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Zinc Injection as a Novel Castration Method and Carry-Over Effects of Growth-Promoting Implants in Beef Cattle

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Zinc Injection as a Novel Castration Method and Carry-Over Effects of Growth-Promoting Implants in Beef Cattle

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ABSTRACT

In experiment 1, crossbred bull calves (n = 31; body weight (BW) = 114.3 ± 26.3 kg; age = 119 ± 18.4 d) were allocated to treatments by BW and birthdate. Twenty-seven bull calves were allocated to 3 injectable castration treatments (n = 9 calves/injectable castration treatment) to reflect 3 dosage levels of zinc. Intact bulls had greater ($P < 0.001$) serum testosterone concentrations compared to bulls injected with zinc. In experiment 2, crossbred beef bulls (n = 180) were blocked by initial BW (337 ± 10.9 kg; 6 blocks) and assigned randomly to 1 of 3 treatments on d 0: 1) INJ; received 1 mL (100 mg Zn) of a Zn solution in each testis, 2) BAN; banded, 3) BUL; intact. Final BW was greater ($P < 0.01$) for INJ (672 kg) and BUL (686 kg) compared to BAN (611 kg). Serum haptoglobin concentration was greater ($P < 0.01$) in INJ compared to BUL and BAN on d 1, 3, 5, and 7. Zinc injection resulted in sterilization but not castration in feedlot bulls although it was efficacious in castration of young bulls at branding. In experiment 3, crossbred beef steers (n = 106; BW = 96 ± 3.9 kg; age = 74 ± 2.0 d) were blocked by parity of dam (≤ 2 or > 2 parities), stratified by BW, calf age, calf sire, cow BW and body condition score to 1 of 4 treatments: 1) RALG, Ralgro, Ralgro, Revalor XS, at branding (D 0), weaning (D 156), and feedlot processing (D 325), 2) COMP, Component E-C, Component TE-G, Revalor XS, 3) N-REV, none, Revalor-G, Revalor XS, and 4) CTRL, no growth-promoting implants administered. Implantation of male calves at branding increased growth performance at weaning. At the end of the stocker phase on d 323, RAGL (330 kg), COMP (324 kg), and N-REV (318 kg) were heavier ($P = 0.02$) compared to CTRL (297 kg). Steers first implanted at weaning (N-REV) gained more during the stocker phase; however, steers implanted at branding had a 6 to 12 kg BW advantage at time of feedlot shipment.

Keywords: castration, zinc, growth-promoting implants, beef calves

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CHAPTER I

INTRODUCTION

Husbandry practices in the beef industry that are associated with pain, discomfort, and distress include castration, dehorning, and branding (Lyles and Calvo-Lorenzo, 2014). Castration derived pain increases in intensity and duration as the age, BW, and testicular size of the calf increases (Chase et al., 1995). Pain and inflammation may also affect the growth rate and efficiency of beef calves (Fisher et al., 1997). There are approximately 15 million castration procedures performed in the United States annually to reduce aggressiveness and sexual activity, facilitate handling, prevent unwanted breeding, and improve the meat quality of cattle (Lyles and Calvo-Lorenzo, 2014).

In Arkansas, only 17% of male calves sold in livestock auctions weighing between 136 and 250 kg were castrated (USDA Agricultural Marketing Service, 2004), and of the bulls placed in feedlots in 2008, 91% were castrated, predominantly by band castration (42%) or surgical castration (44%) (NAHMS, 2014). Ratcliff et al. (2014) found that calves arriving at a stocker receiving facility as intact bulls and castrated gained less weight and had increased incidence of bovine respiratory disease (**BRD**) during the receiving period compared to calves arriving as steers; these cattle were unable to make up for this weight difference during the receiving period or by the end of a 150 day grazing period. Stafford et al. (2002) suggested that surgical castration is more painful initially as evidenced by increased plasma cortisol concentrations.

Economically, castration post-weaning affects profitability by decreasing average daily gain (**ADG**) and increasing susceptibility to BRD (Massey et al., 2011). Currently, no commercially available injection sterilization methods exist for beef cattle in the United States, although there has been a zinc solution utilized in other species, including companion animals.

An injectable sterilization method could be an alternative castration method which could potentially reduce pain, stress, performance loss, and the prevalence of BRD.

Growth-promoting implants are often used by United States. beef producers to increase rate of gain and efficiency of growth; however, the adoption of these strategies varies greatly between sectors of the beef industry. Only 11.9% of cow/calf operations utilize the practice of implantation prior to weaning (NAHMS, 2008); whereas, over 91% of feedlots implant steers weighing less than 318 kg at least once during the finishing phase and over 79% of steers implanted received 2 or more implants (NAHMS, 2013). The effects of previous implantation is well documented; however, concern by operators about the efficacy of cattle implanted multiple times across production sectors compared to cattle implanted only during the finishing phase as per industry standard is of great interest within the industry.

CHAPTER II

REVIEW OF LITERATURE

Overview of Castration

Castration is one of many animal husbandry practices receiving scrutiny and criticism and as consumers' negative perceptions of industry standard practices mainly derives from the humanized opinion of pain and stress inflicted on the animals (Rollins, 2004. However, the European Union (**EU**) has implemented animal welfare regulations in the form of the Treaty of the Functioning of the European Union (**TFEU**) in 2009. Title II, Article 13 of TFEU states,

> In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage" (European Union, 2009).

Therefore, the EU has given individual states the right to further mandate and enforce animal welfare laws beyond what the EU has mandated for all of its countries. The EU's scientific committee on animal health and welfare recommended that castration, dehorning, and hot-iron branding be eliminated from production operations (European Commission, 2001). Council Directive 98/58/EC of 1998 states that animals should not undergo unnecessary pain, suffering or injury. The committee's conclusion on castration states:

> Castration causes severe pain and distress. According to some studies surgical castration seems to be less acceptable from a welfare point of view than Burdizzo or rubber rings. However, those last two techniques can only easily be done on young calves. Local anesthesia or local anesthetic plus systemic analgesia act to reduce the pain (European Commission, 2001).

New Zealand first adopted policy on castration and dehorning in 1960 and renewed this law in the Animals Protection Act of 1999, in which it is an offense for any person to castrate,

any bovine animal, sheep, goat or pig, over the age of 9 mo, unless performed under veterinary supervision; and except for veterinarians, for any person to dehorn, or cause an animal to be dehorned, over the age of 20 mo unless pain is prevented (NAWAC, 2005).

Countries within the EU have further enforced animal welfare laws from what was previously mandated. The United Kingdom prevents non-veterinarians from performing surgical castration on calves older than 2 mo of age without analgesia (MAFF, 1992). This insinuates there is an animal welfare concern among the population associated with castration and without castration animal suffering is reduced or completely mitigated.

The United States government has yet to mandate specific laws on what producers may do to animals in terms of castration, dehorning, and other painful management practices. However, there have been some non-governmental organizations that offer premiums for animals produced with minimal pain. Global Animal Partnership (**GAP**) has enacted a 5-step animal welfare rating standard for beef cattle (GAP, 2009). These standards include a note that calves are ideally castrated before 7 d of age with an emasculator band. At the highest level of the program (Step 5+) cattle are not allowed to be castrated at all. On the opposite end, at the lowest level (Step 1) calves are to be castrated by 6 mo of age (GAP, 2009). A Few beef retailers are using GAP standards including Whole Foods, but the partnership is searching for adoption from other grocers (GAP, 2012). Welfare standards are beginning to percolate into the United States retail sector suggesting a greater potential for more consumer-driven welfare verification programs.

Economics

Buyers of feeder calves typically pay less for bulls than they do steers because the cost to castrate the bull calf and the repercussion of late castration will be placed on the buyer. Also, bulls purchased at live auction markets are plausibly from a ranch that has yet to initiate a

vaccination program; thus, the animal is unprepared for infectious challenges and increasing the chance of detrimental health effects. It is very difficult to determine the appropriate discount for bulls compared to steers around weaning. The ability to quantify the discount under a multitude of conditions would help stocker and feedlot operators maintain a greater level of profitability or recognize opportunities to increase profit margins when excessive discounts are applied. Massey et al. (2011) researched the effect of castration timing on performance, morbidity, and carcass quality and how morbidity affects performance and carcass quality. They calculated price discounts for bulls relative to steers, for both a short backgrounding period and when ownership was retained until slaughter. Coinciding with many others findings, late-castrated bulls exhibited decreased growth performance and increased morbidity relative to early-castrated bulls. The increased morbidity negatively correlated with ADG. However, castration timing and morbidity during the backgrounding period had minimal effect on carcass quality, with morbidity only affecting hot carcass weight, whereas castration timing only affected days to market and hot carcass weight. Massey et al. (2011) determined that on average calves arriving at live auction markets in 2009 weighed 209 kg and bulls should be discounted compared to steer calves. They determined bulls should be discounted $0.11/ kg relative to the same BW steers. The discount increased to $0.12/ kg for 170 kg calves and decreased to $0.08/kg for 250 kg calves. If ownership was retained through slaughter, required discounts changed to $0.14/kg for calves weighing the average of 209 kg. The discount decreased to $0.04/kg for lighter calves weighing 170 kg and increased to $0.17/ kg for the heavy end of calves weighing 250 kg.

Costs of raising bull calves, regardless of the section of the industry from cow-calf producer to packers and all the sectors in-between, increases compared to rearing steer calves. However, the market is constantly changing and the potential for all-natural bull calves may

increase in global demand of beef production with increased consumer concern to know the derivation of their food and is of common practice in many European countries such as Spain and France. If the market continues as it has previously, there is increased profit margins and decreased health risk in rearing, buying, and selling steers compared to bulls. Castration is imperative in this process and the means to do so will continue to be under scrutiny until either the consumer is more educated on the importance of castration or governmental regulations on castration are enforced as they are in other countries. The optimal solution to this issue in maintaining profit margin and meat quality while meeting consumer demands for minimal pain inflicted on the animal is a novel castration technique that can be accepted globally by industry and consumer alike.

Issues raising intact bulls

For thousands of years, meat-producing animals have been castrated to minimize difficulties in management practices rearing intact animals (Trow-Smith, 1957). Problems raising intact males in the cattle industry are aggressive sexual behavior in concert with negative implications on carcass attributes. Intact males should not be grazed with heifers post-weaning as they begin to reach sexual maturity around 9 months and can cause damage to pastures and potentially threaten human safety (Price and Tennesson, 1981; Gregory and Ford, 1983). Heifers exhibit estrus behavior every 18 to 24 d after reaching puberty and becoming fertile (Bonneau and Enright, 1995). Previous research grazing intact males with heifers has shown increased disturbance, stress, risk of injury and unwanted pregnancies (Curran et al., 1965; Roche and Crowley, 1973). Therefore, once cattle reach 9 months of age, intact males and females should be fed separately to minimize negative consequences (Bonneau and Enright, 1995).

At slaughter, intact bulls and heifers present a greater risk of dark cutting and a greater tendency to yield bruised carcasses compared to steers or spayed heifers (Kenny and Tarrant, 1988; Kempster and Lowe, 1993; Scanga et al., 1998). Dark cutting results from stress prior to slaughter, which depletes muscle glycogen stores, thereby reducing glycogen needed to produce lactic acid to reduce muscle pH postmortem (Scanga et al., 1998). A pH greater than 6 increases the light-absorption and water-binding capacity of postmortem muscle and results in an undesirable, dark, firm and dry lean cut surface.

Issues with traditional castration

In the US, castration of non-breeding bulls is common practice, although several disadvantages are associated with castration including the added stress to the animal and castration is a costly and laborious procedure. Morbidity and mortality are added risks from hemorrhage and infection to the calf, whereas economic costs of labor and equipment necessary to castrate intact male calves increase input costs. Welfare and ethical issues related to castration are a concern to the public and may potentially affect consumer perception of beef. This, in concert with greater beef prices compared to other protein sources such as poultry and pork, may have a negative effect on consumer beef demand; however, the largest economic disadvantage is derived from the loss of growth potential and feed conversion rates that are inherent with intact bulls.

Adams and Adams (1992) indicated that gonadal steroids play a critical role in mammalian growth and development. Greater growth performance and conversion of feed to gain, as well as leaner carcass, are all positive attributes associated with feeding intact bull calves rather than steers (Price et al., 1980). Calves castrated post-weaning upon arrival to the feedlot experience a significant reduction in final live weight, ADG, and hot carcass weight when

compared to bull calves (Gonzales et al., 1990; Adams and Adams, 1992; Huxsoll et al., 1998). Previous research by Hannon et al. (1991) reported that calves castrated at 6.5 months of age experienced a reduction in feed intake and weight gain over the subsequent 8 months; thus, steers are commonly implanted with growth promoters in an attempt to mitigate the negative effects of castration on growth and feed conversion efficiency. Robertson et al. (2018) reported that traditional castration methods (surgical or band) at feedlot arrival with or without the addition of an analgesic transiently reduced ADG compared to bulls castrated at birth; however, calves castrated at feedlot arrival that were given an analgesic for pain mitigation had improved overall ADG compared to calves castrated at feedlot arrival that were not administered an analgesic.

Effects of castration

Castration of intact males can either derive from removal of the testicles and epididymides, or banding of the scrotum to occlude blood circulation and subsequent necrosis of the affected tissue occurs, or another treatment which causes the atrophy of the testes. Castration depresses the animal which can vary with calf, behavioral, and physiological status at the time of castration (Hafez, 1993). Male calves reach puberty around 9 months of age, which is accompanied with androgen induced development of male sexual behavior. Many producers castrate male calves prior to puberty and as early as possible to mitigate some of the negative effects of male sexual behaviors. Sterility is the result of castration and prevents the maturation of accessory glands, aggressiveness, and libido. Research by Jago et al. (1997) suggested that surgical castration prior to puberty prevents the development of copulatory behaviors (intromission and ejaculation) and reduces the expression of pre-copulatory behaviors (searching, courtship, and mounting). However, increasing sexual activity of steers from 7 to 17

months of age indicates that testicular removal does not eliminate all sexual behaviors associated with intact males and, in fact, some castrates will mount females (Jago et al., 1997).

Methods of Castration

Three methods of castration are noted by the American Veterinary Medical Association (**AVMA**): physical, chemical, and hormonal (AVMA, 2009). There are a number of specific procedures within these methods in which intact bulls can be castrated. Methods differ from producer to producer and from region to region. A common technique is the traditional method of surgical castration (Coetzee et al., 2010), where a knife or scalpel is used to sever the scrotum, allowing the removal of the testes and part of the spermatic cord. In 2007, 49.2% of US cow-calf operations surveyed utilized surgical castration (testis removal via blade) as castration method. Feedlot operators whom responded to a 1999 survey claimed 48.4% of operations used surgical castration at arrival of intact males (USDA, 2000). Within surgical castration, multiples tools can be utilized to complete castration. Removal of the scrotum may be completed via knife, scalpel, or Newberry Knife. A Newberry Knife, that creates a bilateral excision of medial aspects of the scrotum. The removal of the scrotum can be accomplished readily with a knife or scalpel, although many producers and veterinarians use blades to excise the distal aspects, particularly in heavy intact males (Gilbert and Fubini, 2004). Once the testes are exposed, external testicular fascia are stripped by a knife, scalpel, or gauze pad. The exposed spermatic cords possess challenges in ligating the cord. Ligation of the spermatic cord may be accomplished by using an emasculator that crushes and cuts the spermatic cord. Emasculation followed by suturing the cord, ligating the cord with suture and then cutting the cord, or by a Henderson Castration Tool (Stone Manufacturing, Kansas City, MO) all means of addressing cord ligation. In calves castrated close to birth, ligation is often overlooked as the testes are pulled from the body cavity

until the connective tissue is torn which stretches the spermatic cord until vasculature ruptures (Gilbert and Fubini, 2004). A knife is often used to sever the cord ventral to the testicle.

Another method of castration involves the use of an emasculator to crush and ablate the spermatic cord. Two non-surgical methods include the use of a burdizzo or an elastrator. A Burdizzo emaculatome is applied to each spermatic cord individually and then clamped on each spermatic cord to crush each individually. The theory is the testis will atrophy, but the scrotum will remain intact, as long as the scrotum was not ever crushed (Gilbert and Fubini, 2004). A USDA (2008) survey found that only 3.5% of cow-calf operations claimed to use Burdizzo or clamp methods of castration.

An elastrator, or bander, as means of castration is used on young calves by positioning an elastic band around the neck of the scrotum directly above the testes, cutting off circulation to tissues causing atrophy to begin. Band castration is known as “bloodless castration”, and can be accomplished in multiple ways and at varying weights. In older, more mature calves with larger testes, a ratchet-style applicator secures a latex band around the scrotum (No Bull Enterprise, St. Francis, KS). All banded castration techniques aim to eliminate blood supply to the scrotum and testes to cause necrosis and sloughing within 3 wk (Gilbert and Fubini, 2004). In 2007, 39.5% of cow-calf producers claimed to band castrate calves at less than 3 months of age, and only 7.8% of respondents banded bull calves older than 3 months of age (USDA, 2008). Feedlot operators claimed to band 65.3% of bulls entering the feedlot in a 1999 survey (USDA, 2000).

Previous research by Fisher et al. (2001) compared the effects of band castration and surgical castration on 14 month old beef bulls and found no differences in time spent recumbent or ambulatory between surgical castrates, band castrates, and intact males. Surgical castrates spent less time grazing compared to banded calves, whereas intact bulls exhibited greater time

grazing compared to surgical and banded castrates. Haptoglobin concentrations were greater in surgical castrates than banded and intact bulls on d 1, 2, and 3 post-castration.

There have been a variety of alternative methods to surgical castration including:

1. Lactic acid injection into the parenchyma of the teste resulting in sclerosis and atrophy of the testes (Cohen et al., 1991a, b).
2. The interruption or down-regulation of the hypothalamic-pituitary-gonadal by chronic administration of gonadotropin-releasing hormone (**GnRH**) in high doses (Melson et al., 1986; Ronayne et al., 1993).
3. Active immunization against sex steroids in cattle, sheep, or against boar taint related steroids in male swine (Schanbacher, 1982; Price et al., 1987).

As with any change, there are several negative effects or drawbacks to these methods of castration compared to traditional methods. Efficacy of the castration has had the potential to be inadequate, consumers may have negative attitudes towards certain methods along with governmental regulations may not allow the practice in certain countries. One reason is the potential for residues to be left in the meat prior to entering the food chain.

Alternative castration methods could have a major impact in animal agriculture and food production: to reduce aggressive behavior in bulls, to prevent estrous behavior and fertility in heifers and to decrease the incidence of boar taint in pork (Bonneau and Enright, 1995).

Growth-Promoting Implants

The inability for castrates to produce testosterone at levels comparable to bulls reduces their growth efficiency. In an effort to mitigate this reduction in performance, castrates are often implanted with estrogenic and/or androgenic compounds to increase feed conversion, weight gain, and protein synthesis while inhibiting lipogenesis. Apple et al. (1991) found that synthetic

hormones reduced fat thickness, percentage of internal fat and marbling, while increasing carcass weight and improving carcass confirmation in castrates. Previous research by Lee et al. (1990) indicated the anabolic effects of implants in castrates changed their hormonal status to resemble that of bulls. Growth performance of castrates in the feedlot and at time of slaughter increased when implanted with growth promotors (Perry et al., 1991; Rumsey et al., 1992; Adams et al., 1993).

Both estrogenic and androgenic growth promoting implants exist and, although their mechanisms differ, both improve growth parameters by increasing the synthesis of muscle protein (Hayden et al., 1992). Castrates demonstrate enhanced growth responses when trenbolone acetate (a synthetic androgen) is combined with estrogenic implants (Apple et al., 1991; Hayden et al., 1992), but minimal effects are seen in bulls (Henricks et al., 1988). This coincides with other reports that zeranol, trenbolone acetate and combined progesterone and estradiol benzoate did not improve feed conversion in the feedlot or the carcass traits in bulls (Silcox et al., 1986; Doornenbal et al., 1987; Jones et al., 1991; Adams and Adams, 1992; Adams et al., 1993). Research indicates bulls have sufficient endogenous anabolic steroids for maximizing growth (Lee et al., 1990), and administration of supplemental steroid hormones does not protein anabolism (Adams and Adams, 1992).

Previous research by Lehman et al. (2001) in crossbred suckling calves implanted with approved estrogenic implants comparing varying compounds found that Revalor-G (trenbolone acetate and estradiol) and Ralgro (zeranol) significantly improved rate and accumulative BW gain. Male calves that were castrated and implanted at 2 to 3 months of age had greater ADG the first 50 d after weaning than calves that were castrated and implanted at weaning (Lents et al., 2006). Implanting steer calves during the suckling phase with zeranol has increased weight gain

(Mader et al., 1985). However, Selk (1997) reported that other studies have shown no benefit of implanting young suckling calves (Selk, 1997). Bagley et al. (1989) found that castrating bull calves at birth and implanting them with zeranol increased BW gain by 4% from birth to weaning, and implanting bulls castrated at 4 months of age increased weight gain by 6.7%. Therefore, timing of castration, timing of implantation, or both in concert with one another may cause conflicting results.

Although, the administration of growth implants does not alter growth performance in intact males, it does decrease the testosterone concentrations potentially through a negative feedback mechanism (Lee et al., 1990). The reduction in serum testosterone reduces testicular development and function in implanted bulls, as was noted in bulls implanted with estradiol-17β (Schanbacher, 1984; Calkins et al., 1986), zeranol (Silcox et al., 1986) and progesterone with estradiol benzoate (Adams et al., 1993). Carcass masculinity is reduced by implantation in concert with reduced serum testosterone concentrations (Adams et al., 1993). Schanbacher (1984) indicated that the suppression of testicular growth and development in cattle with implants containing anabolic steroids potentially results from implant-induced attenuation of episodic secretion of GnRH and an associated reduction in gonadotrophin secretion.

Steroid supplementation (progesterone and estradiol benzoate) had no effect on feedlot performance and carcass traits in bulls immunized against GnRH and bulls left intact indicating that growth implants had similar effect to endogenous male hormones in the intact bulls (Adams and Adams, 1992). A later study found that while serum testosterone concentrations were reduced in bulls actively immunized against GnRH, growth and feedlot performance were not improved by implantation (Adams et al., 1993). Research by Huxsoll et al. (1998) implanted 1 month old beef calves in an effort to determine if early implantation would suppress the early

stages of testicular growth and development and complement the effect of immunization against GnRH. Early implantation combined with immunization did not suppress testicular development more effectively than immunization alone and found no benefit to a treatment regimen combining both.

Pain in cattle

Animal welfare is increasing in relevance in recent years as society has become more aware of issues in agricultural practices and then they are subsequently posted via social media websites by activist organizations attempting to harm animal agriculture. The main animal welfare issue associated with castration is most often the pain felt by the animal from the procedure. Every consumer has their own opinion of what pain is stemming from personal experiences and season of life. The International Association for the Study of Pain (**IASP**) defines human pain as: "An unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage" (IASP, 1979).

The perception of pain is the transduction, transmission, modulation, and projection of some sort of painful stimulus (Anderson and Muir, 2005). The physical stimulus of castration is transduced into action potentials by pain receptors on C and A delta nerve fibers. An electrical signal created from nerve action potentials are transmitted to the dorsal horn of the spinal cord. Within the spinal cord, electrical signals are modulated by neurons and transmitted to the brain (Anderson and Muir, 2005). Damage to muscular tissue induces an inflammatory response releasing prostaglandins, histamine, cyclooxygenase, and cytokines. These chemicals induce peripheral sensitization (Woolf and Slater, 2000).

Local anesthetics are commonly used as a pre-emptive analgesia (Anderson and Muir, 2000). Local anesthetics block open Na^+ channels to prevent impulses in response to a painful stimulus (Webb and Pablo, 2009; Coetzee et al., 2011).

There were currently no analgesic drugs approved by the United States Food and Drug Administration (**FDA**) for pain mitigation in food animals (Compendium of Veterinary Products, 2010); however in 2018, Banamine® Transdermal (flunixin transdermal solution) became the first and only United States Food & Drug Administration approved product for pain control in a food producing animals. It is approved for the control of pain associated with foot rot and fever associated with bovine respiratory disease. Flunixin meglumine is a non-steroidal anti-inflammatory drug (**NSAID**) labeled for use in beef cattle for the treatment of pyrexia associated with respiratory disease and mastitis, as well as inflammation associated with endotoxemia (Smith et al., 2008). Non-steroidal anti-inflammatory drugs inhibit one or more processes in the conversion of arachidonic acid to prostaglandins by inhibiting cyclooxygenase (**COX**) enzymes (Boynton et al., 1988). European countries have already mandated the use of local anesthetics at time of castration, while United States producers do not have a clear pathway to administer pain mitigation to food animals; however, the AVMA and AABP have broad recommendations for producers in the United States but no mandates. Yet, the American Medicinal Drug use Clarification Act (**AMDUCA**; FDA, 1994) allows veterinarians in the United States to prescribe drugs that are not labeled for use in a particular species if there is not an approved alternative. Many regulations exist for prescription of extralabel drug use; but a licensed veterinarian must oversee all extralabel drug use (AVMA, 2007).

Withdrawal time must be heeded deriving from the drug label to ensure harmful levels of residues in animals do not enter the food chain. These withdrawal times on food-animal-

approved products must be respected as is the case for non-food-animal-approved products. Producers in concert with veterinarians must document this clearly with individual animal records (AVMA, 2007).

Hormonal influences on bull calf development

Gonadotropin-releasing hormone (**GnRH**) is produced in the hypothalamus and plays a crucial role in the endocrine events within the reproductive process. From the hypothalamus, GnRH travels through the hypophyseal portal blood system to the anterior pituitary, where it is responsible for the release of follicle stimulating hormone (**FSH**) and luteinizing hormone (**LH**). The pulsatile hypothalamic release of GnRH increases with age in pre-pubertal bulls, thereby increasing pulsatile secretion of LH from the anterior pituitary (Rodriguez and Wise 1989). This pulsatile secretion of LH initiates the onset of puberty and testicular maturation in bulls by increasing serum testosterone concentrations (McCarthy et al., 1979; Amann et al., 1986). Luteinizing hormone acts upon the Leydig (interstitial) cells of the testes in the production of androgens. Follicle stimulating hormone stimulates spermatogenesis, with the effect on spermatogonia and sertoli cells in the testes. Sertoli cells also convert testosterone to estradiol under the influence of FSH.

Hormonal changes at puberty

Research by Rodriguez and Wise (1989) indicate that puberty in male beef calves occurs between 4 and 8 months of age. Serum concentrations of LH are low after birth in beef bull calves and slowly begin to increase between 10 and 20 wk of age (McCarthy et al., 1979; Evans et al., 1993; Evans et al., 1996). Concentration of circulating FSH are initially elevated but then decrease between 14 and 30 wk of age (Evans et al., 1996). Secretion of testicular androgens occurs in peaks reflecting the pulsatile release of the pituitary gonadotrophin. Circulating

testosterone concentrations increase gradually from 6 to 35 wk of age and then increase rapidly to 42 wk of age (Evans et al., 1996). Evans et al. (1996) suggested that the early increase in LH secretion, in concert with increased FSH concentrations, stimulated the testes to secrete steroids, indicating testicular maturation and spermatogenesis. When LH is suppressed during the early period, the development of the testes and age at puberty are delayed (Chandolia et al., 1994).

Testosterone is the principle androgen in sexually mature males and is of the utmost importance to researchers and producers, as it is believed that testosterone concentrations correlate with aggressive behavior. Roles of testosterone include: development of secondary sex characteristics, maintenance of the male duct system, expression of sexual behavior, accessory gland functions, function of the tunica dartos muscle in the scrotum, spermatogenesis, and embryonic differentiation of the male duct system and external genitalia (Bearden and Fuquay, 1997). Balthazart (1990) indicated that testosterone induces sexual and aggressive behaviors in males either directly, or through actions of estradiol that derives from aromatization of testosterone in upper brain centers. Testosterone and the previously mentioned hormones (GnRH, LH, and FSH) are antagonistic in their relationship, as high serum concentrations of testosterone inhibit the secretion of those hormones and low levels of testosterone increase their secretion. The interaction of hormones regulating male reproduction are controlled by negative feedback mechanisms.

Immunity in Cattle

The immune system of both cattle and humans is an extremely complex system (Abbas et al., 2015). It can be divided into two branches, innate and adaptive (acquired) immunity, both of which are important in efficient immune response. Innate immunity is the initial defense against foreign pathogens, including physical barriers, such as the skin, and chemicals, such as mucous

and lysozyme. Innate immune cells initiate the inflammatory response while processing and presenting foreign particles (antigens) to the adaptive immune system. Primary cell types of innate immunity include myeloid cells (neutrophils, eosinophils, and basophils) and the macrophage. The methods to assess innate immunity include determining differential cell numbers and measuring the chemotactic and phagocytic activity of neutrophils and macrophages.

In contrast, adaptive immunity is antigen-specific with memory and can be further divided into cellular and humoral branches. Cellular immune system is mediated by T lymphocytes, which, have matured in the thymus, and then live in the lymph nodes and other lymphoid areas. Subclasses of T lymphocytes provide the primary defense against viral infections (T cytotoxic cells), or produce cytokines (T helper cells) that enhance antibody response, regulate immune response level or stimulate macrophages. Laboratory methods of cellular immune responses include in vitro assays of cell proliferation in response to a mitogen, such as phytohemmaglutinin (**PHA**) or concanvallin A, and cytotoxic T lymphocyte response measurements. Many ruminant animal studies utilize an intradermal injection of PHA, which as an in vivo test elicits a reaction with many features of a delayed hypersensitivity response and is believed to correlate with an animal's ability to mount a cell mediated response (Tizard, 2000).

Humoral immune response is synonymous with antibody response and is important for the removal of bacteria, free virus particles, and soluble antigens from the body. B-lymphocytes produce proteins (antibodies) that are antigen-specific. Methods to assess humoral immune responses include isotype-specific and antigen-specific antibody production. The immune system in animals functions to attack substances foreign to the body and destroys them. It can distinguish between 'self' and 'nonself' molecules so that foreign are preserved to be destroyed later. Although, there are instances where the immune system attacks 'self' molecules or tissues

which results in damage to the body. The immune system can be manipulated to regulate attacks on 'self' molecules that can potentially have beneficial effects. On this basis alternative castration methods via vaccines were developed.

