Zinc injection as a novel castration method in beef bulls: effects on performance, behavior, and testosterone and haptoglobin concentration

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ABSTRACT: Crossbred beef bulls ($n = 180$) were blocked by initial BW (337 ± 10.9 kg; six blocks) and assigned randomly to one of three treatments on day 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 (three treatment pens per block and 10 cattle per pen). A subset of 54 animals ($n = 3$ per pen) was fitted with accelerometers on day 0 to quantify behavior variables continuously for 28 d. Testis width and scrotal circumference, and serum haptoglobin (days 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 nonviable whereas BUL testes were normal. Serum testosterone concentrations on day 168 were similar ($P = 0.14$) between INJ and BUL whereas after day 14, BAN was nondetectable; 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 days 1, 3, 5, and 7. Scrotal circumference ($P = 0.08$) and testis width ($P = 0.07$) on day 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 Zn injection. Collectively, Zn injection 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

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J. Anim. Sci. 2018.96:890–901

doi: 10.1093/jas/skx039

The authors sincerely appreciate D. T. Bechtol and A. Waite (AgriResearch Center, Canyon, TX), and A. M. Adams, L. R. Fontenot, R. E. Hudson, E. L. Kaufman, and D. J. Tomczak (West Texas A&M University, Canyon) for their technical assistance with this study.

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Received September 6, 2017.

Accepted November 29, 2017.

INTRODUCTION

Castration is performed on 15 million bulls each year in the United States 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

Before 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 five different dates (truck-load) 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, 4 mL/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 before study initiation, and each metaphylactic treatment was balanced evenly across the three 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 (day -1) and used to determine appropriate BW block allocation to facilitate the randomized complete block design. Blocks were constructed by stratification of day -1 BW, arrival date during backgrounding phase, and a 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 six pens within each treatment were allocated. Cattle were shipped 798 km to the AgriResearch Center feedlot located in Canyon, TX. Upon arrival (day 0), cattle were unloaded and allowed ad libitum access to water and long stem hay. Feedlot processing occurred the following morning on day 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 a 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 the 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 nonsteroidal 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 m² space per 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 h 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 three animals were removed from the dataset: 1 BAN animal was removed due to band failure observed on day 28; 1 BUL animal died on day 56 from an injury not related to treatment; 1

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

Item	Starter diet [†]	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

*Analyses performed by Servi-Tech Laboratories, Amarillo, TX.

[†]Starter fed first 7 d; intermediate fed next 14 d; finisher fed remainder of study.

BUL animal was removed on day 56 due to chute injuries. Animals diagnosed with the 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) florfenicol (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 (three animals from each pen) was selected randomly for repeated blood sampling. Serum haptoglobin (Hp) concentration was determined from samples collected on days 0, 1, 3, 5, 7, and 14; whereas, serum testosterone concentration was determined from samples collected on days 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 $2,060 \times g$ 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 ¹²⁵I 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 days -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 testis was measured for thickness via a digital caliper (Model W80152, Performance Tool, Tukwila, WA). Pens within a block were harvested according to BW and visual appraisal for market readiness. Blocks 5 and 6 were harvested (USDA Establishment #3, Cactus, TX) on day 155; blocks 3 and 4 were harvested on day 176; and blocks 1 and 2 were harvested on day 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 was weighed individually using a digital scale. Testes from 13 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 before 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 includes the main effect of treatment, day, and treatment $\times$ 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 least significant difference method using the PDIFF option in SAS. 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 days 14, 28, 56, and 84 did not differ ($P \geq 0.16$). We observed a tendency ($P = 0.06$) for BW to differ on day 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 day 141 after castration occurred. On day 140 of our study, BW was greater in INJ and BUL compared to BAN ($P < 0.01$). Similarly, BW

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

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

*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.

†Pooled standard error of the mean.

^{a-b}Rows without common superscript letters differ, $P < 0.05$.

was greater in INJ and BUL compared to BAN on day 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 calthood, 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.

The average daily gain was greater in BUL compared to either INJ or BAN from days 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 days 14 to 28 were observed. Cattle on the INJ treatment tended ($P = 0.09$) to have increased ADG from day 28 to 56 compared to BAN whereas BUL was intermediate and did not differ from INJ or BAN. The average daily gain was greater from days 56 to 84 in the INJ and BUL cattle compared to BAN ($P = 0.03$). Similarly, INJ and BUL had greater ADG from days 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, give that 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 days 0 to 14, 14 to 28, 28 to 56, 56 to 84, 84 to 112, and 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 days 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

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

Item	Treatment*			SEM [†]	P-value
	BAN	BUL	INJ		
Dry matter intake, kg/d					
Days 0 to 14	7.0	6.9	6.0	0.4	0.11
Days 14 to 28	9.6	9.7	9.1	0.4	0.51
Days 28 to 56	10.0	9.6	9.4	0.4	0.56
Days 56 to 84	10.2	10.5	9.9	0.3	0.40
Days 84 to 112	10.2	10.5	10.2	0.3	0.54
Days 112 to 140	9.8	10.3	10.0	0.3	0.38
Days 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					
Days 0 to 14	0.17 ^b	0.29 ^a	0.19 ^b	0.03	<0.01
Days 14 to 28	0.18	0.21	0.20	0.03	0.62
Days 28 to 56	0.21	0.24	0.26	0.02	0.13
Days 56 to 84	0.19 ^b	0.23 ^a	0.23 ^a	0.01	0.03
Days 84 to 112	0.15 ^b	0.18 ^a	0.19 ^a	0.007	<0.01
Days 112 to 140	0.15 ^b	0.19 ^{ab}	0.20 ^a	0.02	0.08
Days 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

*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.

