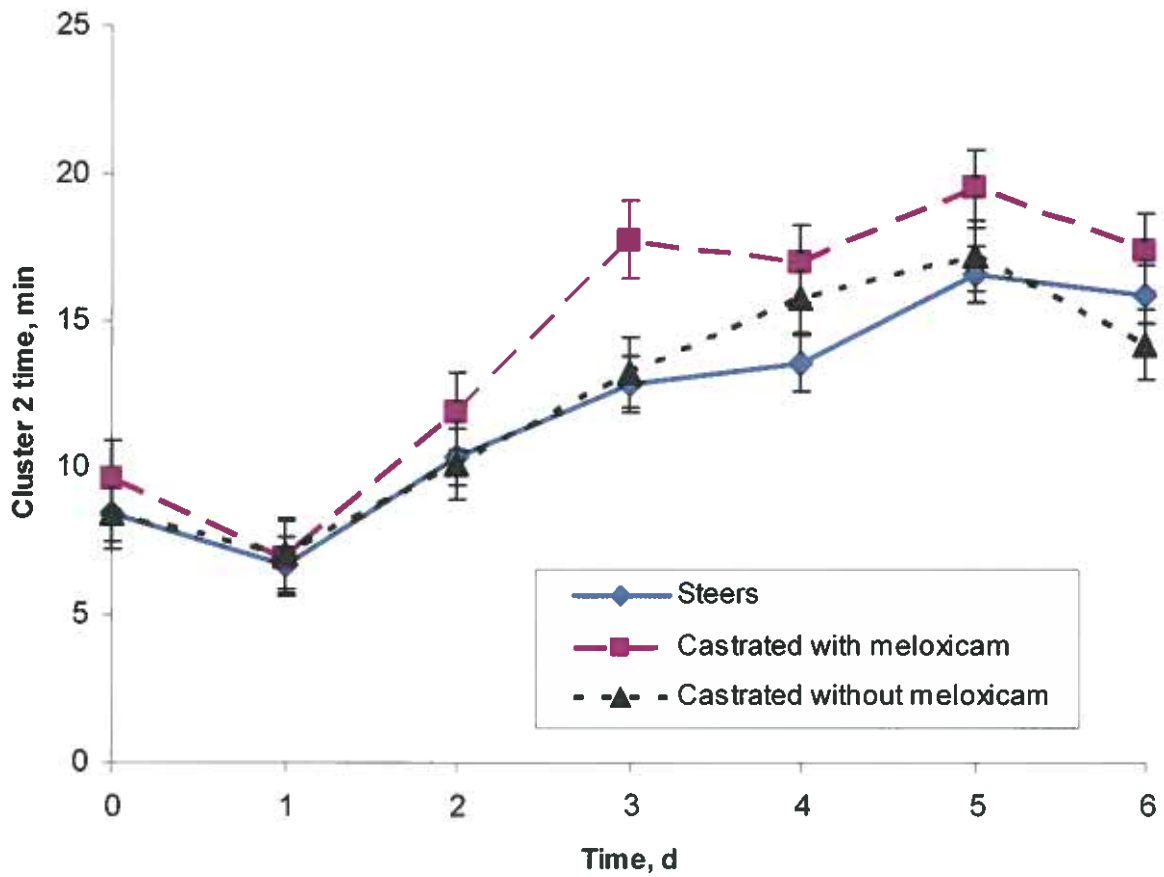


**Figure 34.** Effect of castration at weaning, with or without meloxicam, on cluster 1, mean duration of time (min) spent lying flat on side. Effect of treatment ( $P = 0.36$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.68$ ).



**Figure 35.** Effect of castration at weaning, with or without meloxicam, on cluster 3, mean duration of time (min) spent lying on sternum. Effect of treatment ( $P = 0.22$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.23$ ).





**Figure 36. Effect of castration at weaning, with or without meloxicam, on cluster 2, mean duration of time (min) spent standing. Effect of treatment ( $P = 0.04$ ), day ( $P < 0.0001$ ), treatment x day ( $P = 0.57$ ).**