After vaccination extracellular antigen is taken up by antigen-presenting cells (**APC**) by either receptor-mediated endocytosis via complement receptors (**CD35**) or immunoglobulin (**Ig**) receptors (**CD32**) on their surface or by fluid endocytosis. Antigen-presenting cells are found predominately in the skin, lymph nodes, spleen, in mucosal epithelia, and in the thymus. The APC migrate to lymph nodes via the lymphatic system, to local lymphoid tissue depending on entry site of the antigen into the body.

In the humoral response, B cells may become active either by thymus-independent antigens (either by polyclonal activation of B cell mitogens or by cross-linking antibody molecules on the surface of B cells) or by thymus-dependent antigens. These antigens require T cell help to active the B cell into antibody production. Activation is initiated by the recognition of the processed antigen in a MHCII/peptide complex on the surface of a B cell by a T cell receptor on a T helper cell and co-stimulated through interaction of CD40 with its ligand CD40L (van Kooten and Banchereau, 1997).

Activated B cells express the IL-4 cytokine on the cell surface and is activated by activated T helper cells stimulating the clonal proliferation of B cells. Primed B cells proliferate in response to stimuli from the surrounding follicular dendritic cells (**FDC**) in the germinal center of secondary follicles in the lymph nodes. B cells perish through apoptosis unless they are rescued by either cross-linking of their immunoglobulin molecules by the complexes attached to the FDC, activation of their CD40 receptors, which induces differentiation to memory B cells, or by soluble CD23 which stimulates the cells to produce antibodies (Liu et al., 1991).

B cell Ig genes undergo somatic hypermutation and only cells bearing receptors with high affinity for antigens are selected for survival resulting in affinity maturation of the produced antibody and a 100 to 10,000 fold increase in affinity to the antigen. The B cells also undergo Ig class-switching influenced mainly by T helper cells and their secreted cytokines.

The development of vaccines have 2 different purposes for control of disease and regulating hormone concentrations. Hormone regulating vaccines are directed against self or self-like target molecules and antibody titers must remain high to inactivate an endogenous hormone as its production will usually increase after immunization (Hage-Van Noort et al., 1992). Problems of self-tolerance and immunological cross-reactivity have hindered early research in immunological control. The problem prototype vaccines have with low immunogenicity of the autoantigens have partly been overcome by introducing suitable carrier molecules to optimize presentation of peptides by major histocompatibility complex class II molecules, and by the introduction of adjuvants that are designed to act as lymphocyte mitogens (Dirnhofer et al.,1994).

Behavior

Many of the gender-related behaviors exhibited by bulls are due to testosterone (Dykeman et al., 1982; Katz and McDonald, 1992), with aggressiveness increasing during the peripubertal period (Baker and Gonyou, 1986; Price and Wallach, 1991). Producers may make decisions on culling animals depending on their docility, regardless of their merit in other areas of production. For many years, bulls' aggressive nature has led to an increase in the use of artificial insemination as the means for breeding rather than the traditional natural service not only to increase the genetic potential of the herd but to minimize opportunities for harm to the producer. As the average age of a producer increases, it is imperative for their safety to manage

only the most docile animals to minimize risk of injury. However, the pattern of behavior, along with the type of behavior expressed, varies with time. Previous research by Finnerty et al. (1996) suggested greater sexual than aggressive behavior in 8 to 13 month old bulls reared on pasture, but found the majority of observed behaviors were aggressive pre-harvest at 22 months of age.

Typical aggressive behavior exhibited by bulls accentuates management issues for feedlot operators, this in concert with reduced intramuscular fat deposition and bodily harm from animal to animal quarrels is why most bulls entering the feedlot are castrated upon arrival. In many European countries bulls are left intact and fed out for a shorter duration compared to US cattle. Meat quality deteriorates as an animal ages and bulls that are left intact have a greater chance of being discounted on a grid-based system due to reduced marbling, reduced tenderness, and increases in chute bruises and prevalence of dark cutters. In order for immunocastration to be commercially acceptable, behavior of the injected calves must be similar to that of steer calves rather than intact calves. Finnerty et al. (1996) found that bulls left intact were more active than immunocastrated (against human serum albumin) bulls (age of primary immunization was 1.5 to 2.5 months), both at pasture (8 to 10.8 months of age) and 1 wk prior to slaughter, at approximately 22 months of age. Most of this activity was sexual at younger ages but became more aggressive as they grew older. The high titer group of immunized bulls was more sexually active than the medium titer group or the control bulls at age 11 to 13 months of age. Discussion from the authors suggested that it may reflect delayed sexual maturity and sexual activity in the high titer bulls, due to a prepubertal rise in testosterone concentration.

Jago et al. (1997) found that prepubertal immunocastration (against GnRH) lowered the mean sexual behavior (searching, courtship, mounting, intromission, and ejaculation) between 10 and 17 mo and increased the age of first copulation. Some immunocastrated bulls would

repeatedly mount the cow in estrus but did not protrude the penis so intromission and ejaculation could not occur. The authors suggested that the difference in sexual behavior score for bulls and immunocastrates could be attributed to differences in sexual motivation of the animals (Jago et al., 1997). The developmental delay in ability to copulate in comparison with sexual motivation may be explained by the hypothesis that the mechanisms of ejaculation from the separation of the penis from the prepuce are dependent on greater testosterone production than pre-copulatory sexual behaviors (Sodersten et al., 1980; D'Occhio and Brooks, 1982).

Huxsoll et al. (1998) reported changes in typical bull behavior due to immunocastration (against GnRH). The frequency of spars (head-to-head contact) and butts (head-to-flank contact) initiated did not differ between castrates and bulls that were immunized, and were less than the same measures of aggressive behavior in control bulls. However, the behavior was only evaluated once when cattle were 16 months old, (primary immunization was at 1, 4, or 6 months of age) meaning changes in behavior over the experimental period could not be compared.

Growth performance parameters, including ADG and BW, are imperative in many castration experiments as producers and researchers alike hope to minimize reduced growth with castration while improving meat quality and behavior. Many castration studies in recent years comparing intact males to prepubescent castrated males, regardless of pain mitigation strategy, found negative impacts on ADG (Faulkner et al., 1992; Fisher et al., 2001; Gonzalez et al., 2010). Fisher et al. (1996) failed to find differences in ADG between control and castrated cattle. The greater ADG observed in bulls compared to castrated calves can be attributed to the anabolic property of androgens, especially testosterone (Katz, 2007; Mach et al., 2009). Previous studies (Hedrick et al., 1969; Field, 1971) indicated that bulls grow on average 14 to 17% more than

steers; however, a study by Marti et al. (2013) reported reduced ADG by only 7.5% in castrates compared to intact bulls.

Zinc

Zinc is potentially the most researched trace mineral as it plays a major role in disease resistance and immune responsiveness of humans (Prasad, 2000; Salgueiro et al., 2000). Zinc is an essential component of many enzymes present in animal tissue, including alcohol dehydrogenase, phosphatases, carbonic anhydrase, procarboxypeptidase and cytosolic superoxide dismutase, along with being an essential component of sperm and affecting the motility of sperm (Hidiroglou and Knipfel, 1984). Zinc is considered noncarcinogenic, nonteratogenic, and nonmutagenic, and is essential for normal growth, reproduction and has a beneficial effect on tissues repair and wound healing (Leonard et al., 1987). When activated, the immune system undergoes rapid cell proliferation and protein synthesis, which would require these Zn enzymes.

Zinc influences the activity of thymulin, a hormone that affects the development of lymphocytes in the thymus. Zinc is required to maintain enzymatic activity of inducible nitric oxide synthase and, therefore, nitric oxide production (Mocchegiani et al., 2000). Nitric oxide is important in macrophages for killing bacteria, fungi, and protozoa. A Zn transporter SLC39A8 is potentially a link between regulation of NF-κB activity during innate immune activation and Zn metabolism (Liu et al., 2013). Zinc is imperative to the innate immune system in terms of the skin and other stratified epithelia and is required for normal healing. The supplementation of Zn increases the rate of epithelial tissue repair and maintains cellular integrity in deficient animals.

The importance of Zn in ruminants is supported by the effects of lethal trait A46, which is a rare genetic disorder in dairy cattle resulting in reduced capacity of the intestine to absorb

Zn. Typically, calves that are homozygous for this trait become Zn deficient after birth and will die within 5 mos unless supplemented aggressively with Zn. At birth, calves have normal numbers of functional lymphocyte subpopulations but the activity of these lymphocytes is altered, as the calves become Zn deficient (Perryman et al., 1989). The delay of wound healing and infection are some of the more common causes of mortality in these calves (Machen et al., 1996).

Supplementing steers with Zn oxide and Zn methionine has decreased incidence of footrot in finishing steers (Greene et al., 1988). Brazle (1992) found similar results supplementing Zn methionine in decreasing incidence of footrot but also noted increased growth performance during a grazing period. Previous research suggests that hoof growth and wear is not affected by supplementing Zn methionine but supplementation does improve hoof scores for texture, heel cracks, and interdigital dermatitis (Moore et al., 1988). In a summary of 12 trials, lactating dairy cows supplemented with Zn (180 to 400 mg/d) as Zn methionine have lower somatic cell counts (196 vs. 294 × 10^3 cells/mL; Kellogg et al., 2004). These changes in the incidence of footrot and somatic cell counts may reflect the importance of Zn in maintaining effective epithelial barriers. The incorporation of cysteine into keratin requires Zn (Hsu and Anthony, 1971). The keratin lining of the teat canal protects the teat from bacterial invasion (Nickerson, 1985). Keratin is lost during the milking process and must be regenerated to maintain this protective barrier (Capuco et al., 1992).

In low concentrations, Zn is important for spermatogenesis as it is incorporated in the flagellum in late spermatids and is localized in the outer dense fibers of the spermatozoa tail (Fahim et al., 1993). However, in high concentrations zinc inhibits the division and replication of germ cells and causes the fragmentation of the nucleus and cellular membranes (Fahim et al.,

1993; Bloomberg, 1996).

Castration research using Zn in other animals

Intratesticular injections of Zn gluconate have previously been shown in male dogs to impair spermatogenesis (Oliveira et al., 2007). Previous research by Fahim et al. (1993) injected zinc gluconate neutralized by arginine in the tail of the epididimydes of adult dogs; azoospermia was detected 90 d after treatment. One year after treatment, histological evaluation of the testis and epididimydes revealed that the cellular structure of the testis was preserved, but the rete testis was atrophied. Also, the ductus of the epididimydes was atrophied and fibrous tissue was present, especially in the area of the tail.

Research by Hernandez et al. (2005) in immunocastrated (against GnRH) bulls were similar in that BW did not differ among immunocastrated, castrated, or intact bulls through d 141. Tavarez et al. (2014) reported that immunocastrated (against GnRH) pigs had greater slaughter weights compared to surgically castrated pigs, although surgically castrated pigs possessed greater overall BW at the time of slaughter. Intact males have improved feed efficiency compared to castrated males, and avoiding castration could significantly reduce the amount of biological pollutants excreted by livestock in the environment (Bonneau and Enright, 1995). Similar research in boars by Boler et al. (2011) suggested that immunocastration reduced feed consumption rates with greater, or similar, rates of gain and increased leanness and cutability compared to physically castrated boars. The adverse effects of castration on growth and efficiency can be reduced by administration of growth-promoting implants; however, this is not an option in some countries as it is (Bonneau and Enright, 1995).

Testosterone is important in beef cattle as it is responsible for many characteristics of males, including aggression and enhanced growth characteristics (Field, 1971; Seideman et al.,

1982; Cosgrove et al., 1996), and the ablation of testosterone production by immunocastration techniques result in similar effects to traditional surgical castration (Robertson et al., 1979). . Removal of the source of steroids creates serious economic disadvantages as intact males are more efficient and leaner than castrated males (Charette, 1961; Curran et al., 1965; Harte et al., 1965; Newell and Bowland, 1972; Walstra, 1974; Horstman et al., 1982; Seideman et al., 1982; Walstra and Vermeer, 1993). Similar results were shown in Fagundes et al. (2014), who reported that male cats castrated with zinc gluconate did not differ in serum testosterone concentrations compared to intact males.

Zinc gluconate had previously been used in cats as a means of castration and testis width was smaller in treated cats compared to intact cats on d 60 to 120 but overall testis width did not change over the entire study when comparing treated to non-treated cats (Oliveira et al., 2013). Similarly Hernandez et al. (2005) found intact bulls possessed greater scrotal circumferences than immunocastrated (against GnRH) calves.

Justification

Castration is necessary to reduce aggressive and sexual behaviors and improve meat quality in male beef cattle; thus, castration is a routine management practice performed on approximately 15 million bull calves each year in the US. However, castration causes pain and stress that temporarily reduces growth performance and the performance reduction due to castration is greater in older bulls that are also experiencing weaning stress.

Regardless of the temporary negative effects on growth performance and pain, some producers believe the positive attributes of castration outweigh the negative as evident in the number of castrations completed annually (USDA, 2009). Seidemen et al. (1982) found that intact males exhibited greater growth performance and feed efficiency, but carcass discounts

intact bulls received compared to steers were not included (Faulkner et al., 1992). Castrated males have reduced ADG and dry matter intake (**DMI**) (Fisher et al., 1996), reduced hind leg step length (Gonzalez et al., 2010), increased plasma cortisol levels (Fisher et al., 1996) and increased plasma haptoglobin concentrations compared to intact males or males that were castrated early in life; however, these effects are transient and are not endured throughout finishing.

Scientific research has shown that traditional methods of castration of beef calves results in:

- Reduced ADG
- Increased physiological stress parameters, such as fibrinogen, haptoglobin, and substance P
- Altered behavioral responses
- Immunomodulatory effects
- Increased rates of bovine respiratory disease resulting in greater antibiotic treatment and labor costs and reduced animal well-being

Furthermore, public awareness and concern for the wellbeing of livestock animals has been increasing in the US. Pain management has become an important issue to organizations,like the AVMA and AABP, which are now encouraging the use of pain relief during routine management practices such as castration and dehorning. Although the AVMA position supports reduction or elimination of pain during castration and dehorning, it does not specifically indicate the use of analgesia or anesthesia and reads as follows: “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.”

According to a survey conducted in 2010, surgical castration with a scalpel was found to be the most preferred method by US bovine veterinarians, but only 1 in 5 veterinarians reported using pain relief during castration procedures. Currently, injectable sterilization has had limited use by veterinarians. However, an injectable product consisting of zinc acetate neutralized by L-Histidine (Calviex™) has been cleared by the FDA for investigation in beef and dairy bull calves. In order for the US beef cattle industry to make effective decisions regarding best management practices, additional scientific data are required to determine the most appropriate timing of castration and if injectable sterilization is effective for castration in cattle at different stages of maturity.

Continued scrutiny from consumers and animal rights groups has made attention to animal pain associated with conventional castration techniques an important issue. Recently, the state of New Jersey has implemented rules for castration in cattle and more states could follow their lead. Therefore, novel research is needed to determine the impacts associated with sterilizing calves at different stages of maturity, and if injectable sterilization can minimize the negative physiological and immunological responses in castrated calves of different age. This study is designed to compare cohorts in a feedyard environment and castrated via different methods upon arrival.

Literature Cited

Abbas, A. K., A. H. Lichtman, and S. Pillai. 2015. Cellular and molecular immunology, 8th ed. Elsevier Saunders, Philadelphia, PA.

Adams, T. E., and B. M. Adams. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 70:1691-1698.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakursi. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. 71:811-817.

Amann, R. P., M. E. Wise, J. D. Glass, and T. M. Nett. 1986. Prepubertal changes in the hypothalamic-pituitary axis of holstein bulls. Biol. Reprod. 34:71-80.

Anderson, D. E., and W. W. Muir. 2005. Pain management in ruminants. Vet. Clin. Food Anim. 21:19-31.

Apple, J. K., M. E. Dikeman, D. D. Simms, and G. Kuhl. 1991. Effects of synthetic hormone implants, singularly or in combination, on performance, carcass traits, and longissimus muscle palatability of holstein steers. J. Anim.Sci. 69:4437-4448.

AVMA. 2007. Extralabel Drug Use. Accessed 27 June 2016. http://www.avma.org/reference/amduca/extralabel_brochure.pdf

AVMA. 2009. Welfare implications of castration of cattle. Accessed 27 June 2016. http://www.avma.org/reference/backgrounders/castration cattle bgnd.pdf

Bagley, C. P., D. G. Morrison, J. I. Feazel, and A. M. Saxton. 1989. Growth and sexual characteristics of suckling beef calves as influenced by age at castration and growth implants. J. Anim. Sci. 67:1258-1264.

Baker, A. M., and H. W. Gonyou. 1986. Effects of zeranol implantation and late castration on sexual, agonistic and handling behavior in male feedlot cattle. J. Anim. Sci. 62:1224-1232.

Balthazart, J. 1990. Brain aromatization of testosterone regulates male reproductive behavior in birds. Progress of Comparative Endocrinology. Wiley-Uss Inc. Chichester, pp. 92-98.

Bearden, H. J., and J. W. Fuquay. 1997. Applied Animal Reproduction - 4th Ed. Prentice Hall Inc. New Jersey, USA. pp. 22.

Bloomberg, M. S. 1996. Surgical neutering and non-surgical alternatives. J. Am Vet. Med. Assoc. 208:517-519.

Boler, D. D., L. W. Kutzler, D. M. Meeuwse, V. L. King, D. R. Campion, F. K. McKeith, and J. Killefer. 2011. Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. J. Anim. Sci. 89:2189-2199.

Bonneau, M., and W. J. Enright. 1995. Immunocastration in cattle and pigs. Livestock Production Science 42:193-200.

Boynton, C. S., C. F. Dick, and G. H. Mayor. 1988. NSAID's: an overview. J. Clin. Pharmacol. 28:512-517.

Brazle, F. K. 1992. The effect of zinc methionine in a mineral mixture on gain and incidences of footrot on steers grazing native grass pastures. J. Anim. Sci. 71(Suppl. 1):40.

Calkins, C. R., D. C. Clanton, T. J. Berg, and J. E. Kinder. 1986. Growth, carcass, and palatability traits of intact males and steers implanted with zeranol or estradiol early and throughout life. J. Anim. Sci. 62:625-631.

Capuco, A. V., S. A. Bright, J. W. Pankey, D. L. Wood, R. H. Miller, and J. Britman. 1992. Increased susceptibility to intramammary infection following removal of teat canal keratin. J. Dairy Sci. 75:2126-2130.

Chandolia, R. K., N. C. Rawlings, and A. C. O. Evans. 1994. Inhibition of the early rise of LH secretion in bull calves suppresses testicular development. Biol. Reprod. 50:86.

Charette, L. A. 1961. The effect of sex and age of males at castration on growth and carcass quality of Yorkshire swine. Can. J. Anim. Sci. 41:30-39.

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

Coetzee, J. F., L. N. Edwards, R. A. Mosher, N. M. Bello, A. M. O'Connor, B. Wang, B. KuKanich, and D. A. Blasi. 2011. Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J. Anim. Sci. 90: 1026-1039.

Coetzee J. F., A. L. Nutsch, L. A. Barbur, and R. M. Bradburn. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet. Res. 6:12.

Cohen, R. D. H., B. D. King, E. D. Janzen, and P. S. W. Hunter. 1991a. The efficacy of castration methods and effects of age at castration and implant regime on testicular measurements and testosterone levels of male beef cattle. Can. J. Anim. Sci. 71:1-11.

Cohen, R. D. H., B. D. King, E. D. Janzen, and H. H. Nicholson. 1991b. The effect of castration age, method and implant regime on growth and carcass traits of male beef cattle. Can. J. Anim. Sci. 71:301-309.

Compendium of Veterinary Products. 2010. Compendium of Veterinary Products 13th edition. Bayley, A.J. ed., North American Compendiums INC, Port Huron, MI.

Cosgrove, G. P., T. W. Knight, M. G. Lambert, and A. F. Death. 1996. Effects of post-pubertal castration and diet on growth rate and meat quality of bulls. Proc. N. Z. Soc. Anim. Prod. 56:390-393.

Curran, S., J. P. Crowley, and P. McGloughlin. 1965. The effect of spaying and hormone implantation on the growth and carcass quality of beef heifers. Ir. J. Agric. Res. 4:93-100.

Dirnhofer, S., G. Wich, and P. Berger. 1994. The suitability of human chorionic gonadotropin (hCG)-based birth-control vaccines. Immunol. Today. 15:469-474.

D'Occhio, M. J., and D. E. Brooks. 1982. Threshold of plasma testosterone required for normal mating activity in male sheep. Horm. Behav. 16:383-394.

Doornenbal, H., A. K. W. Tong, J. A. Newman, N. L. Murray, and G. J. Mears. 1987. Blood and serum components and organ weights in steers, bulls and zeranol-implanted bulls. J. Anim. Sci. 64:489-496.

Dykeman, D. A., L. S. Katz, and R. H. Foote. 1982. Behavioral characteristics of beef steers administered estradiol, testosterone and dihydrotestosterone. J. Anim. Sci. 55:1303-1309.

European Commission. 2001. Scientific Commission on Animal Welfare. Welfare of cattle kept for beef production. Accessed 27 June 2016. http://ec.europa.eu/food/fs/sc/scah/out54_en.pdf

European Union. 2009. The EU and animal welfare: policy objectives. Accessed 27 June 2016. http://ec.europa.eu/food/animal/welfare/policy/index_en.htm.

Evans, A. C. O., W. D. Currie, and N. C. Rawlings. 1993. Opioidergic regulation of gonadotrophin secretion in the early prepubertal bull calf. J. Reprod. Fertil. 99:45-51.

Evans, A. C. O., R. A. Pierson, A. Garcia, L. M. McDougall, F. Hrudka, and N. C. Rawlings. 1996. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. Theriogenology 46:345-357.

Fagundes, A. F. F., E. C. S. Oliveira, B. M. Tenorio, C. C. S. Melo, L. T. B. Nery, F. A. B. Santos, L. C. Alves, R. H. Douglas, and V. A. Silva Jr. 2014. Injection of a chemical castration agent, zinc gluconate, into the testes of cats results in the impairment of

spermatogenesis: A potentially irreversible approach for this species? Theriogenology 81:230-236.

Fahim, M. S., M. Wang, M. F. Sutcu, Z. Fahim, and R. S. Yougquist. 1993. Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception 47:107-122.

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

FDA. 1994. Food and Drug Administration. Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994. Accessed 29 June 2016. http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/AnimalMedicinalDrugUseClarificationActAMDUCAof1994/default.htm.

Field, R. A. 1971. Effects of castration on meat quality. J. Anim. Sci. 32:849-858.

Finnerty, M., W. J. Enright, D. J. Prendiville, L. J. Spicer, and J. F. Roche. 1996. The effect of different levels of gonadotropin-releasing hormone antibody titres on plasma hormone concentrations, sexual and aggressive behaviour, testis size and performance of bulls. J. Anim. Sci. 63:51-63.

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

Fisher, A. D., M. A. Crowe, E. M. O'Nuallain, M. L. Monaghan, J. A. Larkin, P. O'Kiely, and W. J. Enright. 1997. Effects of cortisol on in vitro interferon – gamma production, acute-phase proteins, growth, and feed intake in a calf castration model. J. Anim. Sci. 75:1041–1047.

Fisher, A. D., T. W. Knight, G. P. Cosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effects of surgical or banding castration on stress responses and behaviour of bulls. Aus. Vet. J. 79:279-284.

GAP. 2009. Global Animal Partnership. Global Animal Partnership 5-Step™ Animal Welfare Rating Standards for Beef Cattle. Accessed 27 June 2016. http://www.globalanimalpartnership.org/wp-content/uploads/2011/01/5-Step-Animal-Welfare-Rating-Standards-for-Beef-Cattle.pdf

GAP. 2012. Global Animal Partnership. For retailers. Accessed 27 June 2016. http://www.globalanimalpartnership.org/for-retailers/

Gilbert, R. O., and S. L. Fubini. 2004. Surgery of the male reproductive tract. Page 355 in Farm Animal Surgery. S. L. Gilbert and N. G. Ducharme, ed. Saunders, St. Louis, MO.

Gonzales, S., S. Goubau, A. F. Allen, R. J. Mapletoft, R. Cohen, and B. D. Murphy. 1990. Livestock Reproduction in Latin America (IAEA Panel Proceedings Series STI/PUB 833). P. 269-284.

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

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823.

Gregory, K. E., and J. J. Ford. 1983. Effects of late castration, zeranol and breed group on growth, feed efficiency and carcass characteristics of late maturing bovine males. J. Anim. Sci. 56:771-780.

Hafez, E. S. E. 1993. Reproduction in Farm Animals – 6th Ed. Lea and Febiger, Philadelphia, p. 82.

Hage-van Noort, M., W. C. Puijk, W. M. M. Schaaper, D. Kuperus, N. J. C. M. Beekman, H. H. Plasman, H. Lankof, C. J. G. Wensing, and R. H. Meloen. 1992. Immunomodulation of reproductive systems. J. Anim. Reprod. Sci. 28:187-193.

Hannon, K., A. Gronowski, and A. Trenkle. 1991. Relationship of liver and skeletal muscle IGF-1 mRNA to plasma GH profile, production of IGF-1 by liver, plasma IGF-1 concentrations, and growth rates of cattle. Proc. Soc. Exp. Biol. Med. 196:155-163.

Harte, J., S. Curran, and V. E. Vial. 1965. The production of beef from young bulls, I. Ir. J. Agr. Res. 4:189-204.

Hayden, J. M., W. G. Bergen, and R. A. Merkel. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin and cortisol concentrations in growing steers implanted with estradiol-17 P, trenbolone acetate, or estradial-17P plus trenbolone acetate. J. Anim. Sci. 70:2109-2119.

Hedrick, H. B., G. B. Thompson, and F. Krause. 1969. Comparison of feedlot performance and carcass characteristics of half-sib bulls, steers and heifers. J. Anim. Sci. 29:687‑694.

Henricks, D. M., T. Gimenez, T. W. Gettys, and B. D. Schanbacher. 1988. Effect of castration and an anabolic implant on growth and serum hormones in cattle. Anim. Proc. 46:35.

Hernandez, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated *Bos Indicus* bulls. J. Anim. Sci. 83:2901-2907.

Hidiroglou, M., and J. E. Knipfel. 1984. Zinc in mammalian sperm: a review. J. Dairy. Sci. 67:1147-1156.

Horstman, L. A., C. J. Callahan, R. L. Morter, and E. Amstutz. 1982. Ovariectomy as a means of abortion and control of estrus in feedlot heifers. Theriogenology, 17:273-292.

Hsu, J. M., and W. L. Anthony. 1971. Impairment of cystine-35S incorporation into skin protein by zinc-deficient rats. J. Nutr. 101:445-452.

Huxsoll, C. C., E. O. Price, and T. E. Adams. 1998. Testis function, carcass traits and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76:1760-1766.

IASP.1979. Pain terms: a list with definitions and notes on usage. Pain 6**:**249-252.

Jago, J. G., N. R. Cox, J. J. Bass, and L. R. Matthews. 1997. The effect of prepubertal immunization against Gonadatropin-Releasing Hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. 75:2609-2619.

Jones, S. J., R. D. Johnson, C. R. Calkins, and M. E. Dikeman. 1991. Effects of trenbolone acetate on carcass characteristics and serum testosterone and cortisol concentrations in bulls and steers on different management and implant schemes. J. Anim. Sci. 69:1363-1369.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm.Behav. 52:56‑63.

Katz, L. S., and T. J. McDonald. 1992. Sexual behavior of farm animals. Theriogenology 38:239-253.

Kellogg, D. W., M. T. Socha, D. J. Tomlinson, and A. B. Johnson. 2004. Review: effects of zinc methionine complex on milk production and somatic cell count of dairy cows: twelve-trial summary. Prof. Anim. Sci. 20:295-301.

Kempster, A. J., and D. B. Lowe. 1993. Growth performance and carcass characteristics as influenced by genotype and environment. Proc. 44th Annu. Mtg. EAAP, Aarhus, p 2.1:322-323.

Kenny, F. J., and P. V. Tarrant. 1988. The effect of oestrus behaviour on muscle glycogen concentration and dark-cutting in beef heifers. Meat Sci. 22:21-31.

Lee, C. Y., D. M. Henricks, G. C. Skelley, and L. W. Grimes. 1990. Growth and hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. J. Anim. Sci. 68:2682-2689.

Lehman, F. D., T. J. Engelken, and C. R. Boyle. 2001. Case study: weight gain in suckling calves

following Revalor-G®, Ralgro™, or Synovex®-C. Prof. Anim. Sci. 17:54-56.

Lents, C. A., F. J. White, L. N. Floyd, D. L. Gay, and R. P. Wettemann. 2006. Effects of method and timing of castration and the use of an estrogenic growth stimulant on weight gain of bull calves. Prof. Anim. Sci. 22:126-131.

Leonard, A., G. B. Gerber, and F. Leonard. 1987. Mutagenicity, carcinogenicity, and teratogenicity of zinc. Mutat Res. 168:343-348.

Liu, M.-J., S. Bao, M. Galvez-Peralta, C. J. Pyle, A. C. Rudawsky, R. E. Pavlovicz, D. W. Killilea, C. Li, D. W. Nebert, M. D. Wewres, and D. L. Knoell. 2013. The zinc transporter SLC39A8 is a negative feedback regulator of NF-κB through zinc-mediated inhibition of IKK. Cell Rep. 3:386-400.

Liu, Y. J., D. Y. Mason, G. D. Johnson, S. Abbot, C. D. Gregory, D. L. Hardie, J. Gordon, and I. C. MacLennan. 1991. Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur. J. Immunol. 21:1905-1910.

Lyles, J. L. and M. S. Calvo-Lorenzo. 2014. BILL E. KUNKLE INTERDISCIPLINARY BEEF SYMPOSIUM: Practical developments in managing animal welfare in beef cattle: What does the future hold? J. Anim. Sci. 92:5334-5344.

Mach, N., A. Bach, C. E. Realini, M. Font, I. Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behavior, carcass characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81:329‑334.

Machen, M., T. Montgomery, R. Holland, E. Braselton, R. Dunstan, G. Brewer, and V. Yuzbasiyan-Gurkan. 1996. Bovine hereditary zinc deficiency: lethal trait A 46. J. Vet. Diagn. Invest. 8:219-227.

Mader, T. L., D. C. Clanton, J. K. Ward, D. E. Pankaskie, and G. H. Deutscher. 1985. Effect of pre- and postweaning zeranol implant on steer calf performance. J. Anim. Sci. 61:546-551.