†Pooled standard error of the mean.

^{a-b}Rows without common superscript letters differ, $P < 0.05$.

between treatments with corresponding differences in ADG. From days 0 to 14, BUL had greater ($P < 0.01$) G:F than either BAN or INJ, which can be attributed to treatment application on day 0 in BAN and INJ. However, there were no differences ($P \geq 0.13$) in G:F from days 14 to 28 or 28 to 56. From days 56 to 84 and 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 days 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 days 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 (Figure 1). All BUL testis were histopathologically normal. Individual testis was 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 indicate 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 the injection site,

and there may be a systemic effect of Zn dissipating in the blood circulation.

Scrotal Circumference

A treatment $\times$ day interaction was detected for scrotal circumference (Figure 2; $P < 0.01$), which was similar ($P \geq 0.58$) between treatments on day 0. After day 14, all BAN cattle either lost their scrotum or scrotal tissue was necrotic; therefore, they were not used in the analysis thereafter. On days 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 postcastration 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 day 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 days 56, 84, 112, and 140. Final scrotal circumference tended ($P = 0.07$) to be greater in BUL than INJ on day 168. After injection and until day 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 $\times$ day interaction for testis thickness (Figure 3; $P < 0.01$). Similar,

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.

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

Item	Treatment*			SEM†	P-value
	BAN	BUL	INJ		
Testes weight, g‡					
Smallest testis	—	352	242	5.3	<0.01
Largest testis	—	376	313	6.4	<0.01
Total weight	—	727	550	12.0	<0.01

*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.

†Pooled standard error of the mean.

‡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 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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BAN and BUL ($P \leq 0.02$).

to scrotal circumference results, testis thickness increased in INJ cattle directly after administration of Zn from days 0 to 14 and 14 to 28 and then decreased after day 28. On days 1, 3, 5, and 7, testis thickness was greatest in INJ, intermediate in BUL and smallest in BAN ($P \leq 0.05$). After day 14, BAN was removed from the data because their scrotums had detached or become necrotic. From days 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 days 56, 112, and 140. We observed a tendency ($P = 0.08$) for final testis thickness to be greater on day 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 days 60 to 120, but overall testis width did not change over the entire study when comparing treated to nontreated 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 $\times$ day interaction was observed for serum testosterone concentration (Figure 4; $P < 0.01$). Serum testosterone concentrations in BAN cattle were undetectable after day 14 of the study. On days 56 and 112, serum testosterone concentrations were greater ($P < 0.01$) in BUL compared to INJ cattle. However, on days 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 the 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 detectable 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

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 $\times$ day ($P < 0.01$) were detected. * indicates 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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BUL ($P < 0.01$).

possessed serum testosterone concentration similar to BUL suggesting that while sterilization occurred, castration did not.

Serum Haptoglobin

A treatment $\times$ day interaction was detected for serum Hp concentration (Figure 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

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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BAN and BUL ($P < 0.01$).

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 were 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 day 0. However, after treatment administration on day 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 day 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 days 5 and 7 than either BAN or BUL. Final serum Hp concentration on day 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 day 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 day 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 day 0, and this was likely an artifact of the respiratory and clostridial vaccines administered on day 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 $\times$ day interaction occurred for motion index during the first 28 d of the study (Figure 6; $P < 0.01$). Motion index was greater on day 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 day 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 day 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 $\times$ day interaction was detected for time standing (Figure 7; $P < 0.01$). Standing time was greater ($P < 0.01$) in BUL and BAN on day 0 compared to INJ. However, a sharp increase in standing time occurred for INJ cattle on day 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 day 0. On days 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 day 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 before the loss of the testes. For the remainder of the 28 d data collection, standing time did not differ ($P \geq 0.05$) between treatments.

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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BAN and BUL within day ($P \leq 0.05$). \$ indicates 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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BAN and BUL within day ($P \leq 0.05$). \$ indicates BAN differs from INJ and BUL within day ($P \leq 0.05$).

Data suggest that standing time directly after experimental treatments were applied were affected initially but not after day 11.

We observed a treatment $\times$ day interaction for the number of steps taken per day (Figure 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 day 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 day 1; however, as previously reported increased motion index was observed immediately following Zn injection on day 0. No differences ($P \geq 0.08$) were observed in step count from days 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 days 19 to 27 in INJ and BUL compared to BAN cattle. On day 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

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 $\times$ day ($P < 0.01$) were detected. * indicates INJ differs from BAN and BUL within day ($P \leq 0.05$). \$ indicates BAN differs from INJ and BUL within day ($P \leq 0.05$).

day 28, as these variables were likely confounded from handling and removal of the accelerometers at that time.

A treatment $\times$ day interaction was detected for the number of lying bouts (Figure 9; $P < 0.01$). Lying bouts on day 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 days 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 day 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. A 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 days 0 to 112; however, BW was greater in INJ and BUL compared to BAN cattle on days 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

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 $\times$ day ($P < 0.01$). * indicates INJ differs from BAN and BUL within day ($P \leq 0.05$). \$ indicates BAN differs from INJ and BUL within day ($P \leq 0.05$).

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 days 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 day 14. Behavior measurements were greater in INJ and BUL compared to BAN from days 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.

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