MAFF. 1992. Ministry of Agriculture, Fisheries and Food. Summary of law relating to farm animal welfare. Accessed 27 June 2016. http://discovery.nationalarchives.gov.uk/SearchUI/Collection/Display?uri=C10769-

Marti, S., C. E. Realini, A. Bach, M. Perez-Juan, and M. Devant. 2013. Effect of castration and slaughter age on performance, carcass, and meat quality traits of Holstein calves fed a high-concentrate diet. J. Anim. Sci. 91:1129-1140.

Massey, C., K. C. Dhuyvetter, R. V. Llewelyn, and D. A. Blasi. 2011. Castration and morbidity and their effects on performance, carcass quality, and price differentials for bulls and steers. Prof. Anim. Sci. 27:19–28.

McCarthy, M. S., H. D. Hafs, and E. M. Convey. 1979. Serum hormone patterns associated with growth and sexual development on bulls. J. Anim. Sci. 49:1012-1020.

Melson, B. E., J. L. Brown, H. M. Schoenemann, G. K. Tarnavsky, and J. J. Reeves. 1986. Elevation of serum testosterone during chronic LHRH agonist treatment in the bull. J. Anim. Sci. 62:199-207.

Mocchegiani, E., R. Giacconi, M. Muzzioli, and C. Cipriano. 2000. Zinc, infections and immunosenescence. Mech. Ageing Dev. 121:21-35.

Moore, C. L., P. M. Walker, M. A. Jones, and J. W. Webb. 1988. Zinc methionine supplementation for dairy cows. J. Dairy Sci. 71 (Suppl. 1):152.

NAHMS. 2008. Beef 2008–2008. Part 1, Reference of beef cow/calf management practices in the United States, 2007–2008. National Animal Health Monitoring System. USDA. Accessed Jan. 8 2018. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartI_rev.pdf.

NAHMS. 2013. USDA National Animal Health Monitoring System report on the use of growth- promoting implants in US Feedlots. Accessed Jan. 8 2018. https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_is_Implant.pdf

NAWAC. 2005. National Animal Welfare Advisory Committee. 2005. Animal welfare (painful husbandry procedures) code of welfare 2005. Accessed 27 June 2016. http://www.biosecurity.govt.nz/files/regs/animal-welfare/req/codes/painful-husbandry/painful-husbandry.pdf

Nickerson, S. C. 1985. The teat's role in mastitis prevention. Pages 18-29 in Proc. 24th Annual Mtg., Natl. Mastitis Council, Arlington, VA.

Newell, J. A., and J. P. Bowland. 1972. Performance, carcass composition and fat composition of boars, gilts and barrows fed two levels of protein. Can. J. Anim. Sci. 52:543-551.

Oliveira, E. C. S., A. K. F. Fagundes, C. C. S. Melo, L. T. B. Nery, R. G. Revoredo, T. F. G. Andrade, K. Oliveira-Esquerre, J. P. Kastelic, and V. A. Silva Jr. 2013. Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety. Vet. J. 197:307-310.

Oliveira, E. C. S., M. R. Moura, V. A. Silva Jr., C. A. Peixoto, K. L. A. Saraiva, M. J. C. Sa, R. H. Douglas, and A. P. Marques Jr. 2007. Intratesticular injection of a zinc based solution as a contraceptive for dogs. Theriogenology 68:137-145.

Perry, T. C., D. G. Fox, and D. H. Beermann. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of holstein and

beef steers. J. Anim. Sci. 69:4696-4702.

Perryman, L. E., D. R. Leach, W. C. Davis, W. D. Mickelsoen, S. R. Heller, H. D. Ochs, J. A. Ellis, and E. Brummerstedt. 1989. Lymphocyte alterations in zinc-deficient calves with lethal trait A46. Vet. Immunol. Immunopath. 21:239-248.

Prasad, A. S. 2000. Effects of zinc deficiency on immune functions. J. Trace Elem. Exp. Med. 13:1-20.

Price, M.A., S. D. M. Jones, G. W. Mathison, and R. T. Berg. 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. 60:345-358.

Price, C. A., B. A. Morris, and R. Webb. 1987. Reproductive and endocrine effects of active immunization against a testosterone conjugate in the heifer. J. Reprod. Fertil. 81:149-160.

Price, M. A., and T. Tennessen. 1981. Preslaughter management and dark-cutting in the carcasses of young bulls. Can. J. Anim. Sci. 61:205-208.

Price, E. O., and S. J. R. Wallach. 1991. Development of sexual and aggressive behaviors in Hereford bulls. J. Anim. Sci. 69:1019-1027.

Ratcliff, M. D., E. B. Kegley, J. G. Powell, J. Hawley, K. S. Lusby, M. P. Rowe, S. A. Gunter, L. B. Daniels, and D. S. Hubbell III. 2014. Effect of method and timing of castration on newly received stocker cattle. Prof. Anim. Sci. 30:457-465.

Robertson, I. S., J. C. Wilson, and H. M. Fraser. 1979. Immunological castration in male cattle. Vet. Rec. 111:529-531.

Roberts, S. L., J. G. Powell, H. D. Hughes, and J. T. Richeson. 2018. Effect of castration method and analgesia on inflammation, behavior, growth performance, and carcass traits in feedlot cattle. J. Anim. Sci. 96:66-75. https://doi.org/10.1093/jas/skx022

Roche, J. F., and J. P. Crowley. 1973. The long-term suppression of heat in cattle with implants of melengestrol acetate. Anim. Prod. 16:245-250.

Rodriguez, R. E., and M. E. Wise. 1989. Ontogeny of Pulsatile Secretion of Gonadotropin-Releasing Hormone in the Bull Calf During Infantile and Pubertal Development. Endocrinology. 124:248-256.

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

Ronayne, E., W. J. Enright, and J. F. Roche. 1993. Effects of continuous administration of gonadotropin-releasing hormone (GnRH) or a potent GnRH analogue on blood

luteinizing hormone and testosterone concentrations in prepubertal bulls. Domest. Anim. Endocrin. 10:179-189.

Rumsey, T. S., A. C. Hammond, and J. P. McMurtry. 1992. Response to reimplanting beef steers with estradiol benzoate and progesterone: performance, implant absorption pattern, and thyroxine status. J. Anim. Sci. 70:995-1001.

Salgueiro, M. J., M. Zubillaga, A. Lysionek, G. Cremaschi, C. G. Goldman, R. Caro, T. De Paoli, A. Hager, R. Weill, and J. Boccio. 2000. Zinc status and immune system relationship a review. Biol. Trace Elem. Res. 76:193-205.

Scanga, J. A., K. E. Belk, J. D. Tatum, T. Grandin, and G. C. Smith. 1998. Factors contributing to the incidence of dark cutting beef. J. Anim. Sci. 76:2040-2047.

Schanbacher, B. D. 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. Am. J. Physiol. 242:E201-E205.

Schanbacher, B. D. 1984. Pituitary-testicular responses of estradiol-17(5-implanted bull calves to continuous versus pulsatile infusion of luteinizing hormone releasing hormone. J. Anim. Sci. 58:943-948.

Seideman, S. C., H. R. Cross, R. R. Oltjen, and B. D. Schanbacher. 1982. Utilization of the intact male for red meat production: A review. J. Anim. Sci. 55:826-840.

Selk, G. 1997. Implants for suckling steer and heifer calves and potential replacement heifers. In Symposium: Impact of Implants On Performance And Carcass Value Of Beef Cattle. P 40. Oklahoma Agric. Exp. Stn., Stillwater, OK.

Silcox, R. W., J. T. Keeton, and B. H. Johnson. 1986. Effects of zeranol and trenbolone acetate on testis function, live weight gain and carcass traits of bulls. J. Anim. Sci. 63:358-368.

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

Sodersten, P. A., S. Hansen, P. Eneroth, C. A. Wilson, and J. A. Gustafsson. 1980. Testosterone in the control of rat sexual behavior. J. Steroid Biochem. 12:337.

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

Tavarez, M. A., C. L. Puls, A. L. Schroeder, and A. C. Dilger. 2014. Effects of immunocastration and time after second Improvest dose on adipose tissue fatty acid profile on finishing barrows. J. Anim. Sci. 92:3736-3744.

Tizard, I. R. 2000. Veterinary Immunology: an Introduction. W. B. Saunders Co., Philadelphia.

Trow-Smith, R. 1957. A history of British livestock husbandry to 1700. London: Routledge and Kegan Paul, pp.286.

USDA. 2000. NAHMS Part I: Baseline reference of feedlot management practices, 1999. Accessed 27 June 2016. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot99/Feedlot99_dr_PartI.pdf

USDA Agricultural Marketing Service. 2004. Arkansas Extension Service-USDA Market News. Agricultural Marketing Service, USDA, Little Rock, AR.

USDA. 2008. NAHMS Beef 2007-08, Part I: Reference of beef cow-calf management practices in the United States, 2007-08. Accessed 27 June 2016. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartI_rev.pdf

USDA. 2009. National Agricultural Statistics Service Agricultural Statistics 2009. Accessed 27 June 2016. http:// usda.mannlib.cornell.edu/usda/nass/Catt//2000s/2009/Catt-07-24-2009.pdf.

van Kooten, C., and J. Banchereau. 1997. Function of CD40 on B cells, dendritic cells and other cells. Curr. Opin. Immunol. 9:330-337.

Walstra, P. 1974. Fattening of young boars: quantification of negative and positive aspects. Livest. Prod. Sci. 1:187-196.

Walstra, P., and A. W. Vermeer. 1993. Aspects of micro and macro economics in the production of young boars. Proc. 44th Annu. Mtg. EAAP, Aarhus, P 2.2: 324-325.

Webb, A. I., and L. S. Pablo, 2009. Injectable anesthetic agents. In: Veterinary Pharmacology and Therapeutics, 9th ed. Riviere, J.E., Papich, M.G. ed., Wiley-Blackwell, Ames, IA, p. 383.

Woolf C. J., and M. W. Slater. 2000. Neuronal placity: increasing the gain in pain. Science. 288:1765-1769.

CHAPTER III

CASE STUDY: EFFECT OF INJECTABLE CASTRATION REGIMEN ON BEEF BULL CALVES

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ABSTRACT

Castration is performed on bull calves to reduce aggressiveness and sexual activity, improve worker safety, prevent unwanted breeding, and improve meat quality. The objective of this study was to evaluate the effect of a zinc solution as an injectable castration method to bull calves pre-weaning. Crossbred bull calves (n = 31; BW = 115 ± 26.4 kg; age = 119 ± 18.4 d) were allocated to treatments by BW and birthdate. Twenty-seven bull calves were allocated to 3 injectable castration treatments (n = 9 calves/injectable castration treatment) to reflect 3 dosage levels of the zinc solution (Calviex, Cowboy Animal Health, LLC, Plano, TX). On d 0, a single injection of the zinc solution was placed in each testicle. Two bull calves were castrated surgically, and 2 bull calves were left intact until weaning. Calves were weighed on d 0 and on 28-d intervals until they were weaned on d 122. Blood samples and scrotal measurements were obtained on d 0, 28, 56, 83, and 122.There were no effects ($P \geq 0.67$) of Zn solution concentration on BW. A main effect of treatment ($P = 0.005$) showed intact bulls had greater ($P < 0.001$, orthogonal contrast of intact vs. castrated) serum testosterone concentrations than bulls castrated with any method. At weaning, there were no differences in growth, serum testosterone or scrotal thickness due to the concentration of Zn solution used; and the injectable castration method resulted in similar serum testosterone concentrations compared to surgical castration; hence, resulting in successful castration.

Key words: beef bull calves, Calviex, injectable castration, serum testosterone concentration, zinc solution

INTRODUCTION

Husbandry practices in the beef industry that are associated with pain, discomfort, and distress include castration, dehorning, and branding (Lyles and Calvo-Lorenzo, 2014). Castration

derived pain increases in acuteness and duration as the age, BW, and testicular size of the calf increases (Chase et al., 1995). Pain and inflammation may affect the growth rate and efficiency of beef calves (Fisher et al., 1997). It is estimated there are approximately 15 million castration procedures performed in the U.S. annually to reduce aggressiveness and sexual activity, facilitate handling, prevent unwanted breeding, and improve the meat quality of male bovine (Lyles and Calvo-Lorenzo, 2014). In Arkansas, 17% of male calves sold in livestock auctions weighing between 136 and 250 kg were castrated (USDA Agricultural Marketing Service, 2004), and of the bulls placed on feed in feedlots in 2011, roughly 93% were castrated, predominantly by surgical castration (50%) or band castration (33%; USDA, 2011). Ratcliff et al. (2014) indicated that male calves arriving at a stocker receiving facility as intact bulls and then castrated gained less weight and had greater incidence of bovine respiratory disease (**BRD**) during the receiving period compared to calves arriving as steers, and they were unable to make up for this weight difference during the receiving period or by the end of a 150-d grazing period. Stafford et al. (2002) suggested that surgical castration is more painful initially compared to banded castration as evidenced by increased plasma cortisol levels. Economically, castration post-weaning affects profitability by decreasing ADG and increasing susceptibility to BRD (Massey et al., 2011). Currently, no commercially available injection sterilization method exists for beef cattle in the United States, although there have been Zn solutions utilized in other species including companion animals (Oliveira et al., 2013). An injectable sterilization method would be an alternative castration method which could potentially reduce pain, stress, performance loss, and minimize the prevalence of BRD. Therefore, this study was designed to evaluate the utility of an injectable Zn solution at 3 dosage levels on the efficacy of castration of beef bull calves prior to weaning on weight gain, serum testosterone concentrations, and testicle atrophy.

MATERIALS AND METHODS

The current study was conducted in compliance with procedures approved by the University of Arkansas Animal Care and Use Committee (Approval # 14062). On May 15, 2014, 31 beef bull calves (BW = 115 ± 26.4 kg) and their dams were separated from a larger group of cow-calf pairs at the University of Arkansas Division of Agriculture Southwest Research and Extension Center near Hope, Arkansas for evaluation of an injectable castration method in nursing bull calves. The bull calves average birthdate was March 4, 2014 (range of February 4 to April 4). On June 3, 2014, calves were allocated to treatments by BW (average BW = 129 ± 25 kg) and birthdate. Twenty-seven bull calves were allocated to 3 injectable castration treatments (n = 9 calves/injectable castration treatment) with technicians on-site being blinded to treatments. Treatments were arranged to reflect 3 concentrations of Zn solution (Calviex, Cowboy Animal Health, LLC, Plano, TX) solution. These treatments were identified as Inj1 (least concentration of Zn), Inj2, and Inj3 (greatest concentration of Zn). Each treatment was administered as 1 mL of Zn solution in the geometric center of each testis depositing the solution in the parenchyma of the testis. Testes were injected with Zn using a 5 mL syringe with a 20-gauge needle. The injection was administered in the caudal area of the testis, lateral to the caput of the epididimydes and the needle was inserted in a parallel plane relative to the testis (adapted from Oliveira et al., 2007). Two bull calves were castrated to serve as a negative control using a surgical technique with the removal of the bottom third of the scrotum, testes were then pulled from inside the scrotum, and the spermatic cord was severed with a scalpel. Two bull calves were left intact to serve as a positive control until the termination of the study at weaning.

All cattle regardless of treatment were housed together in a single pasture and were rotated across 3, 5-ha paddocks of mixed warm season grasses consisting of bermudagrass

(*Cynodon dactylon [L.]* Pers.), dallisgrass (*Paspalum dilatatum* Poir.), and crabgrass (*Digitaria cilaris*). Calves were gathered from pastures at 0700, separated from dams, and weighed with no further shrink before processing and on 28-d intervals and weaned from dams on September 30, 2014. At each 28-d interim weight collection and at weaning on October 3, 2014 calves were bled via jugular venipuncture and blood was collected into 8.5-mL vacuum tubes (BD Vacutainer SST, Becton Dickson and Co., Franklin Lakes, NJ) that contained spray-coated silica and a polymer gel for serum separation and were immediately placed on ice. Whole blood was centrifuged at 2,060 × *g* for 25 min and serum was removed and stored frozen until analyzed for testosterone concentrations by a commercially available ^{125}I radioimmunoassay kit (ImmuChem™ Double Antibody Testosterone, MP Biomedicals, LLC, Solon, OH). On d 28, 56, 83, and 122 while calves were restrained in a hydraulic chute, the thicknesses of the right testicle and scrotum were measured using a digital caliper (Model W80152, Wilmar Corporation, Tukwila, WA).

Data were analyzed as a one factor completely randomized design using the MIXED and GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Bodyweight and growth performance were analyzed using the GLM procedure of SAS. Serum testosterone concentrations and thicknesses of the right testis and scrotum were analyzed using repeated measures analyses and the MIXED procedure of SAS (SAS Inst. Inc.). Individual animal was the experimental unit with castration method serving as the lone fixed factor in the model. Orthogonal contrasts were used to compare intact vs. castrated, injection vs. surgical method, and linear and quadratic effects for Zn solution concentration.

RESULTS AND DISCUSSION

Zinc is considered noncarcinogenic, nonteratogenic and nonmutagenic; is essential and is a nutrient for normal growth, reproduction and has beneficial effects on tissue repair and wound healing (Leonard et al., 1987). Zinc is an essential component of many enzymes present in animal tissue, including alcohol dehydrogenase, phosphatases, carbonic anhydrase, procarboxypeptidase and cytosolic superoxide dismutase along with being an essential component of sperm and affecting the motility of sperm (Hidiroglou and Knipfel, 1984). In regards to the current study, zinc in physiological concentrations is important for spermatogenesis as it is incorporated in the flagellum in late spermatids and is localized in the outer dense fibers of the spermatozoa tail (Fahim et al., 1993). However, in supraphysiological concentrations zinc inhibits the division and replication of germ cells and causes the fragmentation of the nucleus and cellular membranes (Fahim et al., 1993; Bloomberg, 1996). Previous research by Fahim et al. (1993) injected zinc gluconate neutralized by arginine in the tail of the epididimydes of adult dogs; azoospermia was detected 90 d after treatment. One year after treatment, histological evaluation of the testis and epididimydes revealed that the cellular structure of the testis was preserved, but the rete testis was atrophied. Also, the ductus of the epididimydes was atrophied and fibrous tissue was present, especially in the area of the tail.

There was a main effect of treatment ($P = 0.005$) on serum testosterone concentrations (Figure 1). Intact bulls had greater ($P < 0.001$, orthogonal contrast of intact vs. castrated) serum testosterone concentrations than bulls castrated with any method, and there were no differences ($P = 0.66$, orthogonal contrast of injection vs. surgical castration) due to castration method. Testosterone is important in beef cattle as it is responsible for many characteristics of males including aggression and superior growth characteristics (Field, 1971; Seideman et al., 1982;

Cosgrove et al., 1996), and the ablation of testosterone production by immunocastration techniques result in similar effects to traditional surgical castration (Robertson et al., 1979). There was a treatment × day interaction ($P = 0.0002$) for serum testosterone concentrations, whereas on d 0, all treatments had similar serum testosterone concentrations, and on d 122 intact bulls had greater ($P < 0.05$) serum testosterone concentrations compared to other castrates, regardless of the method used for castration. Testosterone concentrations in bull calves begins to elevate above 1 ng/mL around 20 wk of age regardless of plane of nutrition and increases substantially during puberty (Brito et al., 2007). Intact males are more efficient and leaner than castrate males (Charette, 1961; Curran et al., 1965; Harte et al., 1965; Newell and Bowland, 1972; Walstra, 1974; Seideman et al., 1982; Walstra and Vermeer, 1993), but leaving bull calves intact creates serious economic disadvantages due to a discount in price for intact bulls vs steers (Troxel and Barham, 2012). There were no differences due to Zn solution concentration ($P \geq 0.32$, linear and quadratic contrasts) in serum testosterone concentrations. In contrast, Fagundes et al. (2014) reported that male cats castrated with zinc gluconate did not differ in serum testosterone concentrations compared to intact males.

There was no main effect of treatment ($P = 0.29$) on testis and scrotum thickness (Figure 2). Zinc gluconate had previously been used in cats as a means of castration and yielded similar results such that testis width was smaller in treated cats compared to intact cats on d 60 to 120, but overall testis width did not change over the entire study when comparing treated to non-treated cats (Oliveira et al., 2013). There was a treatment × day interaction for thickness of scrotum and testis ($P = 0.0001$). No change ($P > 0.05$) in the thicknesses of the scrotum and testis of intact bulls were observed from day 28 to 122; however, the thicknesses of scrotums and testes for calves given Zn solutions decreased ($P < 0.05$) from treatment administration to the

end of study. Testicular development increases as gonadotropin hormones (luteinizing hormone and follicle stimulating hormone) rise early in life; however, the greatest increase in testicular growth occurs between 6 and 18 mo of age (Coulter, 1986; Moura and Erickson, 1997). Similar results in a study by Hernandez et al. (2005) found intact bulls had greater scrotal circumferences than immunocastrated calves. There were no differences ($P \geq 0.39$, linear and quadratic contrasts for Zn solution concentrations) in thicknesses of scrotums and testes due to the concentration of Zn solution.

Performance of bulls and steers between application of treatments on June 3 and weaning on September 30 are presented in Table 1. There were no effects ($P \geq 0.64$) of castration or castration method on BW or preweaning ADG. Only 2 intact bulls were used as positive controls along with 2 surgically castrated calves due to limited number of calves and the authors deemed the efficacy of the Zn concentrations were the greatest concern. Results are similar to Brown et al. (2015) in that timing of castration did not affect growth performance variables. Results of research by Hernandez et al. (2005) in immunocastrated bulls were similar in that BW did not differ among immunocastrated, castrated, or intact bulls through d 141. Tavarez et al. (2014) reported that immunocastrated pigs had reduced final BW compared to surgically castrated pigs but immunocastrated pigs had greater HCW indicating a greater dressing percentage in immunocastrated pigs. Intact males have better feed efficiency compared to castrated males and avoiding castration could significantly reduce the amount of biological pollutants excreted by livestock in the environment (Bonneau and Enright, 1995). Similar research in boars by Boler et al. (2011) suggested that immunocastration reduced feed consumption rates with greater or similar rates of gain and increased leanness and cutability compared to surgically castrated boars. Over the course of the experiment, mean ADG was nearly or slightly above 0.9 kg/day for the

initial 2 periods (0.89 ± 0.07 and 0.91 ± 0.10 kg/d, for periods 1 and 2, respectively), yet decreased to 0.74 ± 0.08 kg/d in period 3 and to 0.15 ± 0.07 kg/d in the final period before weaning. The decrease in performance during the late summer was probably due to seasonal deterioration in forage quality and was not related to treatments imposed. Bodyweight at weaning averaged 202.3 ± 13.6 kg.

Although pain was not evaluated in the current study, chemical castration using lactic acid has been shown to cause pain directly after administration as evidenced by increases in plasma cortisol such that pain was similar to that of surgical castration (Fordyce et al., 1989). The authors do not discount the possibility of pain caused by the injection of Zn; however, the low cost, ease of use and cultural acceptance of a castration technique that does not require removal of the testes can make the injection of Zn a valuable method of castration (Levy et al., 2008).

IMPLICATIONS

There were no differences in BW, serum testosterone concentration, and scrotum and testis thickness due to the concentrations of Zn solution used, and the injectable castration method resulted in similar serum testosterone concentrations to calves that had been surgically castrated. Serum testosterone concentration and scrotum and testes thickness were greater in intact bulls than all injectable castrates at weaning. More research is warranted in this field to determine the efficacy and practicality of injectable castration techniques. A solution of Zn could potentially mitigate some of the welfare issues in reducing management and meat quality problems while many of the advantages of intact males still exist. Intratesticular injections of Zn gluconate had previously been shown in male dogs to impair spermatogenesis which correlate to

the finding in our study such that testosterone concentrations were minimized with the application of Zn in young calves.

Table 1. Effect of castration, castration method, and 3 injectable castration solutions on BW and ADG of nursing male calves (n = 31).

	Castration method*						
Item	Inj1	Inj2	Inj3	Surgical	Intact	SE	*P*-value
Observations, n	9	9	9	2	2	NA	NA
Body weight, kg							
D 0	130	131	130	114	125	9.6	0.96
D 28	154	157	154	140	149	10.4	0.97
D 56	181	185	177	166	174	11.5	0.96
D 83	200	200	196	186	194	12.5	0.99
D 122	205	215	200	196	197	14.0	0.94
Average daily gain, kg							
D 0 to 28	0.85	0.94	0.86	0.93	0.85	0.07	0.85
D 28 to 56	0.96	0.97	0.81	0.94	0.90	0.08	0.64
D 56 to 83	0.71	0.82	0.73	0.74	0.71	0.08	0.88
D 83 to 122	0.15	0.22	0.10	0.28	0.10	0.08	0.77
Overall	0.69	0.76	0.65	0.76	0.65	0.07	0.76

*Inj1= least concentration of Zn; Inj2=intermediate concentration of Zn; Inj3=greatest concentration of Zn.

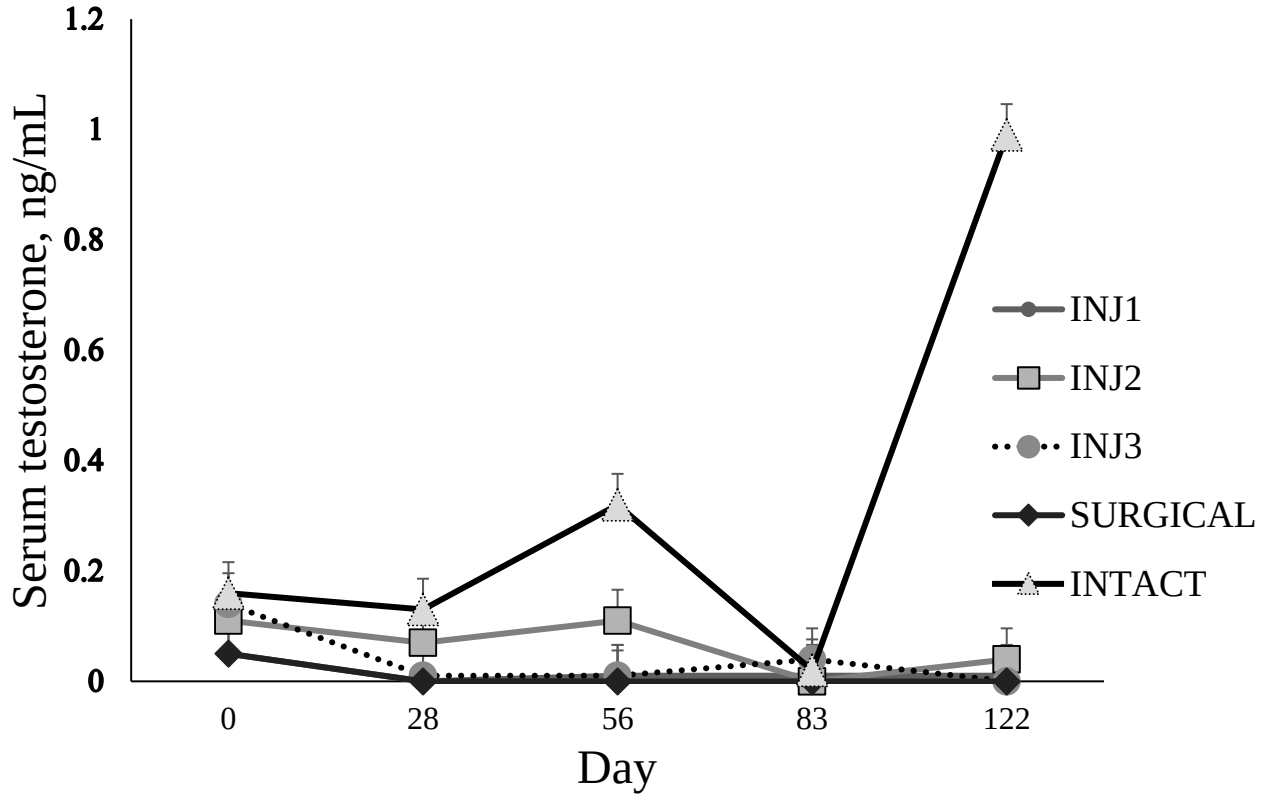

Figure 1. Effect of castration, castration method, and injectable castration Zn concentration on serum testosterone concentrations (least squares means) of nursing male calves (n = 31). Treatment, *P* = 0.005; Day, *P* = 0.002; Treatment × day interaction, *P* = 0.0002. Inj1= least concentration of Zn; Inj2=intermediate concentration of Zn; Inj3=greatest concentration of Zn.

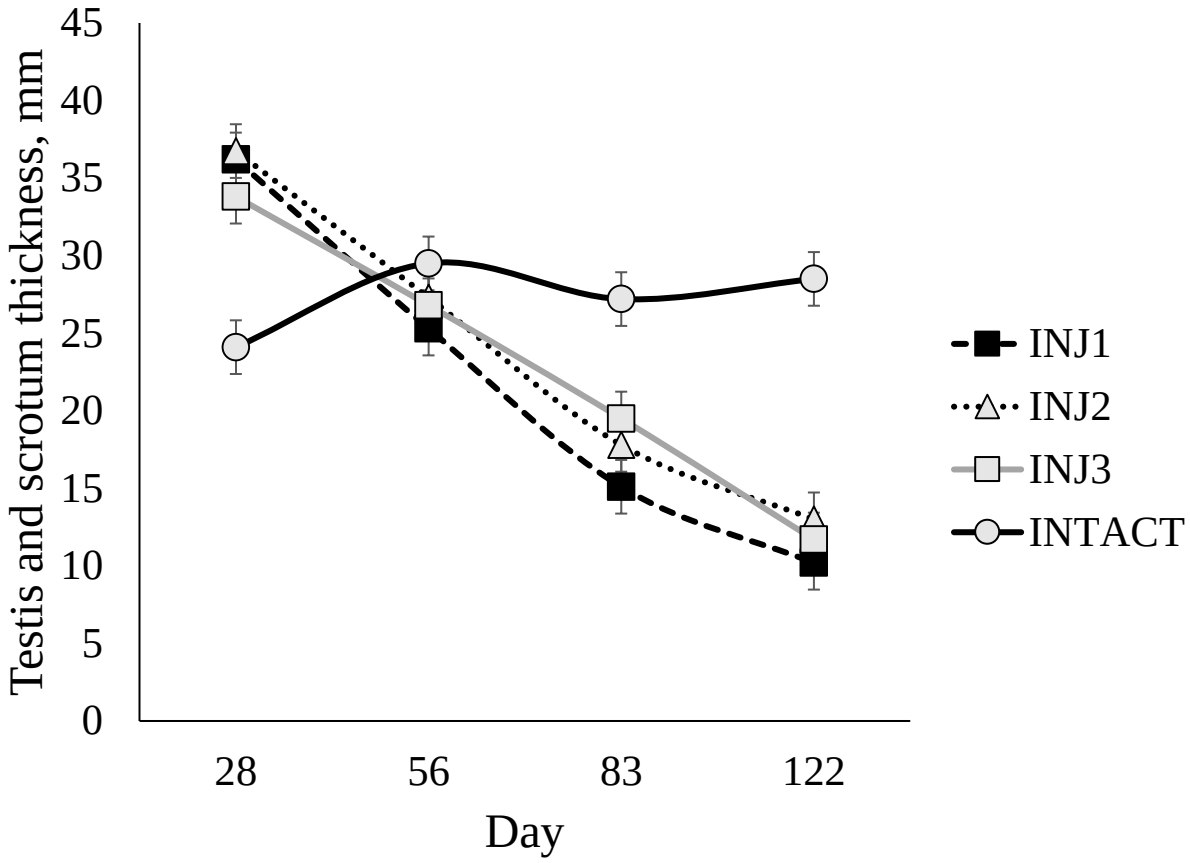

Figure 2. Effect of castration, castration method, and injectable castration Zn concentration on testis and scrotum thickness (least squares means) of nursing male calves (n = 29). Treatment, $P = 0.29$; Day, $P < 0.0001$; Treatment × day interaction, $P < 0.0001$. Inj1= least concentration of Zn; Inj2=intermediate concentration of Zn; Inj3=greatest concentration of Zn.

LITERATURE CITED

Bloomberg, M. S. 1996. Surgical neutering and non-surgical alternatives. J. Am. Vet. Med. Assoc. 208:517-519.

Boler, D. D., L. W. Kutzler, D. M. Meeuwse, V. L. King, D. R. Campion, F. K. McKeith. and J. Killefer. 2011. Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. J. Anim. Sci. 89:2189-2199.

Bonneau, M., and W. J. Enright. 1995. Immunocastration in cattle and pigs. Livest. Prod. Sci. 42:193-200.

Brito, L. F. C., A. D. Barth, N. C. Rawlings, R. E. Wilde, D. H. Crews Jr., P. S. Mir, and J. P. Kastelic. 2007. Effects of nutrition during calfhood and peripubertal period on serum metabolic hormones, gonadotropins and testosterone concentrations, and on sexual development in bulls. Domest. Anim. Endocrinol. 33: 1-18.

Brown, A. C., J. G. Powell, E. B. Kegley, M. S. Gadberry, J. L. Reynolds, H. D. Hughes, J. A. Carrol, N. C. Burdick Sanchez, Y. V. Thaxton, E. A. Backes, and J. T. Richeson. 2015. Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. J. Anim. Sci. 93: 2460-2470.

Charette, L. A., 1961. The effect of sex and age of males at castration on growth and carcass quality of Yorkshire swine. Can. J. Anim. Sci. 41:30-39.

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

Cosgrove, G. P., T. W. Knight, M. G. Lambert, and A. F. Death. 1996. Effects of post-pubertal castration and diet on growth rate and meat quality of bulls. Proc. N. Z. Soc. Anim. Prod. 56:390-393.

Coulter, G. 1986. Puberty and postpubertal development of beef bulls. In: Morrow A, editor. Current therapy in theriogenology. Philadelphia: Saunders p 142-148.

Curran, S., J. P. Crowley, and P. McGloughlin. 1965. The effect of spaying and hormone implantation on the growth and carcass quality of beef heifers. Ir. J. Agric. Res. 4:93-100.

Fagundes, A. F. F., E. C. S. Oliveira, B. M. Tenorio, C. C. S. Melo, L. T. B. Nery, F. A. B. Santos, L. C. Alves, R. H. Douglas, and V. A. Silva Jr. 2014. Injection of a chemical castration agent, zinc gluconate, into the testes of cats results in the impairment of spermatogenesis: A potentially irreversible approach for this species? Theriogenology. 81:230-236.

Fahim, M. S., M. Wang, M. F. Sutcu, Z. Fahim, and R. S. Yougquist. 1993. Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception 47:107-122.

Field, R. A. 1971. Effects of castration on meat quality. J. Anim. Sci. 32:849-858.

Fisher, A. D., M. A. Crowe, E. M. O'Nuallain, M. L. Monaghan, J. A. Larkin, P. O'Kiely, and W. J. Enright. 1997. Effects of cortisol on in vitro interferon – gamma production, acute-phase proteins, growth, and feed intake in a calf castration model. J. Anim. Sci. 75:1041–1047.

Fordyce, G., N. J. Beaman, A. R. Laing, P. B. Hodge, C. Campero, and R. K. Shepherd. 1989. An evaluation of calf castration by intra-testicular injection of a lactic acid solution. Aust. Vet. J. 66:272-276.

Harte, J., S. Curran, and V. E. Vial. 1965. The production of beef from young bulls I. Ir. J. Agr. Res. 4:189-204.

Hernandez, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated *Bos Indicus* bulls. J. Anim. Sci. 83:2901-2907.

Hidiroglou, M., and J. E. Knipfel. 1984. Zinc in mammalian sperm: a review. J. Dairy. Sci. 67:1147-1156.

Leonard, A., G. B. Gerber, and F. Leonard. 1987. Mutagenicity, carcinogenicity, and teratogenicity of zinc. Mutat. Res. 168:343-348.

Levy, J. K., P. C. Crawford, L. D. Appel, and E. L. Clifford. 2008. Comparison of intratesticular injection of zinc gluconate versus surgical castration to sterilize male dogs. Am. J. Vet. Res. 69: 140-143.

Lyles, J. L. and M. S. Calvo-Lorenzo. 2014. BILL E. KUNKLE INTERDISCIPLINARY BEEF SYMPOSIUM: Practical developments in managing animal welfare in beef cattle: What does the future hold? J. Anim. Sci. 92:5334-5344.

Massey, C., K. C. Dhuyvetter, R. V. Llewelyn, and D. A. Blasi. 2011. Castration and morbidity and their effects on performance, carcass quality, and price differentials for bulls and steers. Prof. Anim. Sci. 27:19–28.

Moura, A. A., and B. H. Erickson. 1997. Age-related changes in peripheral hormone concentrations and their relationships with testis size and number of Sertoli and germ cells in yearling beef bulls. J. Reprod. Fertil. 111: 183-190.

Newell, J. A., and J. P. Bowland. 1972. Performance, carcass composition and fat composition of boars, gilts and barrows fed two levels of protein. Can. J. Anim. Sci. 52:543-553.

Oliveira, E. C. S., M. R. Moura, V. A. Silva Jr., C. A. Peixoto, K. L. A. Saraiva, M. J. C. Sa, R. H. Douglas, and A. P. Marques Jr. 2007. Intratesticular injection of a zinc based solution as a contraceptive for dogs. Theriogenology. 68:137-145.

Oliveira, E. C. S., A. K. F. Fagundes, C. C. S. Melo, L. T. B. Nery, R. G. Revoredo, T. F. G. Andrade, K. Oliveira-Esquerre, J. P. Kastelic, and V. A. Silva Jr. 2013. Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety. Vet. J. 197:307-310.

Ratcliff, M. D., E. B. Kegley, J. G. Powell, J. Hawley, K. S. Lusby, M. P. Rowe, S. A. Gunter, L. B. Daniels, and D. S. Hubbell III. 2014. Effect of method and timing of castration on newly received stocker cattle. Prof. Anim. Sci. 30:457-465.

Robertson, I. S., J. C. Wilson, and H. M. Fraser. 1979. Immunological castration in male cattle. Vet. Rec. 111:529-531.

Seideman, S. C., H. R. Cross, R. R. Oltjen, and B. D. Schanbacher. 1982. Utilization of the intact male for red meat production: A review. J. Anim. Sci. 55:826-840.

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

Tavarez, M. A., C. L. Puls, A. L. Schroeder, and A. C. Dilger. 2014. Effects of immunocastration and time after second Improvest dose on adipose tissue fatty acid profile on finishing barrows. J. Anim. Sci. 92:3736-3744.

Troxel, T. R., and B. L. Barham. 2012. Phenotypic expression and management factors affecting the selling price of feeder cattle sold at Arkansas livestock auctions. Prof. Anim. Sci. 28: 64-72.

USDA Agricultural Marketing Service. 2004. Arkansas Extension Service-USDA Market News. Agricultural Marketing Service, USDA, Little Rock, AR.

USDA. 2011. NAHMS Feedlot 2011, Part III: Trends in Health and Management Practices on U.S. Feedlots, 1994-2011. Accessed 04 April 2017. https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_Part%20III.pdf

Walstra, P. 1974. Fattening of young boars: quantification of negative and positive aspects. Livest. Prod. Sci. 1: 187-196.

Walstra, P., and A. W. Vermeer. 1993. Aspects of micro and macro economics in the production of young boars. Proc. 44th Annu. Mtg. EAAP, Aarhus, P 2.2: 324-325.

CHAPTER IV

ZINC INJECTION AS A NOVEL CASTRATION METHOD IN BEEF BULLS: EFFECTS ON PERFORMANCE, BEHAVIOR AND TESTOSTERONE AND HAPTOGLOBIN CONCENTRATIONS

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ABSTRACT

Crossbred beef bulls (n = 180) were blocked by initial BW (337 ± 10.9 kg; 6 blocks) and assigned randomly to 1 of 3 treatments on d 0: 1) INJ; received 1 mL (100 mg Zn) of a Zn solution in each testis, 2) BAN; received blood-restrictive rubber band placed around the dorsal aspect of the scrotum, 3) BUL; bulls with testicles remaining intact in a randomized complete block design (3 treatment pens/block and 10 cattle/pen). A subset of 54 animals (n = 3/pen) were fitted with accelerometers on d 0 to quantify behavior variables continuously for 28 d. Testis width and scrotal circumference, and serum haptoglobin (d 0, 1, 3, 5, 7 and 14) and testosterone concentrations (every 28 d until slaughter) were also determined for the subset. During the slaughter process, testes from INJ and BUL were collected to assess final testes weight and for histopathological evaluation. Data were analyzed using a mixed model ($\alpha = 0.05$); pen served as the experimental unit for all dependent variables. Final BW was greater ($P < 0.01$) for INJ and BUL compared to BAN (672, 686, and 611 kg, respectively; SEM = 4.4). Overall ADG and G:F were greater ($P \leq 0.03$) in INJ and BUL than BAN; whereas, DMI was similar between treatments for the study duration ($P = 0.46$). Histopathological evaluation (n = 13; INJ = 7; BUL = 6) indicated that INJ testes were degenerative and reproductively non-viable whereas BUL testes were normal. Serum testosterone concentrations on d 168 were similar ($P = 0.14$) between INJ and BUL whereas after d 14, BAN were non-detectable; however, initial serum testosterone concentrations were similarly low across treatments. Serum haptoglobin concentration was greater ($P < 0.01$) in INJ than BUL and BAN on d 1, 3, 5, and 7. Scrotal circumference ($P = 0.08$) and testis width ($P = 0.07$) on d 168 tended to be greater for BUL than INJ. Motion index ($P \leq 0.02$) and step count ($P = 0.04$) was greater in BUL and INJ compared to BAN cattle during the 28 d monitoring period. No difference in standing time ($P \geq 0.85$) or lying bouts ($P = 0.35$)

occurred. Zinc injection resulted in sterilization but did not cause complete cessation of testicular function evidenced by testosterone concentrations more similar to BUL than BAN. This resulted in overall increased BW and G:F for INJ vs. BAN, yet the acute phase response was markedly greater directly after injection. Injection of Zn resulted in outcomes more similar to BUL than BAN, implying minimal efficacy of INJ as a castration method in older bulls arriving to the feedlot.

Key words: beef bulls, castration, zinc

INTRODUCTION

Castration is performed on 15 million bulls each year in the U.S. to reduce aggressive and sexual behavior and to improve meat quality (Lyles and Calvo-Lorenzo, 2014). However, castration causes pain and stress that temporarily reduces performance, which is particularly detrimental to the feeding performance in older bulls (Peterson et al., 1989). Previous research has demonstrated that traditional methods of beef cattle castration resulted in: reduced ADG (Fisher et al., 1996), increased serum cortisol, fibrinogen, haptoglobin, and substance P (Fisher et al., 1997; Coetzee, 2011; Roberts et al., 2015), altered behavioral responses (Sutherland, 2011), immunomodulatory effects (Chase et al., 1995; Roberts et al., 2015), and increased rates of bovine respiratory disease requiring greater antibiotic treatment and labor costs (Daniels et al., 2000). However, improved growth performance, feed conversion and red meat yield are positive attributes associated with feeding intact bulls rather than steers (Price et al., 1980).

Two primary methods of castration are used in bovine; however, there was no clear preference between surgical (52.3%) and band (41.1%) castration method used in bulls arriving to U.S. feedlots (USDA, 2013). Currently, injectable castration techniques are not used in beef cattle but they may offer performance or welfare benefit compared to physical castration. An injectable

product consisting of zinc acetate neutralized by L-histidine (Calviex, Cowboy Animal Health, Plano, TX) has been approved by the FDA for proof-of-concept investigation in beef bulls. The authors hypothesized that the use of injectable Zn would result in outcomes similar to the traditional banding method of castration. Hence, the objective of this study was to determine the effects of castration and castration method upon feedlot arrival on growth performance, behavior, and serum testosterone and haptoglobin concentrations.

MATERIALS AND METHODS

Prior to study initiation, animal care and use procedures were independently approved by the University of Arkansas and West Texas A&M University institutional animal care and use committee. Arrival procedures and the backgrounding phase occurred at the University of Arkansas Stocker and Receiving Cattle Unit (Savoy, AR). After treatments were administered, cattle were cared for by AgriResearch Center feedlot (Canyon, TX) personnel.

Arrival Procedures

A total of 207 crossbred beef bulls (BW 297 ± 3.4 kg) acquired from regional auction markets were received on 5 different dates (truckload) at the University of Arkansas Stocker and Receiving Cattle Unit located near Fayetteville, AR. Upon arrival, bulls were ear tagged with a unique identification number, vaccinated against respiratory (Bovi-Shield Gold One Shot, Zoetis, Florham Park, NJ) and clostridial (Covexin 8, Merck Animal Health, Madison, NJ) pathogens, treated for internal parasites with an oral anthelmintic (Valbazen, Zoetis, 4mL /45.5 kg), tested for persistent infection with bovine viral diarrhea virus (BVDV) via ear notch sample submission to a commercial laboratory (Cattle Stats, Oklahoma City, OK), and administered either 1.2 mL/45.5 kg BW of tulathromycin (Draxxin, Zoetis) with a 7-d post-metaphylactic interval (PMI) or 2 mL/45.5 kg BW of gamithromycin (Zactran, Boehringer Ingelheim Vetmedica, Inc., St.

Joseph, MO) with a 5-d PMI. The two different antibiotics administered at processing were compared during the backgrounding phase prior to study initiation and each methaphylactic treatment was balanced evenly across the 3 subsequently described castration treatments.

Backgrounding Phase

Bulls remained at the receiving unit for at least 42 d (range = 42 to 61 d) for the backgrounding phase and 180 bulls were selected for the current study based on BW, treatment history, and time at the stocker and receiving unit. Body weights were obtained on the day of shipment to feedlot (d -1) and used to determine appropriate BW block allocation to facilitate the randomized complete block design. Blocks were constructed by stratification of d -1 BW, arrival date during backgrounding phase, and number of times treated with an antibiotic. The lightest 10 animals within a treatment were allocated to a pen, followed by the 10 next lightest animals, and so on until 6 pens within each treatment were allocated. Cattle were shipped 798 km to the AgriResearch Center feedlot located in Canyon, TX. Upon arrival (d 0), cattle were unloaded and allowed *ad libitum* access to water and long stem hay. Feedlot processing occurred the following morning on d 0 (initial BW = 337 ± 10.8 kg) and included administration of a combination respiratory vaccine (Bovi-Shield Gold One Shot, Zoetis), clostridial-tetanus toxoid (Covexin 8, Merck Animal Health) and the experimental treatments described subsequently. Experimental treatments consisted of: 1) INJ; received 1 mL (100 mg Zn) of a Zn solution in each testis, 2) BAN; received blood-restrictive rubber band placed around the dorsal aspect of the scrotum, 3) BUL; bulls with testicles remaining intact. Zinc was administered as 1 mL of solution containing 100 mg Zn in the geometric center of each testis with the goal of depositing the solution into the parenchyma of the testis. Testes were injected using a 5 mL syringe with a 3.8 cm 20-gauge needle. The injection was administered in the caudal aspect of the testis, lateral

to the caput of the epididymis and the needle was inserted in a parallel plane relative to the testis (adapted from Oliveira et al., 2007). For BAN, the band was applied around the scrotum dorsal to the testes (California Bander, InoSol Co. LLC, El Centro, CA). The elastic band was tightened by hand until adequate tension was applied, then the elastic band was inserted into a metal clip to inhibit the rubber band from sliding, allowing the band to hold tension and eliminate blood flow to the scrotum and testes, causing subsequent necrosis of the scrotum and testes. Cattle on BUL treatment remained intact.

Cattle were removed from their pens and moved to a handling facility and restrained via a hydraulic chute to facilitate processing and medical treatment, BW records, blood sampling, and testes and scrotal measurements. Cattle receiving INJ and BAN treatments had their right leg restrained by attaching a rope and pulling the leg dorsal and posterior to better expose the testes for treatment application administered during initial processing. No local anesthetic or non-steroidal anti-inflammatory drugs were used in the castration regimen. Also, no growth-promoting implants were administered at any time.

Housing and Diets

Study pens were outdoor and naturally lighted and ventilated, had no shade, and had dirt surfaces with 29 m^2 space/animal. Cattle had *ad libitum* access to water and were fed a constant diet regardless of treatment; diet was standard for the AgriResearch Center feedlot (Table 1). Cattle were fed each morning at 0700 and feed offered was increased using clean bunk management methodology (Pritchard and Bruns, 2003). Any feed not consumed was weighed and discarded each 28-d interval and factored into DMI calculations. Samples of supplements and hay were dried at 50°C in a forced air oven to determine DM.

Data from 3 animals were removed from the data set: 1 BAN animal was removed due to band failure observed on d 28; 1 BUL animal died on d 56 from an injury not related to treatment; 1 BUL animal was removed on d 56 due to chute injuries. Animals diagnosed with respiratory disease were treated with antibiotics per feedlot standard operating procedures (n = 8; INJ = 4 and BUL = 4). Antibiotic therapy was: 1) enrofloxacin (Baytril, Bayer, Shawnee Mission, KS); 2) florifenicol (Nuflor, Merck Animal Health); 3) tulathromycin (Draxxin, Zoetis). All antibiotics were administered according to label directions.

Blood Sampling and Behavior Measurements

A subset of 54 cattle (3 animals from each pen) were selected randomly for repeated blood sampling. Serum haptoglobin (Hp) concentration was determined from d 0, 1, 3, 5, 7, and 14; whereas, serum testosterone concentration was determined from d 0, 14, 28, 56, 84, 112, 140, and 168. Blood was collected (approximately 7 mL) via jugular venipuncture into a plain vacuum tube, allowed to clot, then centrifuged at 2,060 × *g* for 20 min at 23°C. Serum was decanted into duplicate aliquots and stored at -20°C for subsequent analysis of serum Hp and testosterone concentrations. Testosterone concentrations were determined by a commercially available ^{125}I radioimmunoassay kit (ImmuChem Double Antibody Testosterone, MP Biomedicals, LLC, Solon, OH) at the University of Arkansas Division of Agriculture Nutrition Laboratory (Fayetteville, AR) with an inter- and intra-assay CV of 7.3 and 3.4%, respectively. Haptoglobin concentrations were determined at the WTAMU Animal Health Laboratory (Canyon, TX) using a commercial, bovine specific sandwich ELISA kit (Immunology Consultants Laboratory, Portland, OR) with an inter- and intra-assay CV of 9.8 and 7.4%, respectively.

Cattle selected for blood sampling were also fitted with accelerometers (IceQube, IceRobotics, Edinburgh, UK) proximate to the metatarsus of the right rear leg during the 28-d period following arrival to record behavioral data (standing duration, step count, lying bouts, and motion index) relative to experimental treatment.

Procedures

Body weights were recorded on d -1, 0, 3, 5, 7, 14, 28, 56, 84, 112, 140, 168, and day of harvest. For the subset of cattle bled and fitted with accelerometers, each time a BW was recorded, scrotal circumference was obtained via tape measure and the right teste was measured for thickness via a digital caliper (Model W80152, Performance Tool, Tukwila, WA). Pens within block were harvested according to BW and visual appraisal for market readiness. Blocks 5 and 6 were harvested (USDA Establishment #3, Cactus, TX) on d 155; blocks 3 and 4 were harvested on d 176; blocks 1 and 2 were harvested on d 197. Upon harvest, testes from INJ and BUL were removed and immediately transported to the WTAMU abattoir for weight determination and gross evaluation. The scrotum was removed, spermatic cords severed, and testis were weighed individually using a digital scale. Testes from thirteen randomly selected cattle (INJ = 7; BUL = 6) were submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (Amarillo, TX) for histopathological evaluation by a board certified pathologist.

Statistical Analyses

Statistical analyses were conducted for all outcome variables in a randomized complete block design. Data were tested for normality using PROC UNIVARIATE and nonparametric data were log-transformed prior to analysis if normality was improved. Performance, behavior, and blood results were analyzed using the mixed models procedure (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC) with treatment as fixed effect and block as the random effect. Pen was

the experimental unit for all dependent variables analyzed. Testosterone, Hp, movement behavior, and testicular measurements used the MIXED procedure with repeated measures. The statistical model include the main effect of treatment, day, and treatment × day interaction with pen as the experimental unit. Day was the repeated statement and the covariance structure with the lowest Akaike information criterion for each dependent variable was used. Least squares means were separated at ($\alpha = 0.05$) via the PDIFF option. Tendencies were declared at $0.05 \leq P < 0.10$ for all dependent variables.

RESULTS AND DISCUSSION

Growth Performance

Initial BW did not differ between treatments (Table 2; $P = 0.99$). Body weights on d 14, 28, 56, and 84 did not differ ($P \geq 0.16$). We observed a tendency ($P = 0.06$) for BW to differ on d 112 with BUL having greater BW compared to BAN whereas INJ was intermediate. Likewise, previous research observations reported by Hernandez et al. (2005) revealed that BW did not differ among immunocastrated, surgically castrated, or intact bulls through d 141 after castration occurred. On d 140 of our study, BW was greater in INJ and BUL compared to BAN ($P < 0.01$). Similarly, BW was greater in INJ and BUL compared to BAN on d 168 ($P < 0.01$). Final BW was greater in INJ and BUL compared to BAN ($P < 0.01$). These results differ from cattle chemically castrated with lactic acid during calfhood, where bulls were heavier at time of harvest compared to castrates regardless of method (surgical or chemical; Cohen et al., 1991b). Although BW was increased for the INJ and BUL treatments, it is important to note that BAN did not receive a growth promoting implant during the finishing period.

Average daily gain was greater in BUL compared to either INJ or BAN from d 0 to 14 (Table 2; $P < 0.01$). Similar results were reported by Cohen et al. (1991a) in late castrated cattle

in that immediately after castration, bulls had a greater ADG compared to chemically castrated cattle. No differences ($P = 0.61$) in ADG from d 14 to 28 were observed. Cattle on the INJ treatment tended ($P = 0.09$) to have increased ADG from d 28 to 56 compared to BAN whereas BUL was intermediate and did not differ from INJ or BAN. Average daily gain was greater from d 56 to 84 in the INJ and BUL cattle compared to BAN ($P = 0.03$). Similarly, INJ and BUL had greater ADG from d 84 to 112, 112 to 140, and 140 to 168 compared to BAN ($P \leq 0.03$). Overall ADG was greater in INJ and BUL compared to BAN$P < 0.01$). During the early portion of the study, ADG was similar in INJ and BAN; however, after the acute pain of INJ cattle subsided their ADG was similar to that of BUL for the remainder of the study whereas BAN was reduced. These data suggest that castration and castration method impacted performance because the INJ and BUL had greater BW and ADG than BAN.

Feed intake and efficiency

Overall DMI was similar between treatments regardless of castration method (Table 3; $P = 0.46$). No differences ($P \geq 0.11$) in DMI were observed from d 0 to 14, d 14 to 28, d 28 to 56, d 56 to 84, d 84 to 112, and d 112 to 140. Dry matter intake was greater in BUL (10.3 kg/d) compared to either BAN (9.3 kg/d) or INJ (9.7 kg/d) from d 140 to 168 ($P < 0.01$). Similar research in boars conducted by Boler et al. (2011) reported that immunocastration reduced feed consumption rates with greater or similar rates of gain and increased leanness and cutability compared to physically castrated boars. In a study comparing castration and castration method in young calves, DMI was similar between castrates, regardless of method, and intact bulls over an 84 d study (Warnock et al., 2012).

Overall G:F was greater in INJ (0.18:1) and BUL (0.18:1) compared to BAN (0.15:1; Table 3; $P = 0.03$). This observation is a function of similar DMI between treatments with

corresponding differences in ADG. From d 0 to 14, BUL had greater ($P < 0.01$) G:F than either BAN or INJ, which can be attributed to treatment application on d 0 in BAN and INJ. However, there were no differences ($P \geq 0.13$) in G:F from d 14 to 28 or d 28 to 56. From d 56 to 84 and d 84 to 112 G:F was greater ($P \leq 0.03$) in BUL and INJ compared to BAN. The INJ cattle had greater ($P = 0.04$) G:F from d 112 to 140 than BAN cattle whereas BUL were intermediate and did not differ ($P = 0.08$) from the other two treatments. Gain to feed ratio from d 140 to 168 was greater in BUL (0.13) and INJ (0.13) compared to BAN (0.08; $P = 0.02$).

Histopathology and Testes Weight

Testis derived from INJ cattle revealed degenerative changes in the testicular tissue with loss of sperm producing spermatogonia and an overall absence of definable sperm formation and maturation, whereas the head of the epididymis lacked stored sperm (Fig. 1). All BUL testis were histopathologically normal. Individual testis were each heavier in BUL vs. INJ, as was the total testes weight (Table 4; $P < 0.01$), indicating testicular atrophy occurred for INJ cattle. Previous research by Fordyce et al. (1989) reported only limited perfusion of testicular parenchyma was achieved in some cattle and/or there was a high rate of leakage in male cattle castrated chemically with lactic acid. Differences in testes weight indicates the effect of INJ; however, INJ did not cause complete atrophy or removal of testicular tissue such that occurs for traditional castration methods. Since there were differences between individual testis weights in INJ, this may illustrate operator error or challenges with administering the injection in a consistent manner. Histopathological variation was greater in INJ compared to BUL indicating that the atrophy of tissue may be specific to injection site, and there may by a systemic effect of Zn dissipating in the blood circulation.

Scrotal Circumference

A treatment × day interaction was detected for scrotal circumference (Fig. 2; $P < 0.01$), which was similar ($P \geq 0.58$) between treatments on d 0. After d 14, all BAN cattle either lost their scrotum or scrotal tissue was necrotic; therefore, they were not used in the analysis thereafter. On d 3, 5, 7, and 14, INJ cattle had the greatest scrotal circumference, BUL was intermediate, and BAN possessed the smallest ($P \leq 0.02$). Cohen et al. (1991a) reported cattle chemically castrated using lactic acid had increased scrotal circumference 7, 14, and 28 d post-castration compared to surgically castrated cattle, although it should be noted that the cattle used in that study were younger than those in the current study. After d 14, scrotal circumference for INJ cattle was less ($P \leq 0.10$) than BUL cattle and remained less for the duration of the study. Scrotal circumference was greater ($P \leq 0.02$) in BUL compared to INJ cattle on d 56, 84, 112, and 140. Final scrotal circumference tended ($P = 0.07$) to be greater in BUL than INJ on d 168. After injection and until d 28, INJ had greater scrotal circumference as a function of the inflammatory response directly after injection. Overall scrotal circumference was affected by castration method; BUL cattle had greater scrotal circumference compared to INJ. Similarly, Hernandez et al. (2005) reported that intact bulls possessed greater scrotal circumferences than immunocastrated cattle.

Testis Thickness

We observed a treatment × day interaction for testis thickness (Fig. 3; $P < 0.01$). Similar, to scrotal circumference results, testis thickness increased in INJ cattle directly after administration of Zn from d 0 to 14 and 14 to 28 and then decreased after d 28. On d 1, 3, 5, and 7, testis thickness was greatest in INJ, intermediate in BUL and smallest in BAN ($P \leq 0.05$). After d 14, BAN was removed from the data because their scrotums had detached or become

necrotic. From d 0 to 28, INJ had greater ($P \leq 0.05$) testis thickness than BUL. Testis thickness was greater ($P \leq 0.05$) in BUL compared to INJ cattle on d 56, 112, and 140. We observed a tendency ($P = 0.08$) for final testis thickness to be greater on d 168 for BUL compared to INJ cattle. Zinc gluconate used in cats as a means of castration yielded similar results; testis width was smaller in treated cats compared to untreated cats on d 60 to 120, but overall testis width did not change over the entire study when comparing treated to non-treated cats (Oliveira et al., 2013). Treatment did impact testis thickness with an initial increase in INJ cattle and then a decline; however, BUL cattle were lesser for the initial 14 d then greater for the remainder of the study.

Serum Testosterone

A treatment × day interaction was observed for serum testosterone concentration (Fig. 4; $P < 0.01$). Serum testosterone concentrations in BAN cattle were undetectable after d 14 of the study. On d 56 and 112, serum testosterone concentrations were greater ($P < 0.01$) in BUL compared to INJ cattle. However, on d 84, 140, and 168, serum testosterone concentrations were similar ($P \geq 0.10$) between BUL and INJ cattle. Research in male cattle castrated chemically with lactic acid concurs with the current study, as bulls had greater plasma testosterone concentrations compared to chemically castrated cattle that had intermediate plasma testosterone concentration, while surgical castrates had the least testosterone concentrations (Cohen et al., 1991a). Fordyce et al. (1989) reported chemically castrated cattle with lesser concentrations of plasma testosterone than cattle with one residual testis and bulls that had both testicles; however, they did not relate plasma testosterone concentration to the weight of residual testicular tissue. Testosterone observations between treatments do not support efficacy of INJ as an alternative castration method in older beef bulls because similar serum testosterone existed between INJ and

BUL, whereas BAN had no detectible production of serum testosterone. In agreement with our observations, male cats castrated with zinc gluconate did not differ in serum testosterone concentrations compared to intact males (Fagundes et al., 2014). Castration is achieved upon the complete removal of testes (or testicular function) and endogenous male androgens; however, INJ cattle possessed serum testosterone concentration similar to BUL suggesting that while sterilization occurred, castration did not.

Serum Haptoglobin

A treatment × day interaction was detected for serum Hp concentration (Fig. 5; $P < 0.01$). Haptoglobin is an acute phase protein that is stimulated by proinflammatory cytokines IL-1, IL-6, and TNF-α and primarily produced by hepatocytes during the acute phase response to inflammation. Haptoglobin migrates to a site of infection via the bloodstream to assist in innate immune response and tissue remodeling and may be an indicator of pain due to the inflammatory response. Although it was not determined in the current study, serum cortisol is a more direct indication of the stress response as it is the final hormone product produced via the HPA axis in response to stressful stimuli. However, cortisol concentrations in cattle that are removed from pens and handled through working facilities are rapidly increased (Faulkner et al., 1992), and pen removal and handling was necessary in the current field study to facilitate blood sample collection. Furthermore, Bretschneider (2005) concluded in a review article on the effects of castration that haptoglobin may be a better indicator of castration-associated stress compared to cortisol because haptoglobin is a more specific indication of stress initiated by the tissue injury and inflammatory response of castration. No differences ($P = 0.91$) in serum Hp concentration were detected on d 0. However, after treatment administration on d 0, serum Hp concentration increased ($P < 0.01$) dramatically in INJ compared to BAN and BUL. Serum Hp concentration in

all treatments was greatest on d 3; INJ cattle had greater ($P < 0.01$) serum Hp concentration (1,404,047 mg/dL) than both BAN (421,508 mg/dL) or BUL (360,427 mg/dL) cattle. Similarly, INJ cattle had greater ($P < 0.01$) serum Hp concentrations on d 5 and 7 than either BAN or BUL. Final serum Hp concentration on d 14 was not different ($P \geq 0.67$) between treatments. This is a function of the acute phase response being resolved after 14-d subsequent to inflammatory procedures administered on d 0. Treatment did affect serum Hp concentration and immediately following treatment administration, a sharp increase in serum Hp concentration was observed for INJ followed by a gradual decline until concentrations were similar between treatments on d 14. Previous research (Ting et al., 2003; Brown et al., 2015; Roberts et al., 2015) demonstrates that serum Hp increases after castration is conducted, especially for the surgical method without a local anesthetic or analgesic. A slight increase in Hp was observed in BUL after d 0 and this was likely an artifact of the respiratory and clostridial vaccines administered on d 0 to all treatments (Stokka et al., 1994; Arthington et al., 2013). We suggest a greater acute phase response for INJ treatment reduced their performance initially; whereas, subsequent testosterone concentrations in INJ and BUL were similarly increased relative to BAN and therefore subsequent growth rates were greater in these treatments.

Behavior

A treatment × day interaction occurred for motion index during the first 28 d of the study (Fig. 6; $P < 0.01$). Motion index was greater on d 0 in INJ and BAN compared to BUL ($P < 0.01$) and may suggest behavior alteration in acute pain experienced immediately following INJ and BAN procedures. Motion index was greater ($P = 0.04$) on d 7 in BUL compared to BAN whereas INJ were intermediate and did not differ with BUL or BAN cattle. Day 14 motion index did not differ ($P \geq 0.75$) between treatments. Motion index on d 21 was greater ($P < 0.01$) in

BUL and INJ compared to BAN indicative of the delayed swelling and inflammation associated with BAN at this time such that overall activity was reduced, possibly to avoid sensation of chronic pain (Molony and Kent, 1997). Motion index was greater in BUL and INJ compared to BAN over the 28 d monitoring period ($P \leq 0.02$). The increase in motion index of BUL and INJ may also be attributed to increased aggressiveness typically observed in intact males as influenced by testosterone production compared to BAN cattle that did not produce testosterone.

A treatment × day interaction was detected for time standing (Fig. 7; $P < 0.01$). Standing time was greater ($P < 0.01$) in BUL and BAN on d 0 compared to INJ. However, a sharp increase in standing time occurred for INJ cattle on d 2 and was greater ($P < 0.01$) than either BUL or BAN cattle. Increased time standing by INJ cattle is likely due to "statue standing" behavior which is an indication of acute pain due to the INJ procedure administered on d 0. On d 10 and 11, BAN cattle spent more time standing compared to BUL ($P \leq 0.05$). The increase in time spent standing in BAN after d 11 may be due to more secondary or chronic pain typically seen in banded animals likely from the testicular tissue beginning to necrose and atrophy prior to loss of the testes. For the remainder of the 28 d data collection, standing time did not differ ($P \geq 0.05$) between treatments. Data suggest that standing time directly after experimental treatments were applied were affected initially but not after d 11.

We observed a treatment × day interaction for the number of steps taken per d (Fig. 8; $P < 0.01$). Day 0 steps were greater ($P \leq 0.03$) for BAN compared to either INJ or BUL. Steps were less ($P < 0.01$) for INJ cattle on d 1 compared to either BUL or BAN cattle. The INJ procedure likely influenced behavior via reduced locomotion to avoid persistent pain which was concomitant with reduced DMI by INJ cattle on d 1; however, as previously reported increased motion index was observed immediately following Zn injection on d 0. No differences ($P \geq 0.08$)

were observed in step count from d 2 to 11. Day 12 steps were greater ($P = 0.03$) in BUL compared to BAN cattle. Step count was greater ($P \geq 0.05$) from d 19 to 27 in INJ and BUL compared to BAN cattle. On d 28, there were no differences ($P \geq 0.81$) in steps regardless of castration method. Step count was variable between treatments at the beginning of data collection, but toward the end of the 28-d observation period, INJ and BUL cattle took more steps than BAN cattle which may be influenced by increased serum testosterone concentrations in INJ and BUL compared to BAN and the associated influence of testosterone on cattle behavior. Alternatively, BAN may have altered their behavior around this time such that less steps were taken in response to delayed pain from tissue necrosis. Differences in step count and motion index were not evident on d 28, as these variables were likely confounded from handling and removal of the accelerometers at that time.

A treatment × day interaction was detected for the number of lying bouts (Fig. 9; $P < 0.01$). Lying bouts on d 0 were greatest ($P < 0.01$) for INJ cattle, intermediate for BAN, and least for BUL. Lying bouts are often indicators of pain directly after castration (Robertson et al., 1994), as noted by the increase in lying bouts of BAN and INJ compared to BUL. However, on d 1, 2, and 3, INJ cattle had fewer ($P \leq 0.05$) lying bouts compared to either BUL or BAN which is likely due to profound swelling that developed around d 1, and indicated by the increased scrotal circumference and testis thickness in INJ that was previously reported. Day 4 to 28 lying bouts were similar ($P \geq 0.05$) between treatments. Number of lying bouts varied between treatments directly after treatment administration but did not differ as the study progressed. Overall, differences observed in behavior variables between treatments suggest different pain responses to INJ or BAN.

Overall Conclusions

Cattle BW was similar between treatments from d 0 to 112; however, BW was greater in INJ and BUL compared to BAN cattle on d 140, 168 and at the end of the experiment. Overall ADG was greater in INJ and BUL cattle compared to BAN. The increased ADG for INJ and BUL is a function of increased serum testosterone concentration later in the feeding period; INJ and BUL cattle had greater testosterone than BAN cattle that had complete removal of their testicles and did not receive exogenous growth hormone in lieu of natural testosterone cessation. Dry matter intake was not different between treatments over the course of the study but overall G:F was greater in INJ and BUL compared to BAN cattle. Haptoglobin concentration was greater in INJ cattle compared to BAN and BUL on d 1, 3, 5, and 7, indicating greater pain and inflammation in INJ compared to BAN and BUL. Scrotal circumference and testis thickness were greater in BUL compared to INJ after swelling subsided on d 14. Behavior measurements were greater in INJ and BUL compared to BAN from d 14 to 28, indicative of the secondary pain/inflammatory response in BAN. Injection of Zn appeared to eliminate spermatogonia and degenerate testes such that they were determined to be reproductively unviable with an overall absence of definable sperm formation and maturation, as the head of the epididymis of INJ lacked stored sperm based upon histopathological observation. Collectively, results from this experiment indicate that the INJ treatment may be a viable option for sterilization, but not castration of older beef cattle because testosterone concentrations were more similar to BUL than BAN.

Table 1. Analyzed nutrient composition of common diets (DM basis)[1].

Item	Starter diet[2]	Intermediate diet	Finisher diet
DM, %	79.2	78.5	78.7
CP, %	19.0	14.4	13.4
NPN, %	3.6	1.9	2.0
Crude fiber, %	18.4	12.3	7.3
TDN, %	69.1	76.6	87.6
NE_m, Mcal/kg	1.61	1.85	2.16
NE_g, Mcal/kg	1.01	1.21	1.50
DE, Mcal/kg	3.04	3.37	3.85
ME, Mcal/kg	2.49	2.77	3.17
Ca, %	1.31	0.60	0.54
P, %	0.35	0.28	0.27
Na, %	0.25	0.15	0.15

[1]Analyses performed by Servi-Tech Laboratories, Amarillo, TX.
[2]Starter fed first 7 d; Intermediate fed next 14 d; Finisher fed remainder of study.

Table 2: Effect of castration and castration method upon feedlot arrival on growth performance in beef cattle.

	Treatment[1]				
Item	BAN	BUL	INJ	SEM[2]	*P*- value
BW, kg					
Initial	337	338	336	10.8	0.99
D 14	354	365	352	11.0	0.64
D 28	377	395	378	13.0	0.57
D 56	435	457	445	12.4	0.48
D 84	490	525	509	12.3	0.16
D 112	531[b]	577[a]	563[ab]	12.8	0.06
D 140	573[b]	632[a]	619[a]	11.2	<0.01
D 168	585[b]	652[a]	641[a]	9.9	<0.01
Final	611[b]	686[a]	672[a]	4.4	<0.01
ADG, kg					
D 0 to 14	1.2[b]	2.0[a]	1.1[b]	0.2	<0.01
D 14 to 28	1.7	2.1	1.9	0.3	0.61
D 28 to 56	2.1[b]	2.3[ab]	2.4[a]	0.1	0.09
D 56 to 84	1.9[b]	2.4[a]	2.3[a]	0.1	0.03
D 84 to 112	1.5[b]	1.9[a]	2.0[a]	0.1	<0.01
D 112 to 140	1.5[b]	2.0[a]	2.0[a]	0.1	0.03
D 140 to 168	0.8[b]	1.3[a]	1.3[a]	0.1	<0.01
Overall	1.4[b]	1.8[a]	1.7[a]	0.1	<0.01

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.
[2]Pooled standard error of the mean.
[a-b]Rows without common letter superscripts differ, $P < 0.05$.

Table 3: Effect of castration and castration method upon feedlot arrival on feed efficiency in beef cattle.

	Treatment[1]				
Item	BAN	BUL	INJ	SEM[2]	*P*- value
Dry matter intake, kg/d					
D 0 to 14	7.0	6.9	6.0	0.4	0.11
D 14 to 28	9.6	9.7	9.1	0.4	0.51
D 28 to 56	10.0	9.6	9.4	0.4	0.56
D 56 to 84	10.2	10.5	9.9	0.3	0.40
D 84 to 112	10.2	10.5	10.2	0.3	0.54
D 112 to 140	9.8	10.3	10.0	0.3	0.38
D 140 to 168	9.3[b]	10.4[a]	9.7[b]	0.2	<0.01
Overall	9.6	10.0	9.5	0.3	0.46
Gain to feed					
D 0 to 14	0.17[b]	0.29[a]	0.19[b]	0.03	<0.01
D 14 to 28	0.18	0.21	0.20	0.03	0.62
D 28 to 56	0.21	0.24	0.26	0.02	0.13
D 56 to 84	0.19[b]	0.23[a]	0.23[a]	0.01	0.03
D 84 to 112	0.15[b]	0.18[a]	0.19[a]	0.007	<0.01
D 112 to 140	0.15[b]	0.19[ab]	0.20[a]	0.02	0.08
D 140 to 168	0.08[b]	0.13[a]	0.13[a]	0.01	0.02
Overall	0.15[b]	0.18[a]	0.18[a]	0.009	0.03

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.
[2]Pooled standard error of the mean.
[a-b]Rows without common letter superscripts differ, $P < 0.05$.

Table 4: Effect of castration and castration method upon feedlot arrival on testes weight in beef cattle.

	Treatment[1]				
Item	BAN	BUL	INJ	SEM[2]	*P*- value
Testes weight, g[3]					
Smallest testis	-	352	242	5.3	<0.01
Largest testis	-	376	313	6.4	<0.01
Total weight	-	727	550	12.0	<0.01

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.

[2]Pooled standard error of the mean.

[3]Testes weight was determined by collecting testes from each BUL and INJ carcass after stunning and exsanguination at the commercial abattoir; testes were severed with a knife, placed in pre-labeled bags and immediately transported to the West Texas A&M University abattoir for weight determination using a digital scale. The testes were allocated into smallest and largest testis per animal prior to statistical analysis.

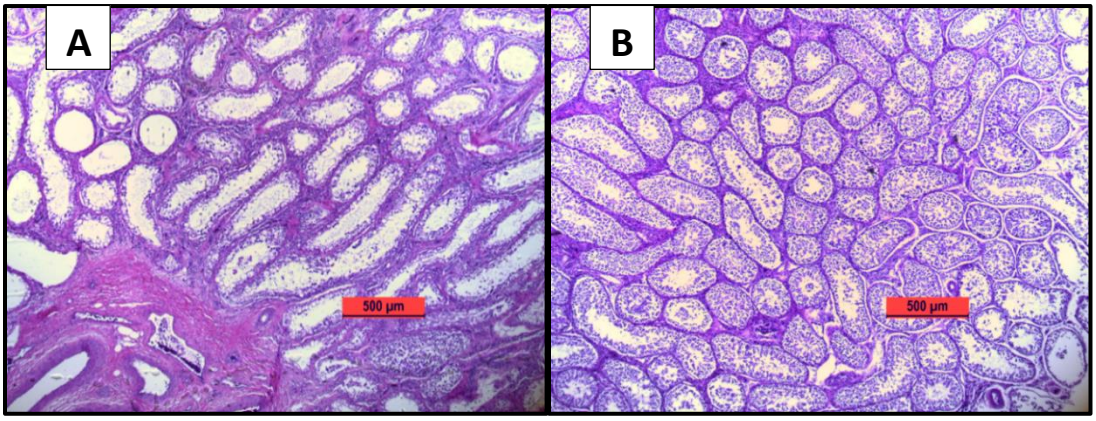

Figure 1. Histopathological representation of testis from INJ (A) and BUL (B) collected upon harvest. INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.

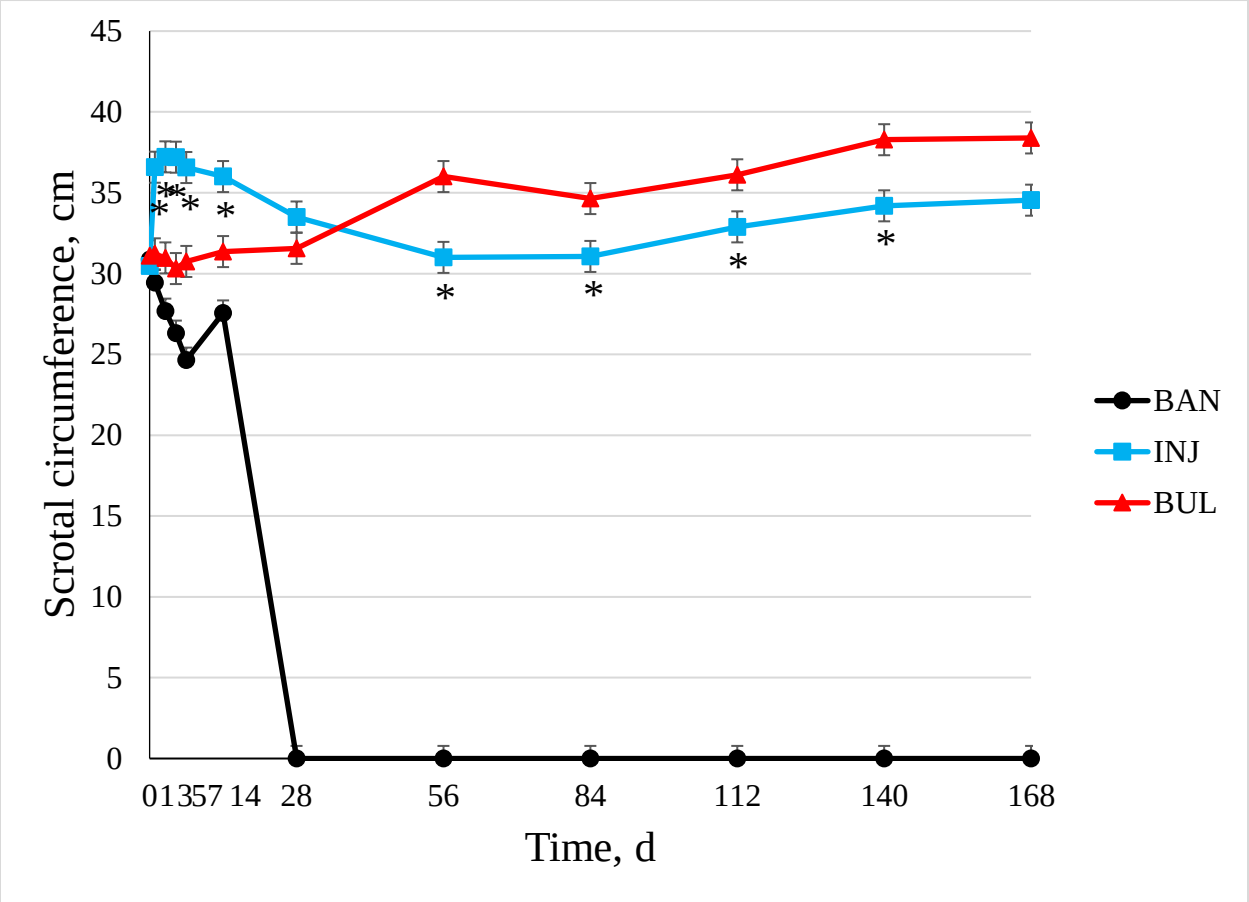

Figure 2. Effect of castration and castration method upon feedlot arrival on scrotal circumference of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P < 0.01$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL ($P \leq 0.02$).

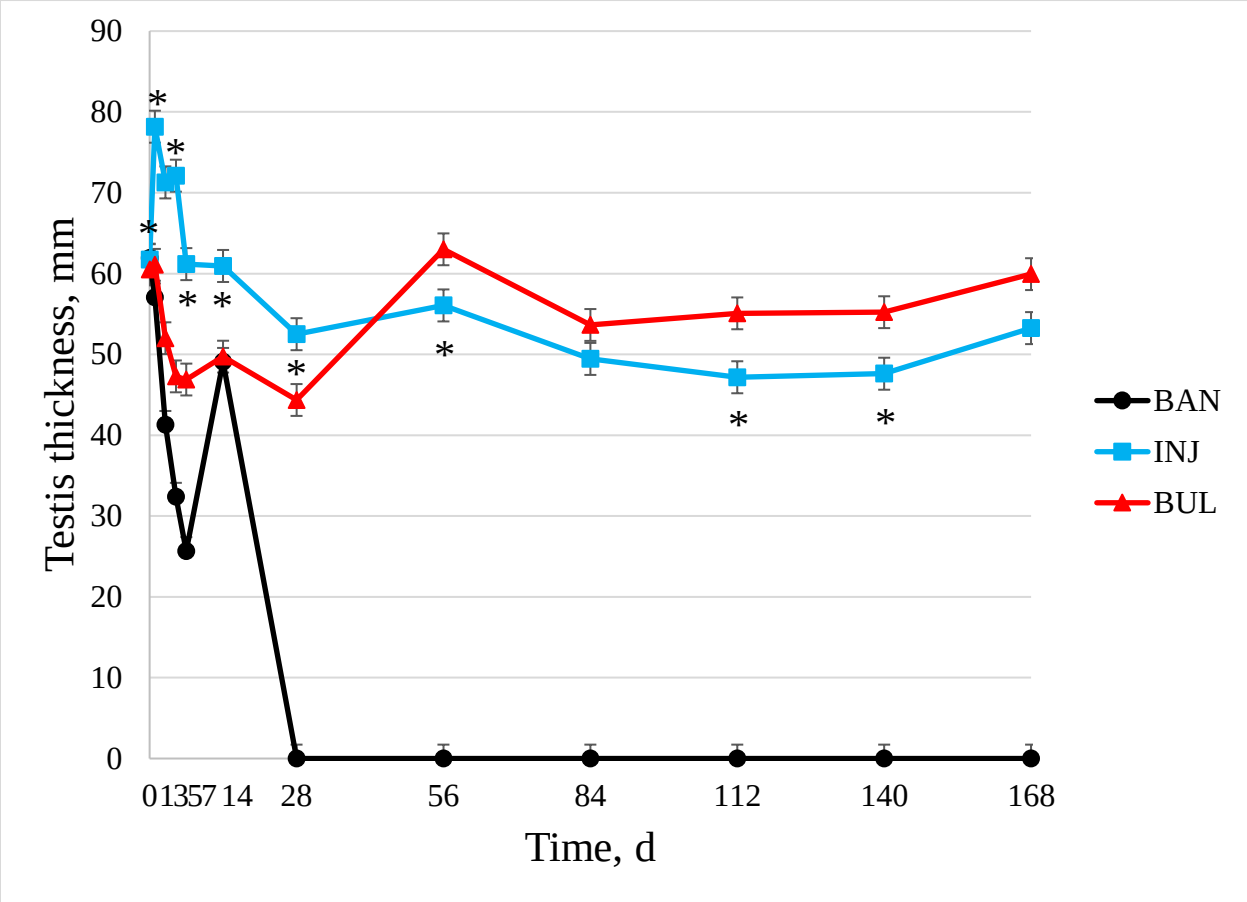

Figure 3. Effect of castration and castration method upon feedlot arrival on testis thickness of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P < 0.01$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL ($P \leq 0.05$).

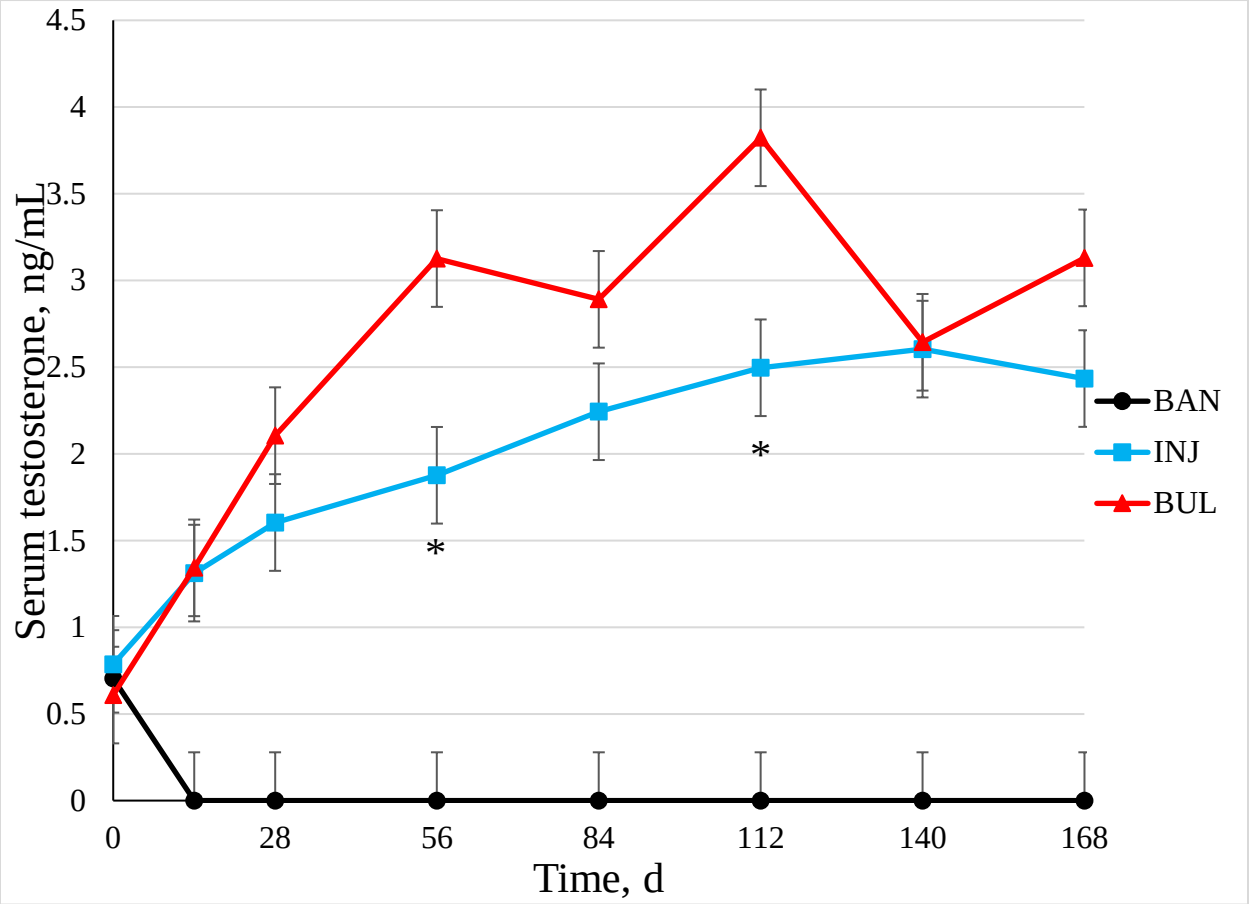

Figure 4. Effect of castration and castration method upon feedlot arrival on serum testosterone concentrations of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P < 0.01$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BUL ($P < 0.01$).

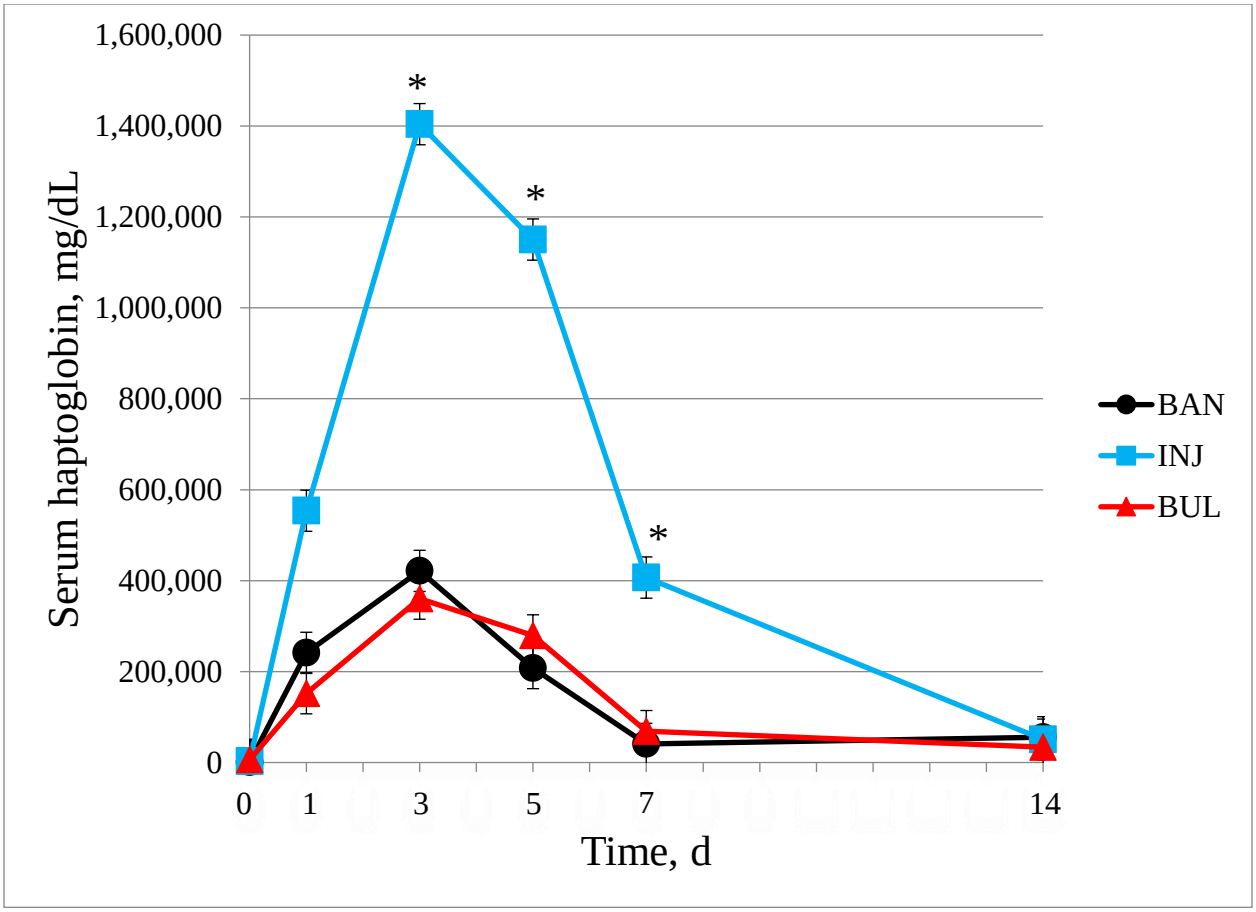

Figure 5. Effect of castration and castration method upon feedlot arrival on serum haptoglobin concentrations of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P < 0.01$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL ($P < 0.01$).

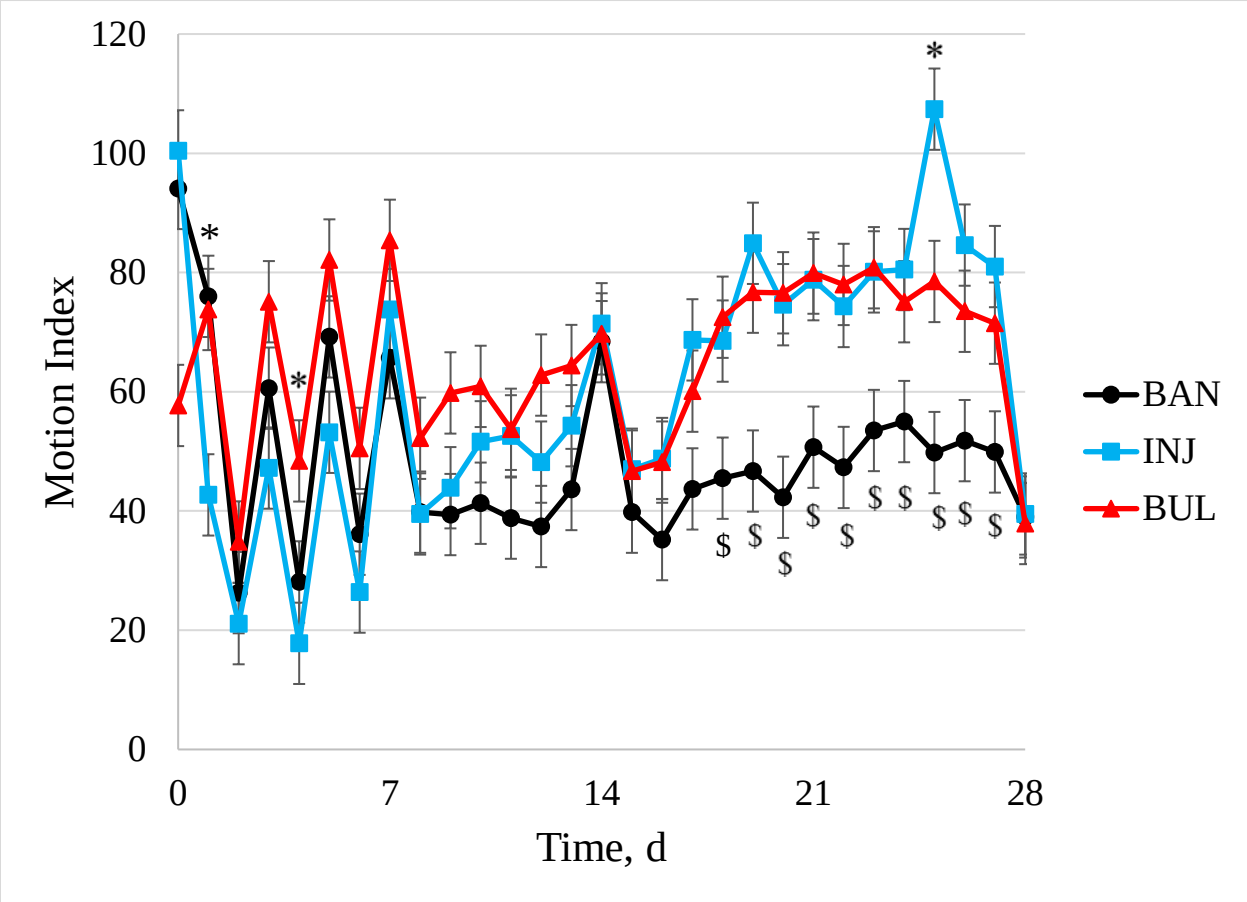

Figure 6. Effect of castration and castration method upon feedlot arrival on motion index of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P < 0.01$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL within day ($P \leq 0.05$). $ BAN differs from INJ and BUL within day ($P \leq 0.05$).

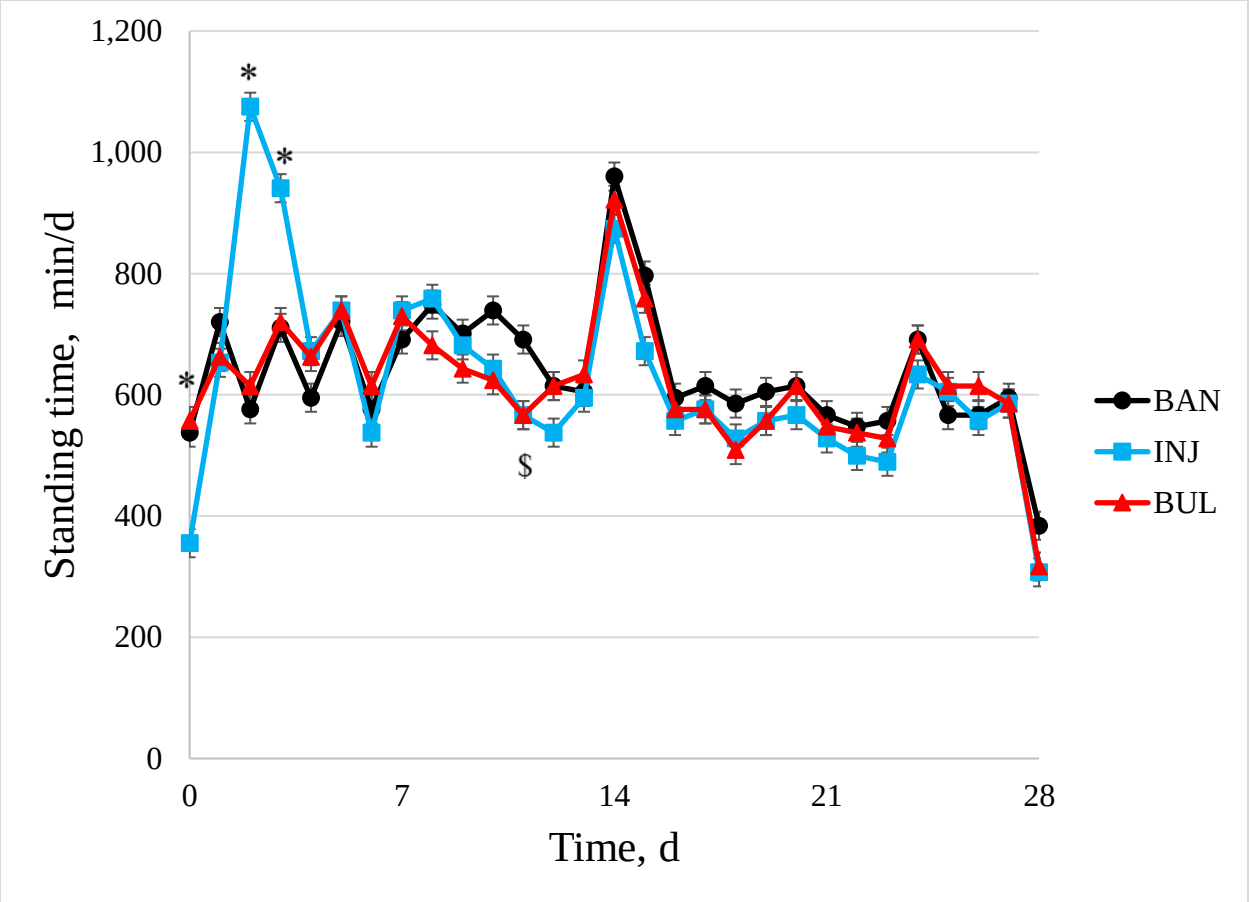

Figure 7. Effect of castration and castration method upon feedlot arrival on standing time of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of day ($P < 0.01$) and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL within day ($P \leq 0.05$). $ BAN differs from INJ and BUL within day ($P \leq 0.05$).

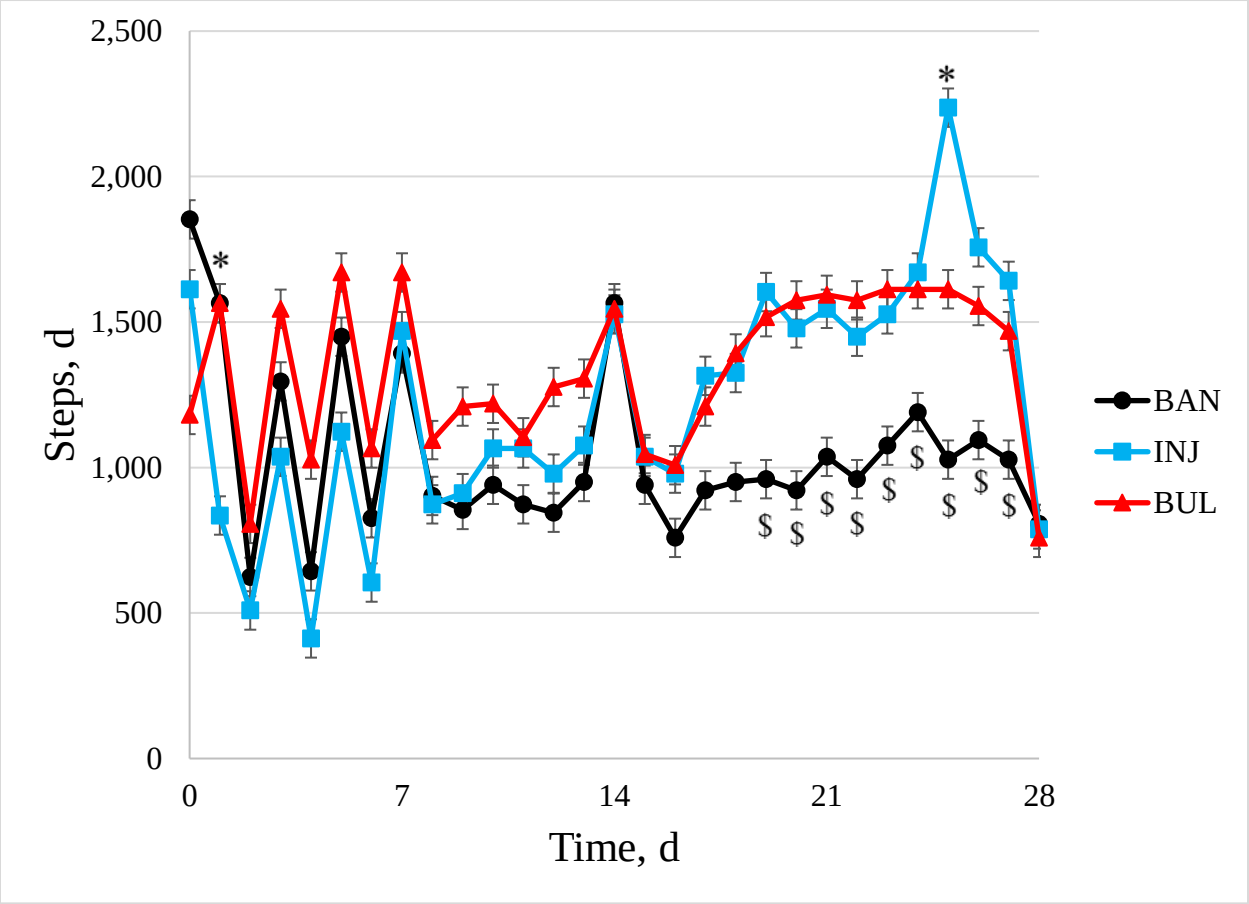

Figure 8. Effect of castration and castration method upon feedlot arrival on steps taken of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of treatment ($P = 0.04$), day ($P < 0.01$), and treatment × day ($P < 0.01$) were detected. * INJ differs from BAN and BUL within day ($P \leq 0.05$). $ BAN differs from INJ and BUL within day ($P \leq 0.05$).

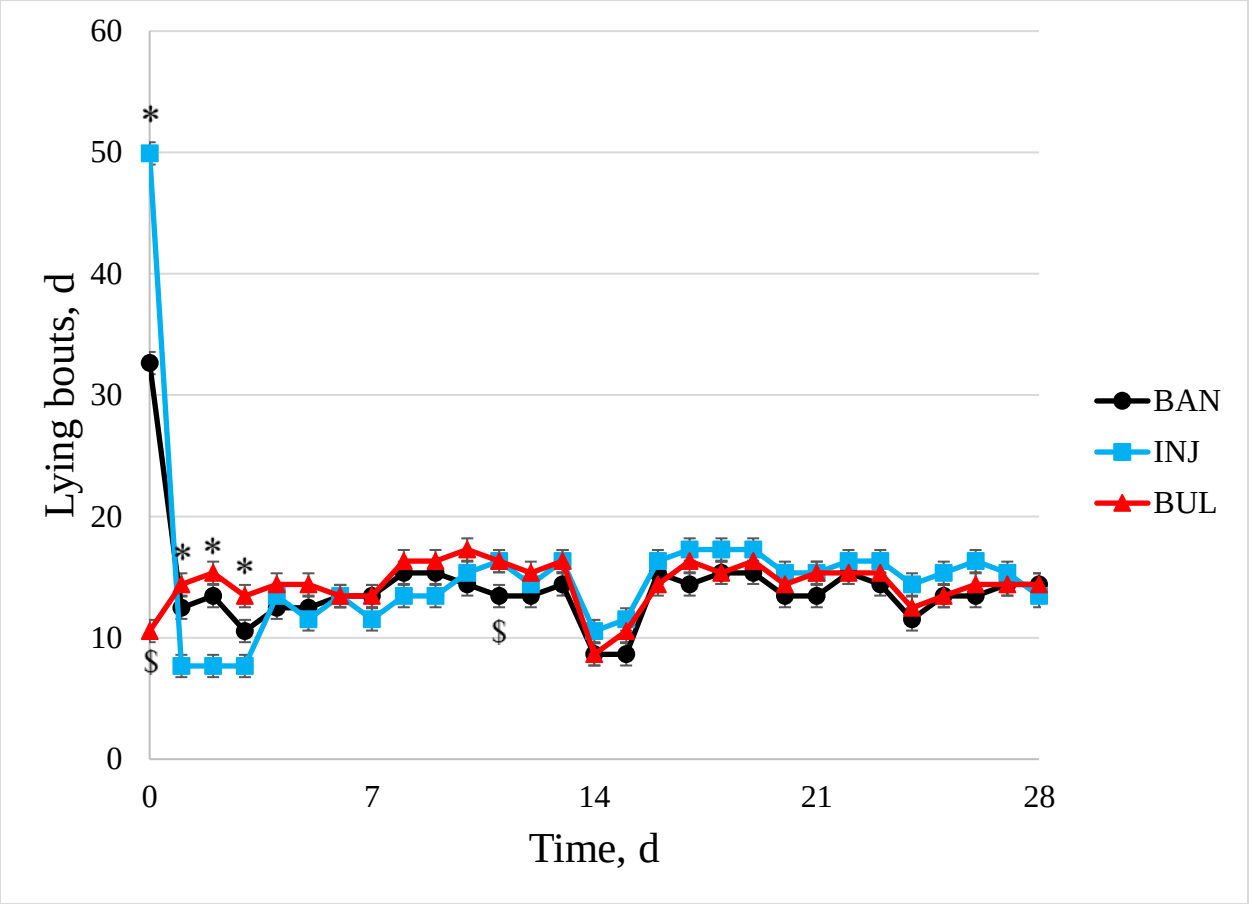

Figure 9. Effect of castration and castration method upon feedlot arrival on lying bouts of beef cattle. BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact. Effect of day ($P < 0.01$) and treatment × day ($P < 0.01$). * INJ differs from BAN and BUL within day ($P \leq 0.05$). $ BAN differs from INJ and BUL within day ($P \leq 0.05$).

LITERATURE CITED

Arthington, J. D., R. F. Cooke, T. D. Maddock, D. B. Araujo, P. Moriel, N. DiLorenzo, and G. C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91:1831-1837. doi:10.2527/jas2012-5724.

Boler, D. D., L. W. Kutzler, D. M. Meeuwse, V. L. King, D. R. Campion, F. K. McKeith, and J. Killefer. 2011. Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. J. Anim. Sci. 89:2189-2199. doi:10.2527/jas.2010-3640.

Bretschneider, G. 2005. Effects of age and method of castration on performance and stress response of beef male cattle: A review. Livestock Sci. 97:89-100.

Brown, A. C., J. G. Powell, E. B. Kegley, M. S. Gadberry, J. L. Reynolds, H. D. Hughes, J. A. Carroll, N. C. Burdick Sanchez, Y. V. Thaxton, E. A. Backes, and J. T. Richeson. 2015. Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. J. Anim. Sci. 93: 2460-2470. doi:10.2527/jas2014-8695.

Chase, C. C., Jr., R. E. Larsen, R. D. Randel, A. C. Hammond, and E. L. Adams. 1995. Plasma cortisol and white blood cell responses in different breeds of bulls: a comparison of two methods of castration. J. Anim. Sci. 73:975–980. doi:10.2527/1995.734975x.

Coetzee, J. F. 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications from cattle production within the United States. Appl. Anim. Behav. Sci. 135:192–213.

Cohen, R. D. H., B. D. King, E. D. Janzen, and P. S. W. Hunter. 1991a. Efficacy of chemical castration and effects of age at castration and implant regime on growth rate, testicular measurements and testosterone levels of beef calves. Can. J. Anim. Sci. 71:1-11.

Cohen, R. D. H., B. D. King, E. D. Janzen, and H. H. Nicholson. 1991b. The effect of castration age, method and implant regime on growth and carcass traits of male beef cattle. Can. J. Anim. Sci. 71:301-309.

Daniels, T. K., J. G. P. Bowman, B. F. Sowell, M. E. Branine, and M. E. Hubbert. 2000. Effect of metaphylactic antibiotics on behavior of feedlot cattle. Prof. Anim. Sci. 16: 247-253.

Fagundes, A. F. F., E. C. S. Oliveira, B. M. Tenorio, C. C. S. Melo, L. T. B. Nery, F. A. B. Santos, L. C. Alves, R. H. Douglas, and V. A. Silva Jr. 2014. Injection of a chemical castration agent, zinc gluconate, into the testes of cats results in the impairment of spermatogenesis: A potentially irreversible approach for this species? Theriogenology 81:230-236.

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

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull cattle. J. Anim. Sci. 74:2336–2343. doi:10.2527/1996.74102336x.

Fisher, A. D., M. A. Crowe, E. M. O'Nuallain, M. L. Monaghan, J. A. Larkin, P. O'Kiely, and W. J. Enright. 1997. Effects of cortisol on in vitro interferon – gamma production, acute-phase proteins, growth, and feed intake in a calf castration model. J. Anim. Sci. 75:1041–1047. doi:10.2527/1997.7541041x.

Fordyce, G., P. B. Hodge, N. J. Beaman, A. R. Laing, C. Campero, and R. K. Shepherd. 1989. An evaluation of calf castration by intra-testicular injection of a lactic acid solution. Aust. Vet. J. 66:272-276.

Hernandez, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated *Bos Indicus* bulls. J. Anim. Sci. 83:2901-2907. doi:10.2527/2005.83122901x.

Lyles, J. L. and M. S. Calvo-Lorenzo. 2014. BILL E. KUNKLE INTERDISCIPLINARY BEEF SYMPOSIUM: Practical developments in managing animal welfare in beef cattle: What does the future hold? J. Anim. Sci. 92:5334-5344. doi:10.2527/jas.2014-8149.

Molony, V., and J. E. Kent. 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. J. Anim. Sci. 75: 266-272. doi:10.2527/1997.751266x.

Oliveira, E. C. S., M. R. Moura, V. A. Silva Jr., C. A. Peixoto, K. L. A. Saraiva, M. J. C. Sa, R. H. Douglas, and A. P. Marques Jr. 2007. Intratesticular injection of a zinc based solution as a contraceptive for dogs. Theriogenology 68:137-145.

Oliveira, E. C. S., A. K. F. Fagundes, C. C. S. Melo, L. T. B. Nery, R. G. Revoredo, T. F. G. Andrade, K. Oliveira-Esquerre, J. P. Kastelic, and V. A. Silva Jr. 2013. Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety. The Vet. J. 197:307-310.

Peterson, E. B., D. R. Stronbehn, G. W. Ladd, and R. L. Willham. 1989. Effects of preconditioning on performance of beef cattle before or after entering the feedlot. J. Anim. Sci. 67: 1678-1686. doi:10.2527/jas1989.6771678x.

Price, M. A., S. D. M. Jones, G. W. Mathison, and R. T. Berg, 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. 60:345-358.

doi.org/10.4141/cjas80-044

Pritchard, R. H., and K.W. Bruns. 2003. Controlling variation in feed intake through bunk management. J. Anim. Sci. 81(E. Suppl. 2):E133-E138.

Roberts, S. L., H. D. Hughes, N. C. Burdick Sanchez, J. A. Carroll, J. G. Powell, D. S. Hubbell, and J. T. Richeson. 2015. Effect of surgical castration with or without oral meloxicam on the acute inflammatory response in yearling beef bulls. J. Anim. Sci. 93: 4123-4131. doi:10.2527/jas.2015-9160.

Robertson, I., J. Kent, and V. Molony. 1994. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Res. Vet. Sci. 56:8–17. doi:10.1016/0034-5288(94)90189-9.

Stokka, G. L., A. J. Edwards, M. F. Spire, R. T. Brandt, and J. E. Smith. 1994. Inflammatory response to clostridial vaccines in feedlot cattle. J. Am. Vet. Med. Assoc. 204:415–419.

Sutherland, M. A. 2011. Painful husbandry procedures and methods of alleviation: a review. Proc. New Zealand Soc. Anim. Prod. 71: 189-194.

Ting, S. T. L., B. Early, J. M. L. Hughes, and M. A. Crowe. 2003. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. J. Anim. Sci. 81:1281-1293. doi:10.2527/2003.8151281x.

USDA. 2013. NAHMS Part III. Trends in Health and Management Practices on U.S. Feedlots, 1994-2011. Page 37. Natl. Anim. Health Monit. Serv., Fort Collins, CO.

Warnock, T. M., T. A. Thrift, M. Irsik, M. J. Hersom, J. V. Yelich, T. D. Maddock, G. C. Lamb, and J. D. Arthington. 2012. Effect of castration technique on beef calf performance, feed efficiency and inflammatory response. J. Anim. Sci. 90:2345-2352. doi:10.2527/jas2011-4511.

CHAPTER V

ZINC INJECTION AS A NOVEL CASTRATION METHOD IN BEEF BULLS: EFFECTS ON CARCASS TRAITS AND CONSUMER ACCEPTABILITY

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ABSTRACT

One hundred and eighty beef bulls (BW = 337 ± 10.9 kg) were blocked by BW (6 blocks) and assigned randomly to 1 of 3 treatments on d 0: 1) INJ; received 1 mL (100 mg Zn) of a Zn solution in each testis, 2) BAN; received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, 3) BUL; bulls with testicles remaining intact. Cattle were grouped by weight block in a randomized complete block design (3 treatment pens/block and 10 cattle/pen) and harvested by block on three separate dates when blocks reached similar BW and visual fat thickness accretion. Striploins were removed from the left carcass sides, vacuum packaged and aged for 14 d, then frozen at -20°C. Frozen striploins were sliced into 2.54-cm-thick steaks and remained frozen until analyses. Steaks (n = 3/animal) were used to assess consumer acceptability via consumer taste panel (n = 152 panelists), Warner-Bratzler shear force, percentage cook loss, and cooked color values. Data were analyzed using the MIXED and GLIMMIX procedures of SAS; pen was the experimental unit for all dependent variables. Hot carcass weights and LM area were greater ($P < 0.01$) for the INJ and BUL treatments compared to BAN. Mean yield grade did not differ between treatments ($P = 0.12$). Percentage of USDA Choice or greater carcasses was greater ($P < 0.01$) for BAN than INJ and BUL treatments. Consumer panelists detected a difference in perceived tenderness; BAN steaks had greater ($P = 0.02$) tenderness scores than BUL steaks whereas INJ steaks were intermediate. Panelists rated juiciness of BAN steaks greater ($P < 0.01$) than either BUL or INJ steaks. Panelists rated beef flavor greater ($P = 0.01$) for BAN and BUL steaks than INJ steaks. Overall acceptability was greater ($P < 0.01$) for BAN compared to INJ steaks whereas BUL steaks were intermediate. Percentage cook loss of striploin steaks ($P = 0.47$) and Warner-Bratzler shear force values ($P = 0.11$) did not differ between treatments. Cooked color lightness values (L*) and redness values (a*) were not

affected ($P \geq 0.23$) by treatment. Striploin steaks from BAN and BUL treatments had greater ($P = 0.02$) yellowness values (b*) than INJ steaks. The ratio of red-to-brown (630:580 nm) of cooked striploin steaks were greater ($P = 0.05$) for INJ than either BAN or BUL treatments. Carcass and palatability outcomes of INJ was more similar to BUL than BAN, suggesting limited efficacy of INJ as a castration method in more mature beef bulls at feedlot entry.

Key words: beef bulls, carcass traits, castration, consumer taste panel, zinc

INTRODUCTION

Alternative castration methods are of increasing importance to producers because consumers are more sensitive toward animal management practices (Lamb et al., 2016). Currently, bulls that arrive at feedlots are castrated (92.5%) predominantly by surgical (50.4%) and banding (42.9%) procedures, with no reported use of chemical castration (NAHMS, 2011). Castration reduces animal aggression by eliminating endogenous testosterone and improves meat quality by increasing intramuscular adipose deposition resulting in greater quality grades and improved tenderness, juiciness, and flavor ratings (Carroll et al., 1975; Calkins et al., 1986). Castration also increases the presence of glycolytic muscle fibers and reduces the frequency of "dark cutting beef" from antemortem stress due to the depletion of muscle glycogen which reduces the presence of lactic acid resulting in increased muscle pH (Scanga et al., 1998). Once more, castration decreases LM area, G:F, ADG, BW and HCW compared to bulls; however, the performance reduction in steers is commonly ameliorated by the use of growth promoting implants containing low dose analogues of testosterone (Price et al., 1980).

Chemical castration utilizing zinc gluconate injection in the testes has been evaluated in companion animals as an alternative to traditional castration methods (Oliveira et al., 2013); however, this method has been minimally explored in beef cattle. An injectable product

consisting of zinc acetate neutralized by L-histidine (Calviex, Cowboy Animal Health, Plano, TX) has been approved by the FDA for proof-of-concept investigation in beef bulls. The objective of this study was to determine the effects of castration and castration method upon feedlot arrival on carcass traits and consumer acceptability of male beef cattle. The authors hypothesized that carcass traits and consumer acceptability of bulls injected with zinc would be similar to the traditionally band-castrated cattle and differ compared to intact bulls.

MATERIALS AND METHODS

Animal care and use procedures and protocol approval, arrival processing procedures, growth performance, behavior, and serum testosterone and haptoglobin concentrations are previously described in a companion study (Ball et al., 2018). There is no animal care and use protocol associated with the post-mortem data reported in this study. The protocol used in this study was approved by the Institutional Review Board (#13-05-713) of the University of Arkansas (Fayetteville, AR). Prior to participation, experimental procedure was explained and a written consent indicating voluntary participation was obtained from each participant. Briefly, the experimental treatments consisted of: 1) INJ; received 1 mL (100 mg Zn) of a Zn solution in each testis, 2) BAN; received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, 3) BUL; bulls with testicles remaining intact. Body weights were obtained on the d of shipment to feedlot (d -1) and used to determine appropriate BW block allocation to facilitate a randomized complete block design. Blocks were constructed by stratification of d -1 BW, arrival date during backgrounding phase, and number of times treated with an antibiotic. The lightest 10 animals within a treatment were allocated to a pen, followed by the 10 next lightest animals, and so on until 6 pens within each treatment were allocated. Pens within block (3/block) were harvested on three separate d according to their projected final BW and visual

appraisal by trained personnel for market readiness. Blocks 5 and 6 were harvested (USDA Establishment #3, Cactus, TX) on d 155; blocks 3 and 4 were harvested on d 176; blocks 1 and 2 were harvested on d 197. Approximate age of cattle at time of harvest was 14 to 18 mo of age; however, cattle were purchased from regional sale barns and exact age of cattle is unknown. Carcasses were evaluated after a 24-h chill period. Harvest floor data, crest height, and lean color score at the 12th rib were recorded by trained personnel (blinded to treatment) from the West Texas A&M University (WTAMU) Beef Carcass Research Center (Canyon, TX). USDA quality and yield grade as well as LM area and preliminary yield grade were determined by a vision camera system (VBG 2000; E+V Technology GmbH, Oranienberg, Germany).

Procurement

Approximately 24 h after slaughter, striploins from the left side of each carcass were obtained from the beef processor and transported to the WTAMU Meat Lab, wet aged for 14 d and then frozen at -20°C. Frozen striploins were transported to the University of Arkansas Red Meat Abattoir, then sliced into 2.54-cm-thick steaks such that a minimum of 3 steaks were obtained from each striploin. One steak was used for cooking loss, Warner-Bratzler shear force, and cooked color; the remaining 2 steaks were used for a consumer taste panel.

Cooking loss

Prior to cooking for determination of Warner-Bratzler Shear Force (WBSF), steaks (n = 120) from blocks 1 through 4 (blocks 5 and 6 were not used for cooking loss analysis) were thawed for 24 h at 4°C, blotted dry, and weighed before cooking on a preheated (204°C) electronic countertop griddle (model 0690005; National Presto Industries, Inc., Eau Claire, WI). Steaks were turned every 4 min until they reached an internal temperature of 71°C in the geometric center as determined by a digital thermometer (C28 K Type; Comark Instruments,

Beaverton, OR). Steaks were allowed to cool to room temperature (23°C) before being reweighed to calculate cooking loss [100- ((cooked weight/raw weight after thawing) × 100)].

Warner–Bratzler Shear Force

Steaks (n = 180) used for determination of WBSF values as a proxy for tenderness were cooked using the same procedures as previously described, and after cooking and cooling to room temperature (23°C), six 1.27 cm-diameter cores were removed from each steak parallel to the muscle fiber orientation. Each core was sheared perpendicular to the longitudinal positioning of the muscle fibers in the geometric center of the sample using a WBSF device attached to an Instron Universal Testing Machine (model 4466; Instron Corp., Canton, MA), equipped with a 50-kg load cell and a crosshead speed of 24.9 cm/min. Shear force values (reported in kgF) were the average of 6 cores from each steak.

Cooked Color

Before WBSF cores were removed, each steak was sliced laterally and instrumental color (L*, a*, b*) was determined immediately. Cooked color values for each steak were determined from an average of 3 randomly placed readings with a Hunter MiniScan XE Plus (Hunter Associates Laboratory, Reston, VA) using Illumina A with a 9-mm aperture and a 10° observation angle. Red-to-brown was calculated as the reflectance ratio of 630 to 580 nm (representing the change of denatured myoglobin during cooking to either metmyoglobin or denatured myoglobin). Instrumental cooked color values were used to calculate hue angle (representing a change from the true red axis) as: $\tan^{-1}(b^*/a^*)$, chroma (representing the total color) as: $(a^{*2}+b^{*2})^{1/2}$. The colorimeter was calibrated against standard black and white tiles before data collection.

Con*sumer Taste Panel*

Consumer panels (15 sessions; 152 panelists) were conducted on a single day at the University of Arkansas Food Science and Sensory Laboratory (Fayetteville, AR). Consumers were screened before panel participation; prerequisites for participation included male or female (18 to 55 yr old) consumers who consumed beef and liked steak. Each panelist was assigned a random number and instructed to sit at a designated monitor to complete each sample ballot. Panels were conducted with 8 to 12 consumers/session; each session lasted approximately 20 min. Consumer panelists were provided a Styrofoam tray containing a napkin, fork, water, and 3 saltine crackers. The water and saltine crackers were available to each panelist to cleanse their palette between each sample and they were instructed to do so prior to consumption of each sample. Steaks were cooked on electronic countertop griddles (model G767; Farberware, Fairfield, CA) and turned every 4 min until an internal endpoint temperature of 71.1°C was achieved. Internal temperature was monitored using digital thermometers (model 51 TI Thermometer; Fluke Corp., Everett, WA) placed in the geometric center of each steak. For each session, steaks (minimum of one per treatment) were cooked and allowed to rest for 3 to 5 min before cutting. Each cooked steak was trimmed of the outside edges, excess muscle, and fat before cutting into 1cm × 1cm × cooked steak thickness. Pieces were randomly identified with a 3-digit code for assessment and held in a warmer (model MP-941; Henny Penny Corp., Eaton, OH) at 62.8°C for approximately 10 min during each sensory evaluation session. Samples were presented to consumer panelists in randomized order and each panelist evaluated their steak piece at his/her own pace for overall scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely). Tests were conducted under fluorescent

lights with partitioned booths to isolate panelists. Questions included consumer likeness of tenderness, juiciness, flavor, off-flavor (yes/no), off-flavor score (if off-flavor = yes, then the same 1-9 likert scale described previously was used), and overall acceptability. The off-flavor yes/no was utilized to determine if consumers detected what they perceived as an off-flavor in the steak samples and then, of those who detected an off-flavor, where did it rank on the likert scale.

Statistical Analyses

Statistical analyses were conducted for all outcome variables in a randomized complete block design. Data were tested for normality using PROC UNIVARIATE and nonparametric data were log-transformed prior to analysis if normality was improved. If normality was not improved, initial geometric means were utilized. Quantitative carcass and steak variables were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment as the lone fixed effect and block as the random effect. Categorical carcass and ordinal scale consumer panel data were analyzed using PROC GLIMMIX of SAS. Pen was the experimental unit for all dependent variables analyzed. Means were separated at an α of 0.05 using the PDIFF option in SAS. Statistical significance was declared at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$ for all dependent variables.

RESULTS AND DISCUSSION

Carcass Characteristics

The percentage of cattle grading USDA Choice was greater (Table 1; $P < 0.01$) in BAN (55.6%) than either BUL (28.3%) or INJ (23.9%). Conversely, the percentage of cattle grading USDA Select or USDA Standard were greater ($P < 0.01$) in BUL (71.7%) and INJ (76.1%) than in BAN (44.4%). No cattle graded USDA Prime nor were there any cattle assigned as “no-rolls”.

All cattle were A maturity with no ossification of the lumbar or thoracic vertebrae with distinct separation of the sacral vertebrae. Quality grades are assigned based on marbling and skeletal maturity and can be affected by non-genetic factors including pre-feedlot nutrition and health. Castration increases marbling and therefore quality grade compared to bulls due to reduced testosterone concentrations in steers (Field, 1971). No growth implants were used during the current study because the authors anticipated similar results between BAN and INJ compared to BUL and did not want the effects of implants of either BAN or INJ to confound castration treatment. A review by Duckett and Pratt (2014) reported that use of growth implants reduced marbling scores between 3 and 12% depending on the type of implant and number of times implanted compared to a non-implanted control. Therefore, had we implanted BAN, their quality grades may have been comparable to BUL and INJ. As expected, castration via BAN affected quality grade as a function of increased fat deposition compared to INJ and BUL. The injection of Zn did not alter quality grade from that of BUL; similar quality grades noted in BUL and INJ is indicative of limited efficacy in INJ to reduce male characteristics associated in bulls compared to steers.

There were no differences (Table 1; $P = 0.30$) between treatments in the percentage of cattle grading USDA yield grade 1. According to the USDA (2016), only 5.7% of beef carcasses grade USDA 1; therefore, the results in the current study concur as a small percentage of cattle graded USDA 1, which explains the absence of treatment differences. However, the percentage of cattle grading USDA yield grade 2 were greater ($P = 0.04$) for INJ (59.1%) than BAN (27.6%) whereas BUL (37.4%) were intermediate. The increase in percentage of USDA yield grade 2 in INJ compared to BAN is relative to the antagonistic relationship between quality and yield. Intramuscular fat deposition (marbling) is the lowest priority of the 4 fat depots to occur in cattle:

internal, subcutaneous, and intermuscular fat deposition, respectively, occur prior. Hence, cattle with greater marbling often have greater subcutaneous fat, which negatively affects their yield grade. There was a tendency ($P = 0.06$) for the percentage of cattle grading USDA yield grade 3 to be greater for BAN (51.8%) than either BUL (28.2%) or INJ (27.7%). There were no differences ($P = 0.13$) between treatments for the percentage of cattle grading either USDA 4 or 5; however, it should be noted that only 4.2% of INJ were USDA 4 or 5 compared to BAN (17.0%) or BUL (23.1%) and a larger number of animals is likely required to statistically resolve the numerical difference in percentage of carcasses grading USDA 4 or 5. The percentage of cattle that graded USDA 4 or 5 was greater than the national average (USDA 4 = 11.0% and USDA 5 = 1.8 %; USDA 2016) and is indicative of greater fat deposition and/or reduced muscle accretion.

Hot carcass weights were greater for INJ (404 kg) and BUL (414 kg) compared to the BAN (366 kg) treatment (Table 2; $P < 0.01$). Tavarez et al. (2014) reported that immunocastrated pigs had reduced final BW compared to surgically castrated pigs; however, immunocastrated pigs had greater HCW compared to surgically castrated pigs indicating a greater dressed carcass yield in immunocastrated pigs. Previous research in chemically (lactic acid) castrated bulls reported that bulls had greater hot carcass weights compared to chemically castrated cattle (Cohen et al., 1991). Similarities in HCW between INJ and BUL correlates with similarly increased serum testosterone concentrations (Ball et al., 2017) and was phenotypically confirmed via noticeably increased male characteristics possessed by both INJ and BUL treatments. Crest height was greater ($P = 0.01$) in BUL (25.1 cm) and INJ (25.7 cm) compared to BAN (20.8 cm). Crest height is a phenotypic indicator of the effects of testosterone; thus, the increase in crest height observed for BUL and INJ compared to BAN was expected due to the difference in

testosterone concentrations reported in the companion study (Ball et al., 2017). Dressed carcass yields were not affected ($P = 0.72$) by treatment. Bulls typically have greater dressed carcass yields compared to steers due to increased muscling. However, that did not occur in the current study, likely due to INJ not causing complete cessation of testicular function as noted by serum testosterone not completely ablated by INJ. Marbling scores were greatest in BAN (Small ^{15}) compared to either INJ (Slight ^{61}) or BUL (Slight ^{70}; $P < 0.01$). The current study concurs with Pérez-Linares et al. (2017) where Holstein bulls immunocastrated with Bopriva (Zoetis) on 4 different occasions had greater marbling scores than bulls treated with a saline placebo. It should be noted that serum testosterone was significantly reduced in the immunocastrated treatment evaluated in the previously mentioned study; whereas, in the current study serum testosterone was similar for INJ and BUL prior to harvest. Lean color scores and fat depth were similar ($P \geq 0.17$) between treatments. Because cattle were harvested with similar visual assessment of fat deposition, the similar preliminary yield grade was expected. Conversely, LM area was greater ($P < 0.01$) in BUL (101.3 cm^2) and INJ (98.1 cm^2) than BAN (87.7 cm^2). Cohen et al. (1991) reported adjusted rib-eye area of bulls was greater compared to surgical castrates and also greater compared to chemically castrated (lactic acid) cattle in one year of a two-year study. Longissimus muscle area is highly correlated to carcass weight which is supported by the current study; however, BAN cattle were not implanted with analogues of testosterone as per standard industry practices which presumably would have increased both HCW and LM area. Similar LM area in BUL and INJ supports the increase in serum testosterone concentration in INJ and BUL compared to BAN, indicative of limited castration efficacy in INJ. Estimated percentage of kidney, pelvic, and heart fat was similar ($P = 0.99$) between treatments as was expected due to similar external fat deposition between treatments. Previous research by Pérez-Linares et al.

(2017) report increased KPH fat in immunocastrated bulls compared to placebo bulls left intact which differs from the current study. Numeric yield grade did not differ ($P = 0.12$) between all treatments. Our carcass variable observations suggest limited efficacy of INJ to reduce male characteristics via castration as indicated by similar characteristics of BUL and INJ compared to BAN.

Consumer Taste Panel

Consumer taste panelists (n = 152) were predominantly female (71.7%); age varied from 18 to 24 yr old (8.6%), 25 to 34 yr old (25.8%), 35 to 45 yr old (17.2%), 46 to 54 yr old (23.2%), 55 to 65 yr old (13.3%), and over 65 years old (11.9%). The majority of panelists (55%) had an annual income less than $50,000; however, 38.1% earned at least $60,000 annually, and only 17.2% of panelist earned less than $20,000 annually. Consumer panelists detected a difference in perceived tenderness; BAN steaks had greater ($P = 0.02$) tenderness than BUL steaks whereas INJ steaks were intermediate (Table 3). Panelists rated juiciness greater ($P < 0.01$) in BAN steaks than either BUL or INJ steaks. Flavor was rated by panelists as greater ($P = 0.01$) in BAN and BUL steaks than INJ steaks. Overall acceptability was greater ($P < 0.01$) for BAN steaks than INJ steaks whereas BUL steaks were intermediate. Consumers preferred BAN steaks to INJ and BUL steaks indicative of taste preferences of traditionally castrated beef compared to BUL or INJ, which possessed phenotypic male characteristics. Panelists deemed steaks from INJ to be similar to BUL whereas BAN was more favorable to consumers for each variable. Consumer detection of off-flavor was greater ($P < 0.01$) in INJ (28.46%) than in either BAN (17.29%) or BUL (16.13%) steaks. The injection of zinc negatively affected perceived off-flavor of steak compared to BAN or BUL indicating a potential effect of INJ on steak flavor. Of those panelists that deemed an off-flavor, the likeability of the off-flavor was not different ($P = 0.35$) between

treatments. Results from the consumer taste panel indicate a preference for steak from BAN compared to BUL or INJ; however, it should be noted that almost all of the beef consumed in the U.S. derived from males is from steers, thus these consumers were unaccustomed to steak from bulls and likely has an effect on their perception of flavor attributes.

Cook Loss and Tenderness

Percentage cook loss of striploin steaks did not differ ($P = 0.47$) between treatments (Table 4). Cooking loss of steaks influences product yield as well as profitability (Kondjoyan et al., 2013). In agreement with the current study, Costa et al. (2007) reported no differences in cooking loss in steaks from crossbred (Nelore × Charolais) steers and bulls; Vaz and Restle (2000) reported similar results in Hereford bulls and steers. Warner-Bratzler shear force values did not differ ($P = 0.11$) between treatments (BAN = 3.7, BUL = 4.0, and INJ = 3.9 kgF) but numerical differences suggest slight increase in tenderness for BAN. Previous research on immunocastrated bulls concur with the current study as several studies have reported no differences in shear force values of beef from *B. taurus* in the feedlot due to castration or method (Cook et al., 2000; Miguel et al., 2014). The threshold of acceptable tenderness (< 4.55 kgF) utilized in the current study was based on findings reported by Tatum et al. (1999) and consensus established at the National Beef Tenderness Conference. Treatment did not affect ($P = 0.21$) the percent of WBSF values less than the threshold (< 4.55 kgF) of acceptable tenderness; however, 94.5, 85.2, and 81.8% of BAN, INJ, and BUL were less than the tenderness threshold, respectively. Conversely, Costa et al. (2007) reported steers to have significantly improved tenderness values compared to bulls. A larger sample size may have been required to statistically resolve the numerical differences in tenderness observed from the current study.

Cooked Color

Cooked color values of striploin steaks indicate that lightness values (L*) were not different ($P = 0.44$) between treatments (Table 4). Intact males typically have lesser L* values (fresh or cooked color) compared to castrates due to increased pH often seen in bulls compared to steers (Page et al., 2001); however, the current study does not agree with previous research. Redness values (a*) did not differ ($P = 0.23$) between castration treatments. Yellowness values (b*) of striploin steaks were greater ($P = 0.02$) for BAN and BUL than INJ which is likely due to more myoglobin oxidation in INJ. There was a tendency for chroma values to be greater ($P = 0.10$) in BUL compared to INJ whereas BAN was intermediate. There were no differences ($P = 0.59$) in hue angle values of striploin steaks. The ratio of red-to-brown (630:580 nm) of cooked striploin steaks was greater ($P = 0.05$) in INJ compared to either BAN or BUL. Red-to-brown ratio indicates reduced myoglobin denaturation in INJ compared to BAN or BUL. Internal cooked color is highly correlated to muscle pH and myoglobin concentration; greater muscle pH protects myoglobin from denaturation during cooking which results in an undercooked appearance (Trout, 1989; Hunt et al., 1999).

In conclusion, the percentage of cattle grading USDA Choice or better and overall consumer acceptability was increased for BAN compared to INJ and BUL, whereas INJ and BUL cattle had greater HCW, LM area, and crest height than BAN. Hence, BUL and INJ possessed similar carcass characteristics and consumer acceptability compared to BAN, resulting in disagreement with our hypothesis. Shear force, cooking loss, and instrumental cooked color values were minimally affected by castration or method of castration. The INJ treatment evaluated in the current study may be a viable option for sterilization, but not castration of more mature beef cattle upon feedlot entry because meat quality, consumer acceptability, and

testosterone concentrations were more similar to BUL than BAN. However, INJ may have value in a natural market setting that does not allow the use of growth implantation, places merit on carcass yield rather than quality, and where sterilization is desirable.

Table 1. Effect of castration and method of castration on USDA quality and yield grade in male beef cattle.

	Treatment[1]				
Item	BAN	BUL	INJ	SEM[2]	*P*-value
Quality grade, %					
Select or Standard	44.4[b]	71.7[a]	76.1[a]	6.4	<0.01
Choice	55.6[a]	28.3[b]	23.9[b]	6.4	<0.01
USDA Yield grade, %					
1	1.9	9.4	8.7	3.3	0.30
2	27.6[b]	37.4[ab]	59.1[a]	8.4	0.04
3	51.8[a]	28.2[b]	27.7[b]	7.7	0.06
4 or 5	17.0	23.1	4.2	6.4	0.13

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.
[2]Pooled standard error of the mean.
[a-b]Rows without common letter superscript differ, $P < 0.05$.

Table 2. Effect of castration and method of castration on carcass traits in male beef cattle.

	Treatment[1]				
Item	BAN	BUL	INJ	SEM[2]	*P*-value
HCW, kg	366[b]	414[a]	404[a]	3.7	<0.01
Dressed carcass yield, %	60.0	60.4	60.0	0.4	0.72
Marbling score[3]	415[a]	370[b]	361[b]	9.3	<0.01
Lean color score[4]	4.98	5.10	5.00	0.05	0.19
Fat thickness, cm	1.47	1.37	1.17	0.1	0.17
LM area, cm^2	87.7[b]	101.3[a]	98.1[a]	1.9	<0.01
KPH, %	2.03	2.03	2.03	0.03	0.99
Yield grade	3.32	3.15	2.81	0.16	0.12
Crest, cm	20.8[b]	25.1[a]	25.7[a]	1.0	0.01

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.

[2]Pooled standard error of the mean.

[3]300 = Slight[00]; 400 = Small[00]; 500 = Modest[00]

[4]Lean color score (brightness); 1 = light pink, 2 = pink, 3 = dark pink, 4 = light cherry red, 5 = cherry red, 6 = dark red, 7 = very dark red {1/3 dark cutter}, 8 = maroon {2/3 dark cutter}, 9 = dark maroon {full dark cutter}

[a-b]Rows without common letter superscript differ, $P < 0.05$.

Table 3. Effect of castration and method of castration on consumer taste panel outcomes in male beef cattle.

	Treatment[1]				
Item[2]	BAN	BUL	INJ	SEM[3]	*P*-value
Tenderness	6.68[a]	6.16[b]	6.34[ab]	0.1	0.02
Juiciness	6.67[a]	6.01[b]	6.08[b]	0.1	<0.01
Flavor	6.44[a]	6.41[a]	6.02[b]	0.1	0.01
Acceptability	6.53[a]	6.26[ab]	5.99[b]	0.1	<0.01
Off-Flavor, %	17.29[b]	16.13[b]	28.46[a]	2.4	<0.01
Off-Flavor Score	5.27	4.88	4.73	0.2	0.35

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.
[2]Hedonic scale: 1 = dislike extremely to 9 = like extremely.
[3]Pooled standard error of the mean.
[a-b]Rows without common letter superscript differ, $P < 0.05$.

Table 4. Effect of castration and method of castration on cook loss, tenderness, and cooked color in male beef cattle.

	Treatment[1]				
Item[2,3]	BAN	BUL	INJ	SEM[4]	*P*-value
Cook loss, %	22.2	21.2	22.3	0.7	0.47
WBSF[5], kg force	3.7	4.0	3.9	0.1	0.11
WBSF < 4.55 kg force, %	94.5	81.8	85.2	5.0	0.21
L*	57.5	56.8	57.7	0.5	0.44
a*	17.1	17.6	16.9	0.3	0.23
b*	16.8[a]	16.9[a]	16.4[b]	0.1	0.02
Chroma	24.1[ab]	24.4[a]	23.7[b]	0.3	0.10
Hue angle	44.7	44.0	44.4	0.5	0.59
Red-to-brown	2.2[b]	2.2[b]	2.4[a]	0.1	0.05

[1]BAN = bulls that received blood-restrictive rubber band placed upon the dorsal aspect of the scrotum, INJ = bulls that received 1 mL (100 mg Zn) of a Zn solution in each testis, BUL = bulls with testicles remaining intact.
[2]Cook loss = 100 – ((cooked weight/ raw weight) * 100); WBSF = mean kgF of 6 cores per steak.
[3]L^* = lightness, a^* = redness, b* = yellowness, Chroma = intensity of light, Hue angle = distance from true red axis, Red-to-brown = spectral ratio of 630:580.
[4]Pooled standard error of the mean.
[5]Warner-Bratzler shear force.
[a-b]Rows without common letter superscript differ, $P < 0.05$.

LITERATURE CITED

Ball, J. J., E. B. Kegley, T. E. Lawrence, S. L. Roberts, J. G. Powell, and J. T. Richeson. 2018. Zinc injection as a novel castration method in beef bulls: Effects on performance, behavior and testosterone and haptoglobin concentration. J. Anim. Sci. 96:890-901. https://doi.org/10.1093/jas/skx039

Calkins, C. R., D. C. Clanton, T. J. Berg, and J. E. Kinder. 1986. Growth, carcass and palatability traits of intact males and steers implanted with zeranol or estradiol early and throughout life. J. Anim. Sci. 62:625-631. doi:10.2527/jas1986.623625x.

Carroll, F. D., W. C. Rollins, K. A. Wagnon, and R. G. Loy. 1975. Comparison of beef from bulls and DES implanted steers. J. Anim. Sci. 41:1008-1013. doi:10.2527/jas1975.4141008x.

Cohen, R. D. H., B. D. King, E. D. Janzen, and H. H. Nicholson. 1991. The effect of castration age, method and implant regime on growth and carcass traits of male beef cattle. Can. J. Anim. Sci. 71:301-309.

Cook, R. B., J. D. Popp, J. P. Kastelic, S. Robbins, and R. Harland. 2000. The effects of active immunization against gnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J. Anim. Sci. 78:2778–2783. doi:10.2527/2000.78112778x.

Costa, C., P. R. L. Meirelles, S. Savastano, M. B. Arrigoni, A. C. Silveira, R. O. Roça, and G. B. Mourão. 2007. The impact of castration on the quality of yearling beef cattle meat. J. Vet. Med. Anim. Sci. 14:115-123.

Duckett, S. K., and S. L. Pratt. 2014. MEAT SCIENCE AND MUSCLE BIOLOGY SYMPOSIUM: Anabolic implants and meat quality. J. Anim. Sci. 92:3-9. doi:10.2527/jas2013-7088.

Field, R. A. 1971. Effects of castration on meat quality and quantity. J. Anim. Sci. 32: 849-858. doi:10.2527/jas1971.325849x.

Hunt, M. C., O. Sørheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64:847–851. doi:10.1111/j.1365-2621.1999.tb15925.x.

Kondjoyan, A., S. Oillic, S. Portanguen, and J. B. Gros. 2013. Combined heat transfer and kinetics model to predict cooking loss during heat treatment of beef meat. Meat Sci. 95:336-344.

Lamb, G. C., V. R. G. Mercadante, D. D. Henry, P. L. P. Fontes, C. R. Dahlen, J. E. Larson, and N. DiLorenzo. 2016. Invited Review: Advantages of current and future reproductive technologies for beef cattle production. Prof. Anim. Sci. 32:162-171. http://dx.doi.org/10.15232/pas.2015-01455.

Miguel, G. Z., M. H. Faria, R. O. Roca, C. T. Santos, S. P. Suman, A. B. G. Faitarone, N. L. C. Delbem, L. V. C. Girao, J. M. Homem, E. K. Barbosa, L. S. Su, F. D. Resende, G. R. Siquerira, A. D. Moreira, and T. V. Savian. 2014. Immunocastration improves carcass traits and beef color attributes in Nelore and Nelore × Aberdeen Angus crossbred animals finished in feedlot. Meat Sci. 96:884-891.

NAHMS. 2011. Part III. Trends in Health and Management Practices on U.S. Feedlots, 1994-2011. Page 37. Natl. Anim. Health Monit. Serv., Fort Collins, CO.

Oliveira, E. C. S., A. K. F. Fagundes, C. C. S. Melo, L. T. B. Nery, R. G. Revoredo, T. F. G. Andrade, K. Oliveira-Esquerre, J. P. Kastelic, and V. A. Silva Jr. 2013. Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety. Vet. J. 197:307-310.

Page, L. K., D. M. Wulf, and T. R. Schwotzer. 2001. A survey of beef muscle color and pH. J. Anim. Sci. 79: 678–687. doi:10.2527/2001.793678x.

Pérez-Linares, C., L. Bolado-Sarabia, F. Figueroa-Saavedra, A. Barreras-Serrano, E. Sánchez-López, A. R. Tamayo-Sosa, A. A. Godina, F. Ríos-Rincón, L. A. García, and E. Gallegos. 2017. Effect of immunocastration with Bopriva on carcass characteristics and meat quality of feedlot Holstein bulls. Meat Sci. 123:45-49. http://dx.doi.org/10.1016/j.meatsci.2016.08.006.

Price, M.A., S. D. M. Jones, G. W. Mathison, and R. T. Berg, 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. 60:345-358.

Scanga, J. A., K. E. Belk, J. D. Tatum, T. Grandin, and G. C. Smith. 1998. Factors contributing to the incidence of dark cutting beef. J. Anim. Sci. 76:2040-2047. doi:10.2527/1998.7682040x.

Tatum, J. D., K. E. Belk, M. H. George, and G. C. Smith. 1999. Identification of quality management practices to reduce the incidence of retail beef tenderness problems: Development and evaluation of a prototype quality system to produce tender beef. J. Anim. Sci. 77:2112–2118. doi:10.2527/1999.7782112x.

Tavarez, M. A., C. L. Puls, A. L. Schroeder, and A. C. Dilger. 2014. Effects of immunocastration and time after second Improvest dose on adipose tissue fatty acid profile on finishing barrows. J. Anim. Sci. 92:3736-3744.

Trout, G. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium triplyphosphate, and cooking temperature. J. Food Sci. 54:536–544. doi:10.1111/j.1365-2621.1989.tb04644.x.

USDA. 2016. National Summary of Meats Graded. https://www.ams.usda.gov/sites/default/files/media/FY2016GradeVolumeReport.pdf. (Accessed 12 September 2017).

Vaz, F. N., and J. Restle. 2000. Qualitative aspects of the carcass and meat of Hereford males, intact or castrated, slaughtered at fourteen months of age. Braz. J. Anim. Sci. 29:1894-1901.

CHAPTER VI

CARRY-OVER EFFECTS OF GROWTH PROMOTING IMPLANTS AT BRANDING OR WEANING ON SUBSEQUENT FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS IN BEEF STEERS

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ABSTRACT

Crossbred beef steers (n = 106; BW = 96 ± 3.9 kg; Age = 74 ± 2.0 d) were blocked by parity of dam (≤ 2 or > 2 parities), stratified by BW, calf age, calf sire, cow BW and BCS and were assigned randomly to 1 of 4 treatments: 1) RALG, administered Ralgro at branding (D 0), Ralgro at weaning (D 156), and Revalor XS at feedlot processing (D 325), 2) COMP, administered Component E-C at branding, Component TE-G at weaning, and Revalor XS at feedlot processing, 3) N-REV, no growth-promoting implant at branding, administered Revalor-G at weaning, and Revalor XS at feedlot processing, and 4) CTRL, no growth-promoting implants administered during any phase of production. Body weights were recorded periodically throughout the study, blood was collected on a subset of calves until feedlot processing, and carcass data were collected on all calves. Quantitative data were analyzed using a mixed model and qualitative data were analyzed using the Glimmix procedure of SAS. At weaning on d 156, there was no main effect (P = 0.19) of implant treatment; however, there was a tendency (P = 0.08, Implant vs. none orthogonal contrast) for greater BW in implanted steers (RALG = 242, COMP = 236 kg) compared to non-implanted steers (CTRL = 225 kg) at weaning. Overall ADG during calfhood (d 0 to 156) was greater (P = 0.03) in RALG (0.91 kg) and COMP (0.90 kg) compared to CTRL (0.82 kg). Implantation of male calves at branding, regardless of implant type, increased growth performance prior to weaning. At the end of the stocker phase on d 323, RALG (330 kg), COMP (324 kg), and N-REV (318 kg) were heavier (P = 0.02) compared to CTRL (297 kg). Overall stocker ADG from d 156 (weaning) to 323 (feedlot shipment) was greater (P < 0.01) in N-REV (0.50 kg) and COMP (0.46 kg) compared to CTRL (0.38 kg) while RAL (0.43 kg) did not differ (P > 0.05). Steers implanted with a growth implant containing 8 mg estradiol and 40 mg trenbolone acetate at weaning that had not previously been implanted at

branding (N-REV) gained the most during the stocker phase compared to steers that had been previously implanted at branding (RALG and COMP) although steers implanted twice had a 6 to 12 kg BW advantage at time of feedlot shipment.

Key words: BUN, Growth implant, NEFA, Steers

INTRODUCTION

Growth-promoting implants are often used by U.S. beef producers to increase rate of gain and efficiency of growth; however, the adoption of growth technologies varies greatly within sectors of the beef industry. Only 11.9% of cow/calf operations utilize the practice of growth-promoting implants prior to weaning (NAHMS, 2008); whereas, over 91% of feedlots implanted steers weighing less than 318 kg at least once during the finishing phase and over 79% of steers implanted received 2 or more implants during the finishing period (NAHMS, 2013). The adoption of growth-promoting implant varies; however, they are approved for use in cattle of all ages to enhance growth during nursing, growing, and finishing phases of production. Although benefits of growth-promoting implants are well documented, repetitive use of implants through multiple phases of the beef production cycle may be detrimental to meat tenderness and carcass quality (Tatum, 1993; Morgan, 1997; Roeber et al., 2000). Duckett and Andrae (2001) summarized that nursing and stocker-phase implants have minimal carryover effects on subsequent finishing and carcass performance. However, in an extensive study by Platter et al. (2003) on the repetitive use of various growth implants on beef carcass quality found that lifetime implant protocols increased growth performance, instrumental tenderness, and hot carcass weight; however, marbling score and desirability of consumers was reduced. The effects of previous growth implantation is well documented; however, concerns exist regarding the efficacy growth implants administered multiple times in varying production sectors compared to

cattle implanted only during the finishing phase is of great interest within the industry as consumers begin to mold cattle production practices.

The objective of the study was to evaluate commercial growth-promoting implants at branding or weaning and the effect of previous implantation at branding, weaning, or both on subsequent growth performance, blood urea nitrogen concentrations (**BUN**), non-esterified fatty acids (**NEFA**), and carcass characteristics in beef steers. The authors hypothesized that previous implantation would improve final BW at time of harvest; however, feedlot ADG would be greater in calves implanted only once prior and that carcass characteristics would not be affected by previous implantation.

MATERIALS AND METHODS

Animal care and use procedures were approved by the University of Arkansas institutional animal care and use committee prior to study initiation (Approval # 17043). Crossbred beef bull calves (n = 106; BW = 96 ± 3.9 kg) from the University of Arkansas Cow-Calf unit located near Fayetteville, AR were used to determine the effect of implant strategy on growth performance, BUN and NEFA concentrations, and carcass characteristics. At traditional branding time (Age = 74 ± 2.0 d), and to coincide with insertion of controlled internal drug-release (CIDR, Zoetis, Kalamazoo, MI) in dams, bull calves and their dams were separated from other cow-calf pairs at the University of Arkansas Division of Agriculture Cow-Calf Unit. Bull calves were blocked into 2 groups by dam parity (≤ 2 or > 2 parities) and managed as such for study duration.

On d -7, bull calves were weighed, tested for the prevalence of persistent infection with bovine viral diarrhea virus (**PI-BVDV**) via ear notch (Cattle Stats, Oklahoma City, OK), vaccinated against clostridial (5 mL SQ, Covexin 8; Merck Animal Health, Madison, NJ) and

respiratory (2 mL SQ, Titanium 5+PH-M; Elanco Animal Health, Greenfield, IN) pathogens, and were castrated. Bulls were castrated using a surgical technique with the removal of the bottom third of the scrotum, testes were then pulled from inside the scrotum, and the spermatic cord was severed with a scalpel. Dams were weighed and a body condition score (**BCS**) was assigned on d -7. All calves were negative for PI-BVDV. Within block, calves were stratified by d -7 BW, calf age, calf sire, cow BW and BCS and were assigned randomly to 1 of 4 treatments: 1) RALG, administered Ralgro (Merck Animal Health, 36 mg zeranol) at branding (d 0), Ralgro at weaning (d 156), and Revalor XS (Merck Animal Health, 200 mg trenbolone acetate and 40 mg estradiol) at feedlot processing (d 325), 2) COMP, administered Component E-C (Elanco Animal Health, 100 mg progesterone and 10 mg estradiol with 29 mg tylosin tartrate) at branding (d 0), Component TE-G (Elanco Animal Health, 40 mg trenbolone acetate, 8 mg estradiol, and 29 mg tylosin tartrate) at weaning (d 156), and Revalor XS (Merck Animal Health, 200 mg trenbolone acetate and 40 mg estradiol) at feedlot processing (d 325), 3) N-REV, no growth-promoting implant at branding (d 0), administered Revalor-G (Merck Animal Health, 40 mg of trenbolone acetate and 8 mg estradiol) at weaning (d 156), and Revalor XS (Merck Animal Health, 200 mg trenbolone acetate and 40 mg estradiol) at feedlot processing (d 325), and 4) CTRL, no growth-promoting implants administered during any phase of production (Table 1). Experimental treatments were administered on d 0 with an implantation apparatus designed for each specific implant. Implants were placed subcutaneously in the center one-third of the posterior aspect of the pinna of the ear. Dry ears were implanted without cleaning and ears that were wet or contaminated with manure or mud were scrubbed with a solution of chlorhexidine (Nolvasan, Zoetis) before implanting. The stylets on each implantation apparatus were disinfected in chlorhexidine solution after each animal was treated.

Steers were weighed on d 0 (branding), 57, 98, 156 (weaning), 170, 198, 233, 268, 303, 323 (feedlot shipment), 325 (feedlot arrival), 353, 381, and 409. A subset of 48 steers (4 steers/treatment/block) were selected randomly for repeated blood sampling to assess BUN and NEFA concentrations through weaning. Periodic blood sampling occurred on d 0, 57, 98, 156, 198, 233, 268, 303, and 323. Blood was collected (approximately 7 mL) via jugular venipuncture into evacuated tubes (BD Inc., Franklin Lakes, NJ) and sera were harvested to determine BUN and NEFA content concentrations. After blood was collected into the plain vacuum tube, it was allowed to clot, then centrifuged at 2,060 × g for 20 min and serum was decanted into duplicate aliquots and stored at -20°C for subsequent analysis. A serum aliquot was analyzed for BUN using a commercially available colorimetric assay kit (Teco Diagnostics, Anaheim, CA) with an inter- and intra-assay CV of 5.9 and 2.8%, respectively. Nonesterified fatty acid concentrations were analyzed using a commercially available colorimetric assay kit (Wako Chemicals USA Inc., Richmond, VA) with an inter- and intra-assay CV of 9.3 and 5.1%, respectively.

Steers were reared with their dams within block for the entirety of calfhood. Mature cows (> 2 parities) and steers were rotationally grazed in large pastures of varying sizes consisting of primarily toxic endophyte infected tall fescue and bermudagrass as well as supplemented hay when forages became limiting (Table 2). Similarly, young cows (≤ 2 parities) and their steers were rotationally grazed in large pastures of varying sizes consisting of primarily toxic endophyte infected tall fescue, triticale, and bermudagrass as well as supplemented hay when forages became limiting. Young cows were also supplemented 0.5 to 1% BW of corn gluten pellets until weaning to increase body condition to increase likelihood of re-breeding (Table 2). Three steers died during calfhood and deaths were not related to treatment, these were subsequently removed from the data set thereafter (RALG = 1, and N-REV = 2).

At weaning (d 156), steers were removed from dams and weaned via fenceline weaning such that steers and cows were adjacent to one another between pastures. On d 156, steers were implanted depending on treatment as previously described. Steers were also vaccinated on d 156 (weaning) for respiratory (2 mL subcutaneous (**SQ**), Titanium 5, Elanco) and clostridial pathogens (5 mL SQ, Covexin 8, Merck), a pour-on insecticide applied for horn flies (15 mL/steer, StandGuard, Elanco), dewormed (6 mL SQ Block 1 and 5 mL SQ Block 2, Dectomax, Zoetis). On d 170, steers were revaccinated against clostridial (Covexin 8, Merck) and respiratory (Titanium 5, Elanco) pathogens. Steers were moved to 2 separate 10-ha pastures consisting of primarily toxic endophyte-infected tall fescue and bermudagrass based on calfhood block assignments and were offered ad libitum access to bermudagrass hay and water. Steers were supplemented 0.5% BW of corn gluten pellets each d from weaning (d 156) to feedlot shipment (d 323) based on the most recent mean BW determined within block. During the stocker phase, 70 steers were treated for pinkeye and 5 were treated for footrot per standard operating procedures of the unit. Two steers were removed from study during the stocker phase due to lameness not related to treatment (RALG = 1, and COMP = 1).

At the completion of the stocker phase (d 323), cattle were shipped 798 km to the West Texas A&M University (**WTAMU**) Research Feedlot on d 324. Upon arrival (d 325), steers were processed at the WTAMU Research feedlot. Cattle were vaccinated against clostridial pathogens and *H. somnus* (5 mL SQ, Bar-Vac 7/Somnus, Boehringer Ingelheim, St. Joseph, MO), dewormed (1 mL/ 34 kg BW SQ, Noramectin, Norbrook Inc., Overland Park, KS) and steers that were implanted at weaning (d 156) were implanted again at feedlot processing with Revalor-XS (Merck). Revelor-XS is a time release, long-acting growth implant. Therefore, RALG, COMP, and N-REV were implanted at feedlot processing while CTRL was not.

Steers were fed in 2 pens by block for the duration of the feedlot phase; therefore, DMI and G:F were not attainable as treatments were commingled within block. Steers were harvested on 2 separate d by block according to their final BW and visual appraisal by trained personnel for market readiness. Block 1 (dams >2 parities) was harvested (USDA Establishment M245E, Amarillo, TX) on d 465 and block 2 (dams ≤ 2 parities) was harvested on d 493. Carcasses were evaluated after a 24-h chill period. Harvest floor data, and lean color score at the 12th rib were recorded by trained personnel (blinded to treatment) from the WTAMU Beef Carcass Research Center (Canyon, TX). The USDA quality and yield grades as well as LM area and preliminary yield grade were determined by a vision camera system (VBG 2000; E+V Technology GmbH, Oranienberg, Germany).

Statistical analyses were conducted for all outcome variables in a randomized complete block design. Data were tested for normality using PROC UNIVARIATE and nonparametric data were log-transformed prior to analysis if normality was improved. Performance, and quantitative carcass measurements were analyzed using the mixed models procedure (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC) with treatment as fixed effect and block as the random effect. Serum BUN and NEFA concentrations were log-transformed and used the MIXED procedure with repeated measures. Categorical carcass data were analyzed using PROC GLIMMIX of SAS. Orthogonal contrasts were used to compare implanted vs. non-implanted steers for each production phase. Calf was the experimental unit for all dependent variables analyzed. Means were separated at an α of 0.05 using the PDIFF option in SAS. Statistical significance was declared at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$ for all dependent variables.

RESULTS AND DISCUSSION

Calfhood Phase

Due to N-REV and CTRL both not being implanted during the calfhood phase and thus, being treated the same, their means were pooled to represent steers that had not been implanted and were denoted as CTRL for each dependent variable only through calfhood.

On d 0 when branding age growth-promoting implants were administered in RALG and CTRL, BW was not different ($P = 0.90$) between treatments (Table 3; RALG = 98, COMP = 96, and CTRL = 96 kg). There was no effect ($P = 0.87$) of calfhood growth-promoting implant on d 57 BW. On d 98, RALG and COMP began to separate in terms of BW from CTRL (8 and 7 kg increase, respectively); however, there was no statistical difference ($P = 0.53$) at this time. At weaning on d 156, there was no main effect ($P = 0.19$) of treatment; however, there was a tendency ($P = 0.08$) for greater BW in implanted steers (RALG = 242, COMP = 236 kg) compared to non-implanted steers (CTRL = 225 kg) at weaning. It is important to note that at weaning, RALG and COMP had BW $\geq$ 11 kg compared to either of the treatments yet to receive a growth-promoting implant. Similar results were reported by Bayliff et al. (2017) where suckling calves were implanted with Ralgro at branding and Revalor-G at weaning or not implanted at all and results indicated that by weaning that calves implanted with Ralgro had 3.2% (8 kg) increase in weaning weight compared to non-implanted controls. There was a limited number of animals available for this study and thus the replication of the study was limited as well a wide variation in BW from d 0 (SEM = 4.1 kg) to weaning on d 156 (SEM = 7.0 kg) increasing the likelihood of a type II error occurring. Both RALG and COMP currently cost $1.37/dose, and that investment would yield cow-calf producers 11 to 17 kg BW advantage (4.6 to 7.5 % BW increase) compared to non-implanted steers at weaning. This is important as

according to the NAHMS (2008), only 9.4% of cow-calf producers administer growth-promoting implants in non-replacement calves prior to weaning, and this percentage is reported to continually decline in recent years (Seeger et al., 2011).

Average daily gain was not affected ($P = 0.59$) by growth-promoting implant treatment from d 0 to 57 (Table 3). From d 57 to 98, ADG was greater ($P = 0.04$) in implanted steers at branding compared to non-implanted steers. There was an increase ($P = 0.05$) in ADG from d 98 to 156 in RALG and COMP compared to CTRL. Overall ADG during calfhood (d 0 to 156) was greater ($P = 0.03$) in RALG (0.91 kg) and COMP (0.90 kg) compared to CTRL (0.82 kg). Therefore, an 0.09 kg increase in ADG during calfhood (156 d) for steers implanted compared to steers that were not implanted. Average daily gain may be a better indicator of growth-promoting implant efficacy as Selk (1997) suggests ADG is the most accurate measurement as steers vary in terms of age at time of weaning.

A treatment × day interaction was not detected ($P = 0.27$) for serum BUN concentrations during calfhood (Table 4). A main effect of treatment was noted ($P = 0.01$) as CTRL had greater serum BUN concentration during calfhood compared to COMP while RALG was intermediate. A main effect of day was also detected for serum BUN concentrations on d 156 (weaning) BUN concentrations were greatest ($P < 0.01$), followed by d 57 and 98, the least serum BUN concentrations were observed on d 0. Increased serum BUN concentrations is highly correlated with dietary protein intake and greater BUN is expected as BW and plane of nutrition increase. There was not a treatment × day interaction ($P = 0.86$) for serum NEFA concentrations. Furthermore, there was no main effect ($P = 0.54$) of implant treatment on serum NEFA concentrations. There was however, a main effect ($P < 0.01$) of day for serum NEFA concentrations as d 57 was greatest, followed by d 98, and then d 0 and 156 were the least.

Stocker phase

At weaning on d 156, there was an increase ($P = 0.08$) in BW in implanted steers compared to non-implanted steers; however, it is important to note on d 156 RALG (Ralgro) and COMP (Component TE-G) were re-implanted, N-REV (Revalor G) was implanted for the first time, and CTRL were not implanted (Table 5). At revaccination on d 170, there was no effect ($P = 0.40$) of treatment nor of orthogonal contrast of implant vs. none ($P = 0.21$). On d 198, RALG (284 kg) had a greater ($P = 0.04$) BW compared to N-REV (264 kg) and CTRL (257); COMP (278 kg) was not different ($P > 0.05$) from any other treatment. Body weights on d 233 and 268 followed a similar pattern as RALG (303 kg) tended to have a greater ($P = 0.09$) BW compared to CTRL (276 kg) on d 233 and was greater ($P = 0.04$) on d 268. On d 303, RALG (331 kg) and COMP (323 kg) were heavier ($P = 0.05$) compared to CTRL; whereas, N-REV was 19 kg heavier than CTRL but N-REV was statistically similar ($P > 0.05$) to all other treatments. Implanted steers on d 303 were heavier ($P = 0.01$) compared to CTRL. At the end of the stocker phase on d 323, RALG (330 kg), COMP (324 kg), and N-REV (318 kg) were heavier ($P = 0.02$) compared to CTRL (297 kg). Steers that were implanted once (N-REV) at weaning were 21 kg heavier than CTRL; whereas, steers that were implanted twice (branding and weaning) were 33 (RALG) and 27 kg (COMP) heavier. Although not statistically different, steers implanted previously at branding (RALG and COMP) maintained a 12 and 6 kg BW advantage, respectively, compared to steers implanted once at weaning (N-REV).

Average daily gain from d 156 (weaning) to 170 (revaccination) was not affected ($P = 0.39$) by treatment or by administration of growth-promoting implant at weaning (Table 5, $P = 0.49$). From d 170 to 198 and d 198 to 233, ADG was not different ($P \geq 0.30$) between treatments. However, ADG was improved ($P = 0.01$) from d 233 to 268 in the steers that were

implanted compared to the non-implanted CTRL. There was no effect ($P = 0.33$) of treatment on steer ADG from d 268 to 303. Average daily gain from d 303 to 323 was greater ($P = 0.01$) in COMP compared to RALG and CTRL while N-REV was not different between any treatment. Due to severe heat and drought conditions during the study, ADG from d 268 to 303 and d 303 to 323 (feedlot shipment) was < 0.20 kg/d regardless of treatment. Overall stocker ADG from d 156 (weaning) to 323 (feedlot shipment) was greater ($P < 0.01$) in N-REV (0.50 kg) and COMP (0.46 kg) compared to CTRL (0.38 kg) while RALG (0.43 kg) was not different from any other treatment. Steers implanted with a growth implant at weaning that had not previously been implanted at branding (N-REV) gained the most efficiently during the stocker phase compared to steers that had been previously implanted at branding (RAL and COM) although steers implanted twice had a 6 to 12 kg BW advantage at time of feedlot shipment.

There was no treatment × day interaction ($P = 0.45$) for serum BUN concentrations during the stocker phase portion of the current study (Table 6). A main effect of treatment was not detected ($P = 0.75$) for serum BUN concentrations during the stocker phase. There was a main effect ($P < 0.01$) of day for serum BUN concentrations as d 156 (weaning) was the greatest compared to d 303 and d 233 was the least. A treatment × day interaction for serum NEFA concentrations was not detected ($P = 0.99$) during the stocker phase. Similar to serum BUN, there was not a main effect ($P = 0.85$) of treatment for serum NEFA concentrations. However, there was a main effect ($P < 0.01$) of day for serum NEFA concentrations as d 156 had the greatest serum NEFA concentration and d 323 was the least serum NEFA concentrations compared to all other d. During times of stress such as weaning, the mobilization of adipose tissue occurs to aid in energy maintenance needed to combat a negative energy balance and a

reduction in immunity in stressed steers which is evident by the greater serum NEFA concentration at weaning compared to the end of the stocker phase.

Feedlot phase

Body weights upon feedlot arrival were greater ($P = 0.02$) in RALG (331 kg), COMP (332 kg), and N-REV (321 kg) compared to the non-implanted CTRL (299 kg, Table 7). Thus at the beginning of the feedlot phase, steers implanted once previously during weaning (N-REV) were 22 kg heavier and steers that were implanted twice previously at branding and weaning (RALG and COMP) were 32 and 33 kg heavier, respectively. On d 353, 28 d into the feedlot phase, RALG, COMP, and N-REV maintained a greater ($P = 0.01$) BW compared to CTRL. Body weights on d 381 were markedly greater ($P < 0.01$) in RALG (454 kg), COMP (449 kg), and N-REV (437 kg) compared to CTRL (398 kg). There was an increase ($P < 0.01$) in BW on d 409 as RALG, COMP, and N-REV were heavier compared to CTRL. Implanted calves regardless of implant or when implanted, were heavier ($P < 0.01$) on d 437 and d 455 compared to non-implanted calves.

Feedlot ADG from d 325 to 353 was not affected ($P = 0.22$) by treatment; however, implanted steers tended to have a greater ($P = 0.06$, implant vs. none orthogonal contrast) ADG from d 325 to 353 compared to non-implanted steers (Table 7). Average daily gain was greater ($P < 0.01$) from d 353 to 381 in RALG (2.27 kg), COMP (2.18 kg), and N-REV (2.13 kg) compared to CTRL (1.70 kg). There was an increase ($P < 0.01$) in ADG from d 381 to 409 as RALG, COMP, and N-REV gained more efficiently compared to CTRL. Average daily gain was increased ($P < 0.01$) from d 409 to 437 in COMP compared to either N-REV or CTRL. From d 437 to 455, ADG was greater ($P = 0.02$) in all implanted calves (RALG, COMP, and N-REV) compared to CTRL.

IMPLICATIONS

This study was not able to differentiate efficacy between implant formulations or frequency. At time of harvest, steers that were implanted at branding regardless of implant (RALG and COMP) were numerically heavier compared to steers first implanted at weaning (N-REV); however, with limited replications, the difference was not significant. This study corroborates many other studies that growth-promoting implants are efficacious in improving weight gain in steers. Implanting steers within different sectors (branding or weaning) may not negatively affect growth performance as steers move to the next phase of production, which is an industry dogma that cattle have the greatest response to a growth implant after the first administration of an implant and all subsequent implants have reduced potency compared to the first.

Table 1. Experimental treatments at branding, weaning, and feedlot processing.

	Treatment[1]			
Item	RALG	COMP	N-REV	CTRL
Production phase				
Branding	Ralgro	Component E-C	_____	_____
Stocker	Ralgro	Component TE-G	Revalor G	_____
Feedlot	Revalor XS	Revalor XS	Revalor XS	_____

[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[2]Pooled standard error of the mean.

Table 2. Analyzed nutrient composition of diets, DM basis

	Calfhood		
Nutrient	Corn gluten	Forage	Hay
DM, %	89.8 ± 1.3	24.0 ± 2.7	90.4 ± 0.3
CP, %	21.8 ± 0.5	19.4 ± 2.2	10.8 ± 0.7
NDF, %	43.1 ± 1.0	64.4 ± 4.8	72.4 ± 1.2
ADF, %	10.3 ± 0.4	31.5 ± 3.0	40.5 ± 1.6
Ash, %	11.0 ± 0.1	21.0 ± 1.4	15.1 ± 1.7
Cu, mg/kg	5.3 ± 0.2	10.9 ± 0.7	8.3 ± 0.5
Zn, mg/kg	86.9 ± 2.3	53.4 ± 6.1	57.7 ± 7.1
Mn, mg/kg	13.8 ± 0.5	125.4 ± 15.4	107.3 ± 26.9
Fe, mg/kg	121.7 ± 3.8	314.7 ± 49.4	142.5 ± 32.9
B, mg/kg	6.9 ± 0.02	4.9 ± 0.4	6.1 ± 1.2
Ca, %	0.03 ± 0.003	0.5 ± 0.02	0.4 ± 0.04
P, %	1.1 ± 0.02	0.4 ± 0.03	0.4 ± 0.03
K, %	1.4 ± 0.01	2.4 ± 0.4	2.4 ± 0.2
Mg, %	0.4 ± 0.006	0.2 ± 0.01	0.3 ± 0.06
S, %	0.4 ± 0.008	0.2 ± 0.02	0.2 ± 0.02
Na, %	0.1 ± 0.008	0.02 ± 0.006	0.04 ± 0.005

Table 3. Effect of implantation regimen on growth performance in beef steers during calfhood.

Item	Treatment[1]			SEM[2]	*P*- value	
	RALG	COMP	CTRL		Treatment	Implant vs. none
Body weight, kg						
D -7	93	93	92	4.0	0.98	0.89
D 0	98	96	96	4.1	0.90	0.76
D 57	130	128	127	4.5	0.87	0.66
D 98	167	166	159	5.7	0.53	0.27
D 156	242	236	225	7.0	0.19	0.08
Average daily gain, kg						
D 0 to 57	0.56	0.56	0.56	0.03	0.59	0.31
D 57 to 98	0.90	0.92	0.79	0.05	0.11	0.04
D 98 to 156	1.25[a]	1.21[ab]	1.12[b]	0.04	0.05	0.02
D 0 to 156	0.91[a]	0.90[a]	0.82[b]	0.03	0.03	0.01

[a-b]Rows without common letter superscripts differ, $P < 0.05$.
[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[2]Pooled standard error of the mean.

Table 4. Effect of implantation at branding on serum BUN and NEFA concentrations in beef steers during calfhood.

Item	Treatment[1]			SEM[2]	*P*- value		
	RALG	COMP	CTRL		Treatment	Day	Treatment × day
BUN, mg/dL							
D 0[c]	6.8	7.0	7.8				
D 57[b]	10.2	9.4	9.8				
D 98[b]	8.6	9.2	10.2				
D 156[a]	14.6	13.9	15.3	0.7	-	<0.01	0.27
Main effect[3]	10.1[ab]	9.3[b]	10.8[a]	0.4	0.01		
NEFA, μg/L							
D 0[c]	423.9	427.4	423.2				
D 57[a]	585.0	608.6	599.4				
D 98[b]	485.1	576.1	547.3				
D 156[c]	421.8	424.6	470.2	43.2	0.54	<0.01	0.86

[a-c]Colums without common letter superscripts differ, Main effect of day, $P < 0.05$.
[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[3]Main effect of treatment; COM less than CON, $P < 0.01$.

Table 5. Effect of implantation regimen on body weight in beef steers during the stocker phase.

Item	Treatment[1]				SEM[2]	*P*- value	
	RALG	COMP	N-REV	CTRL		Treatment	Implant vs. none
Body weight, kg							
D 156	242	236	225	224	7.8	0.34	0.08
D 170	248	242	235	230	7.7	0.40	0.21
D 198	284[a]	276[ab]	264[b]	257[b]	7.0	0.04	0.04
D 233	303[a]	292[ab]	283[ab]	276[b]	7.9	0.09	0.06
D 268	331[a]	320[ab]	314[ab]	298[b]	8.0	0.06	0.02
D 303	331[a]	323[a]	318[ab]	299[b]	8.1	0.05	0.01
D 323	330[a]	324[a]	318[a]	297[b]	7.9	0.02	<0.01
Average daily gain, kg							
D 156 to 170	0.44	0.46	0.69	0.44	0.11	0.39	0.49
D 170 to 198	0.84	0.78	0.75	0.71	0.05	0.33	0.18
D 198 to 233	0.55	0.46	0.54	0.46	0.04	0.30	0.24
D 233 to 268	0.70[ab]	0.72[a]	0.82[a]	0.57[b]	0.05	0.02	0.01
D 268 to 303	0.07	0.13	0.19	0.10	0.04	0.33	0.63
D 303 to 323	-0.09[b]	0.16[a]	0.06[ab]	-0.12[b]	0.07	0.01	0.05
D 156 to 323	0.43[bc]	0.46[ab]	0.50[a]	0.38[c]	0.02	<0.01	<0.01

[a-b]Rows without common letter superscripts differ, $P < 0.05$.
[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[2]Pooled standard error of the mean.

Table 6. Effect of implantation at weaning on serum BUN and NEFA in beef calves until feedlot entry.

Item	Treatment[1]				SEM[2]	*P*- value		
	RALG	COMP	N-REV	CTRL		Treatment	Day	Treatment × day
BUN, mg/dL[3]								
D 156[a]	14.6	13.9	14.4	16.1				
D 198[b]	11.4	11.6	11.1	11.8				
D 233[c]	9.9	11.0	9.2	10.0				
D 268[ab]	12.7	13.1	12.5	12.8				
D 303[b]	13.5	12.4	11.7	11.0				
D 323[ab]	12.9	13.5	12.8	11.7	0.8	0.75	<0.01	0.45
NEFA, μg/L								
D 156[a]	421.8	424.6	510.6	433.1				
D 198[c]	271.0	268.5	298.4	274.1				
D 233[bc]	286.8	305.3	323.0	288.5				
D 268[c]	244.5	295.5	254.8	354.1				
D 303[b]	307.6	340.1	326.2	329.7				
D 323[d]	178.5	203.3	190.6	204.9	30.7	0.85	<0.01	0.99

[a-d]Columns without common letter superscripts differ, Main effect of day, $P < 0.05$.
[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[2]Pooled standard error of the mean for the interaction.

Table 7. Effect of implantation regimen on average daily gain in beef steers during the feedlot phase.

Item	Treatment[1]				SEM[2]	*P*- value	
	RALG	COMP	N-REV	CTRL		Treatment	Implant vs. none
Body weight, kg							
D 325	331[a]	332[a]	321[a]	299[b]	8.0	0.02	<0.01
D 353	390[a]	388[a]	377[a]	350[b]	9.1	0.01	<0.01
D 381	454[a]	449[a]	437[a]	398[b]	9.9	<0.01	<0.01
D 409	520[a]	514[a]	499[a]	453[b]	10.5	<0.01	<0.01
D 437	569[a]	566[a]	546[a]	491[b]	11.4	<0.01	<0.01
D 455	610[a]	602[a]	591[a]	531[b]	11.2	<0.01	<0.01
Average daily gain, kg							
D 325 to 353	2.27	2.18	2.12	1.94	0.11	0.22	0.06
D 353 to 381	2.27[a]	2.18[a]	2.13[a]	1.70[b]	0.09	<0.01	<0.01
D 381 to 409	2.30[a]	2.24[a]	2.16[a]	1.87[b]	0.08	<0.01	<0.01
D 409 to 437	1.83[ab]	1.98[a]	1.75[b]	1.50[c]	0.08	<0.01	<0.01
D 437 to 455	1.61[a]	1.62[a]	1.68[a]	1.36[b]	0.08	0.02	<0.01

[a-c]Rows without common letter superscripts differ, $P < 0.05$.
[1]RALG = administered Ralgro at branding (D 0), Ralgro at weaning (D 156) and Revalor XS upon feedlot entry (D 325), COMP = administered Component E-C at branding (D 0), Component TE-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), N-REV = no growth-promoting implant at branding (D 0) and administered Revalor-G at weaning (D 156) and Revalor XS upon feedlot entry (D 325), and CTRL = no growth-promoting implants administered during any phase of production.
[2]Pooled standard error of the mean.

LITERATURE CITED

Bayliff, C. L., M. D. Redden, J. R. Cole, A. L. McGee, C. Stansberry, M. E. Corrigan, W. Burdett, and D. L. Lalman. 2017. Effects of Ralgro at branding and Revalor-G at weaning on growth performance of steer calves. Prof. Anim. Sci. 33:108-112. DOI: http://dx.doi.org/10.15232/pas.2016-01519

Duckett, S. K., and J. G. Andrae. 2001. Implant strategies in an integrated beef production system. J. Anim. Sci. 79(E-Suppl.):E110–E117.

Morgan, J. B. 1997. Implant program effects on USDA beef carcass quality grade traits and meat tenderness. Pages 147–154 in Proc. OSU Symp.: Impact of Implants on Performance and Carcass Value of Beef Cattle, Oklahoma Agric. Exp. Stn., Stillwater, OK.

NAHMS. 2008. Beef 2008–2008. Part 1, Reference of beef cow/calf management practices in the United States, 2007–2008. National Animal Health Monitoring System. USDA. Accessed Jan. 8 2018. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartI_rev.pdf.

NAHMS. 2013. USDA National Animal Health Monitoring System report on the use of growth- promoting implants in US Feedlots. Accessed Jan. 8 2018. https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_is_Implant.pdf

Platter, W. J., J. D. Tatum, K. E. Belk, J. A. Scanga, and G. C. Smith. 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. J. Anim. Sci. 81:984-996.

Roeber, D. L., R. C. Cannell, K. E. Belk, R. K. Miller, J. D. Tatum, and G. C. Smith. 2000. Implant strategies during feeding: Impact on carcass grades and consumer acceptability. J. Anim. Sci. 78:1867–1874.

Seeger, J. T., M. E. King, D. M. Grotelueschen, G. M. Rogers, and G. S. Stokka. 2011. Effect of management, marketing, and certified health programs on the sale price of beef calves sold through a livestock video auction service from 1995 through 2009. J. Am. Vet. Med. Assoc. 239:451‑466.

Selk, G. 1997. Implants for suckling steer and heifer calves and potential replacement heifers. Pages 40‑49 in Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle. P-957. Oklahoma Agric. Exp. Stn., Stillwater, OK.

Tatum, J. D. 1993. The effects of anabolic implants on beef quality traits. Meat Focus Int. 2:71–74.

CHAPTER VII

CONCLUSION

In young bulls at branding, there were no differences in body weight, serum testosterone concentration, and scrotum and testis thickness due to the concentrations of Zn solution used, and the injectable castration method resulted in similar serum testosterone concentrations to calves that had been surgically castrated. Serum testosterone concentration and scrotum and testes thickness were greater in intact bulls than all injectable castrates at weaning. Injection of Zn in feedlot bulls appeared to eliminate spermatogonia and degenerate testes such that they were determined to be reproductively unviable with an overall absence of definable sperm formation and maturation, as the head of the epididymis of INJ lacked stored sperm based upon histopathological observation. Collectively, results from this experiment indicate that the INJ treatment may be a viable option for sterilization, but not castration of older beef cattle because testosterone concentrations were more similar to BUL than BAN. The INJ treatment evaluated in the current study may be a viable option for sterilization, but not castration of older beef cattle because meat quality, consumer acceptability, and testosterone concentrations were more similar to BUL than BAN. However, INJ may have value in a natural market setting that does not allow the use of growth implantation, places merit on carcass yield rather than quality, and where sterilization is desirable. Growth-promoting implants in beef cattle work and are efficacious in increasing BW and ADG; however, the current study was not able to differentiate efficacy between implant formulations or frequency.

CHAPTER VIII

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Dr. Paul Beck

FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee (IACUC)

DATE: September 8, 2014

SUBJECT: IACUC APPROVAL
Expiration date: September 7, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED protocol 14062: *'Using Complementary Forages and Grazing Management to Improve Efficiency of Cow-Calf Operations'*
In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing(via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond September 7, 2017 you must submit a new protocol prior to that date. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

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UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Beth Kegley

FROM: Craig N. Coon, Chairman

DATE: 9/14/15

SUBJECT: IACUC Approval

Expiration Date: Sep 27, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 16018 "Evaluation of treatment protocols for bovine respiratory disease in high-risk, newly received beef calves" to begin September 28, 2015.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Sep 27, 2018 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Jeremy Powell
FR: Craig Coon
Date: December 14th, 2016
Subject: IACUC Approval
Expiration Date: December 12th, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17043:** *Comparison of production management strategies in male beef calves.*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond December 12th, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Beth Kegley, Jeremy Powell, Jase Ball, Peter Hornsby, and Toby Lester. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem