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Chemical sterilisation of *Bos indicus* bull calves following intratesticular injection of zinc acetate: Effects on growth and concentrations of testosterone

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ABSTRACT

The aim of this study was to determine the effects in *Bos indicus* calves of intra-testicular injection of either saline ($n = 9$) or one of two doses of zinc acetate ((ZA1, 57.75 mg, $n = 10$, or ZA2, 71.75 mg, $n = 10$) or surgical castration ($n = 9$) on circulating concentrations of testostosterone and liveweight. Human chorionic gonadotrophin (hCG, 1500 IU) was administered 202 and 525 days after treatment on Day 0 and animals were slaughtered on Day 860. In animals left intact treatment with ZA reduced mean serum concentrations of testosterone (Saline: 5.58 ± 0.79 ng/mL, ZA1: 1.28 ± 0.27 ng/mL, ZA2: 1.01 ± 0.17 ng/mL; $P < 0.001$) and concentrations 48 h following administration of hCG. The maximum concentration of testosterone recorded throughout the study in six out of 19 animals treated with ZA was $\leq$0.21 ng/mL. Treatment with ZA did not significantly affect live weights or carcass weights or result in any detectable scrotal lesions. Animals with concentrations of testosterone $\geq$1.0 ng/mL exhibited greater liveweights throughout most of the study and yielded heavier carcass weights (340.9 ± 7.02 versus 309.3 ± 6.17 kg, $P = 0.002$). It is concluded that a single, intra-testicular administration of either 57.75 mg or 71.75 mg of ZA was able to similarly reduce circulating concentrations of testosterone without significantly affecting liveweights or carcass weights. Treatment with ZA can result in variation in circulating concentrations of testosterone which could lead to differences in behaviour, liveweights and carcass characteristics.

1. Introduction

Castration of calves within the cattle industry is widely practised to modify behaviour, carcass characteristics and to prevent unwanted pregnancies. Castrated males are less aggressive, safer and easier to manage and are at reduced risk of incurring injuries than steers (Price et al., 2003; Stafford and Mellor, 2005; Katz, 2007). Intact bulls, however, have greater liveweight gains and feed efficiency and so castration can reduce potential production advantages (Field, 1971; Wainewright et al., 2011). Controlling the exposure of breeding cows only to selected sires can be particularly difficult in extensively managed beef herds. This is the case in Northern Australia where it is difficult to maintain effective fencing across a vast area which is frequently challenged by natural events such as floods and fire (Petherick, 2006). Surgical castration remains as the predominant method of managing male cattle in these environments to restrict unwanted matings although concerns over the welfare of the practice within

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the community persist (Petherick, 2006). Development of alternative methods of castration which are not adverse to animal welfare could be more acceptable to consumers.

Sterilization using chemical substances injected into the testes has been attempted as an alternative to surgical castration in a variety of domestic and laboratory animal species, including bulls (Fordyce et al., 1989; Canpolat et al., 2006), with variable responses being reported (Kutzler and Wood, 2006; Massei and Miller, 2013). Intra-testicular administration of zinc gluconate are effective in inducing sterility in dogs (Oliveira et al., 2007; Soto et al., 2009) with no or a small percentage (<4%) of dogs being reported to experience pain or discomfort during or after injection (Tepsumethanon et al., 2005; Soto et al., 2007; Oliveira et al., 2012). Injection site reactions requiring treatment are also uncommon, being evident in <4% of cases (USFDA, 1993; Levy et al., 2008). Effects of treatment with zinc gluconate on circulating concentrations of testosterone have been variable. In Beagle dogs treated with zinc gluconate at 6 months of age, a 41% to 52% decrease in serum concentrations of testosterone was recorded during the study but within 24 months of treatment concentrations in 70% (21/30) of dogs returned to values that were similar to control animals following treatment (USFDA, 1993). In rats treated with zinc tannate, circulating concentrations of testosterone were not significantly decreased following intra-testicular injection of 3 mg but were decreased by 79% after increasing the dose to 6 mg (Fahim et al., 1982). When cats were treated with a dose of zinc gluconate equivalent to nearly twice the dose recommended for dogs with a testis of similar width, a 94% reduction in concentrations of testosterone was observed 120 days after treatment (Fagundes et al., 2014). Treatment of mature bears (*Ursus americanus)* with zinc gluconate (19.7–32.8 mg), however, failed to result in a significant reduction in circulating concentrations of testosterone (Brito et al., 2011) These results suggest that suppressive effects on testosterone synthesis may be dose dependent, variable and may be incomplete with suppression in the synthesis of testosterone being more likely when larger doses are administered.

There are currently no reports on the use the intra-testicular administration of zinc-containing compounds to chemically castrated bulls and its effects on growth, hormone and carcass characteristics in bulls. Use of zinc acetate (ZA; mass % = 29.8) compared to current available formulations of zinc gluconate (mass % = 14.4; Zeuterin™, Ark Sciences, 13.1 mg/mL zinc) would enable a more concentrated delivery of zinc to the testes and could improve the likelihood that serum concentrations of testosterone are suppressed following treatment. Administration of different doses may also result in different circulating concentrations of testosterone after treatment which could affect behaviour, liveweights and carcass characteristics. Zinc, when injected in small quantities into meat-producing animals, should not pose a danger to human health. It therefore offers the possibility of being a single use chemical sterilant in cattle that could be offered as an alternative to surgical castration and may reduce circulating concentrations of testosterone. The aim of this study was to investigate the effects of administering two different doses of the potential intra-testicular sterilant ZA to bull calves on circulating concentrations of testosterone and the live weight of *B. indicus* bulls following treatment. Effects on semen quality and testicular changes have been reported separately (Cavalieri et al., 2015).

2. Materials and methods

The experimental protocol and procedures used in this study were approved by the James Cook University Animal Ethics Committee (approval number: A1304).

2.1. Location, treatment and nutrition

Animals were located at the James Cook University, Tropical Veterinary Research Station, Fletcherview (latitude 19°53′4″ S; longitude 146°10′43″ E) located in the dry tropics, of Northern Queensland, Australia. The climate is characterised by a hot, wet summer period (wet season) and a warm, dry winter period (dry season) with average annual rainfalls of 600 mm. Cattle grazed a mixture of native perennial, native legume and exotic improved pasture species. Brahman calves ($n = 38$), 5 to 6 months of age were first weighed and randomly assigned to treatments on Day -7 of the study. After balancing treatments for liveweight treatments were administered on Day 0. Calves ($n = 9$) were surgically castrated by removal of each testis from the scrotum, following two parallel incisions through the scrotal skin with a scalpel blade (Castration) in accordance with standard industry practice (Newman, 2007). Calves in the control group (Saline, $n = 9$) had each testis injected with 1 mL of saline (0.9% NaCl) solution. Other calves had each testis injected with 1 mL of a solution containing ZA at a concentration of 57.75 mg/mL (ZA1; $n = 10$, 17.2 mg Zn) or 71.75 mg/mL (ZA2; $n = 10$, 21.4 mg Zn).

Intra-testicular injections were administered with a 25-gauge needle, 25 mm in length. The scrotal skin was first swabbed with a povidone iodine antiseptic scrub. The needle was inserted into the dorsal half of the testis, through the scrotal skin. Small quantities of the solution (approximately 0.2 mL) were injected in one location and then the needle was repositioned adjacent to that location and another similar quantity was injected. This procedure was repeated with the intent of avoiding the deposition of product in one location only but instead fanning the substance across the dorsal pole of the testis to inject in the region of the *ductuli efferentes*. Calves were released to pasture immediately after treatment. Calves were observed on pasture within 2 to 5 h of application of treatments for any abnormalities of gait and behavior. Animals were also observed while on pasture 24 and 48 h after treatment.

After administering treatments on Day 0 bulls could not be recovered for data collection between Days 545 to 775 due to local flooding. Two bulls (ZA2 treatment) could not be found when remaining stock were recovered and by the time of slaughter. From Days 787 to 859 after treatment bulls were maintained in a feedlot (72 days) and then slaughtered (Day 860). At the feedlot the diet was fed *ad-libitum* and contained steam flaked corn (17.2%), wheat (17.2%), a mineral supplement (4.1%), molasses (13.0%), whole cottonseed (6%), cottonseed meal (7.2%), corn silage (12.0%) and sorghum hay (23.3%). The dry matter content

of the diet was 76.4%, crude protein 14.0%, nutrient detergent fibre 30.5%, with an energy content of 11.2 MJ ME/kg dry matter. Bulls were maintained within a single group within the feedlot.

2.2. Measurements and blood samples

Liveweights and blood samples were collected on Days -7, 34, 62, 132, 201, 263, 343, 448, 524 and 783. Blood samples were collected from the tail vein or artery, into plain evacuated tubes between 0830 and 1300 h. Serum was harvested following centrifugation (3000 x *g* for 15 minutes) and frozen until the time of assay for concentration of testosterone.

A stimulation test using human chorionic gonadotrophin (hCG; Chorulon, Intervet Australia Pty Ltd, Bendigo East; 1500 IU, IM)) was performed on Days 202 and 525 after treatment. A blood sample was initially collected the day before hCG was administered. On the following day, all animals were treated with hCG within a 1-h period. A second blood sample was then collected 48 ± 1 h after administration of hCG.

2.3. Testosterone assay

Plasma concentrations of testosterone were measured using an extraction tritiated radioimmunoassay (Rosales Nieto, 2013) using antiserum (20C-CR2140R; Fitzgerald Industries International, North Acton, MA, USA) against testosterone. Cross-reactions, as specified by the manufacturer, were 100% with testosterone, 0.5% with androstenedione and 0.01% with dehydroepiandrosterone. The sensitivity of the assay was 0.05 ng/mL. Intra- and inter-assay coefficients of variation for standard serum pools of 0.14, 0.34 and 2.13 ng/mL were 7.1% and 8.5%, 6.2% and 7.9%, and 4.9% and 6.4%, respectively.

2.4. Statistical analyses

Statistical analyses were conducted using the statistical software package IBM SPSS Statistics 19 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corporation). Repeated measures analysis of variance was used to assess the effects of treatment on body weights and plasma concentrations of testosterone. The model included the main effects of Treatment, Day and the interaction of Treatment and Day. If Mauchly's test indicated violation of the assumption of sphericity, probability values were obtained after degree of freedom were adjusted using the Greenhouse-Geisser statistic. Where the main effect of Treatment, or the Treatment by Time effect were significant ($P < 0.05$) Tukey's HSD test was used as the mean separation procedure. Logarithmic transformation of concentrations of testosterone was conducted before analysis to reduce the variance.

Comparison of mean concentrations of testosterone within treatments before and after administration of hCG were performed using a paired *t*-test. Average daily gains, carcass weights and monetary values were compared using ANOVA with treatment entered as a fixed factor. In animals treated with ZA scatter plots and regression analyses were used to explore associations between the total testicular mass at the time of slaughter and concentrations of testosterone 48 h after administration of hCG. Linearisation of the regression line was achieved by $\log_{10}$ transformation of both the dependent and independent variables. Values of $P \leq 0.05$ were considered significant. Means are presented along with the SEM.

3. Results

3.1. Data exclusions

During the course of the study two bulls (ZA2 treatment) were not recovered after a flooding event, one bull (Saline treatment) died at the feedlot from respiratory disease and two other bulls (Saline and ZA1 treatments) were removed during the feedlot period after acquiring bovine respiratory disease, treated with antibiotics and maintained on pasture until the time of slaughter (Day 870). Data from all of these animals were not included in the assessment of carcass characteristics or liveweight gain during the feedlot period (Table 1) but were included in the assessment of hormone concentrations and liveweights before they entered the feedlot. The carcass weights of the two animals that were removed from the feedlot due to respiratory disease were >3SD less than the mean of the weights for their respective groups. Data for one animal in the ZA1 treatment group were removed from all analyses as the mean concentration of testosterone over the duration of the study, total testicular weight, liveweight and carcass weight at slaughter for this animal were within 1 SD of the mean of the saline-treated bulls. The maximum number of progressively motile and morphological sperm collected over the course of the study (Cavalieri et al., 2015) was also >3 SD greater than the mean of other animals that were treated with ZA. This animal was therefore deemed to be a treatment failure. It was likely that an incomplete dose of ZA was inadvertently administered to this animal.

3.2. Animal behaviour

Within 2 to 5 h of treatment some calves treated with ZA were observed to exhibit stiffness of gait and occasionally kick with a hind leg at the flank region. Others were observed to be sitting in ventral recumbency when most other calves were standing. One calf was observed to be lying in lateral recumbency but would rise and walk away when approached. Stiffness of gait was observed in some of the animals treated with ZA 24 h following treatment but was not observed 48 h after treatment. The apparent adverse responses which were consistent with a degree of abdominal pain were not anticipated at the time and were not quantified or categorised by the dose of ZA administered.

3.3. Body weights

Mean body weights of animals between the start of treatment and when animals exited the feedlot (Day 858) are depicted in Fig. 1. Assessment of bodyweights over time indicated that mean bodyweight increased over time

Table 1
Carcass characteristics, monetary value and daily gain of bull calves treated with intratesticular injections of saline or one of two doses of zinc acetate (ZA1, 57.75 mg or ZA2, 71.75 mg) or surgically castrated.

	Saline	Castrated	ZA1	ZA2	*P*
n	7	9	8	8	
Carcass weight (kg)	342.9 ± 12.6	304.6 ± 9.0	330.2 ± 7.8	331.6 ± 11.2	0.068
Gross value ($AUD)	1046.02 ± 25.0	987.66 ± 17.6	1015.25 ± 14.7	1047.65 ± 21.6	0.112
Value per kg ($AUD)	3.06 ± 0.05	3.26 ± 0.05	3.08 ± 0.05	3.17 ± 0.06	0.042
Daily gain (kg/day)	2.28 ± 0.13	2.24 ± 0.13	2.31 ± 0.16	2.43 ± 0.17	0.838

($P < 0.001$) but effects due to treatment ($P = 0.625$) or a treatment by time interaction ($P = 0.260$) were not detected. No significant change in body weight of animals ($P > 0.05$) in all of the treatment groups was observed between Days 343 to 524 and this coincided with a cumulative total rainfall during this period of only 25.6 mm (Fig. 1).

3.4. Concentrations of testosterone

Concentrations of testosterone in serum following treatment are depicted in Fig. 2. An interaction of treatment with day ($P < 0.001$) was found for concentrations of testosterone. Concentrations of testosterone in the

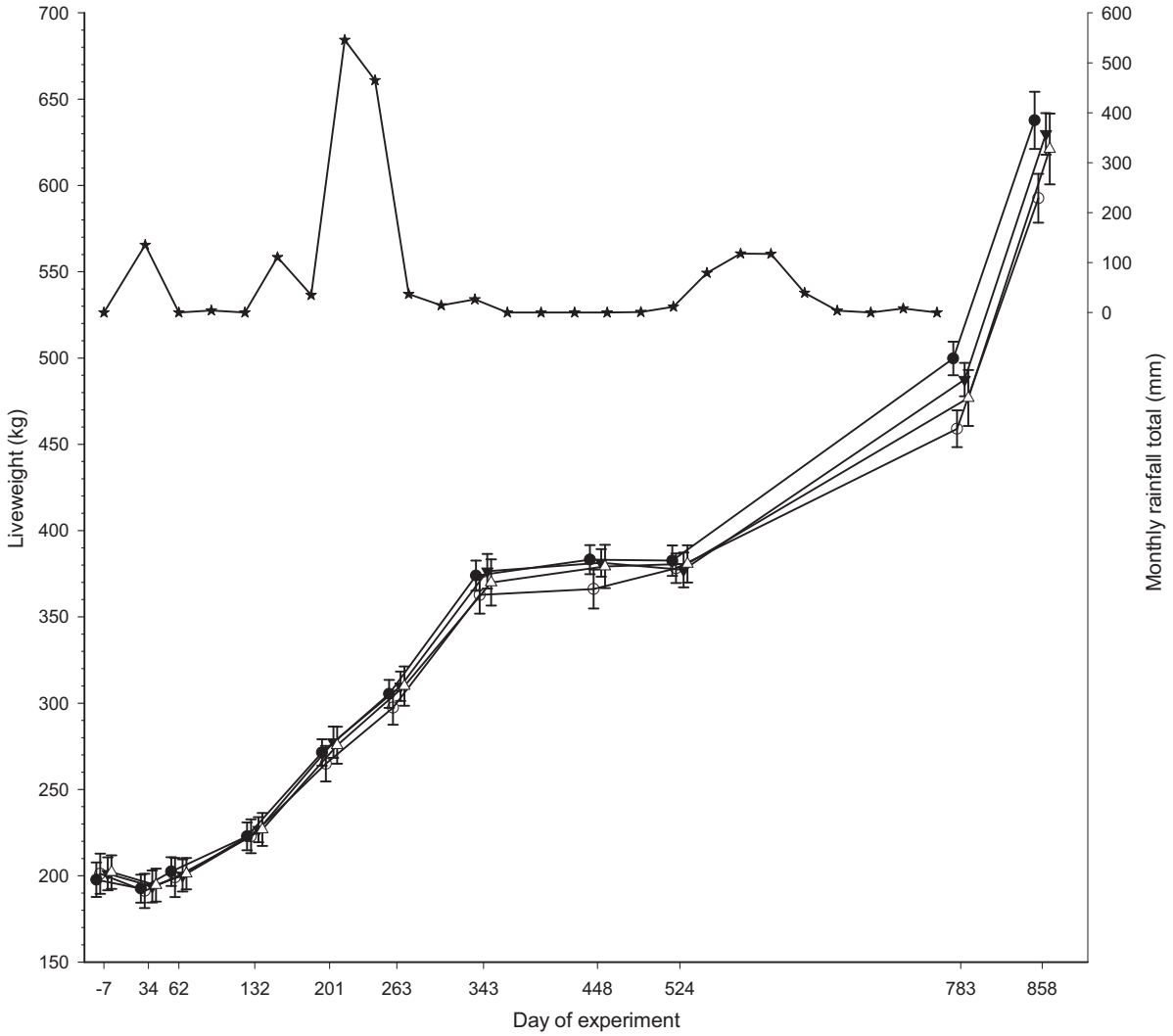

Fig. 1. Effect of treatment with an intratesticular injection of saline (•), zinc acetate (ZA1, 57.75 mg, ▼ or ZA2, 71.75 mg, △) or surgical castration (◯) on Day 0 on mean ± SEM body weight. Monthly rainfall total (mm) during the experimental period is also shown (*). Bulls were maintained on pasture up to Day 786. From Days 787 to 859 bulls were maintained in a feedlot (72 days) and then slaughtered (Day 860).

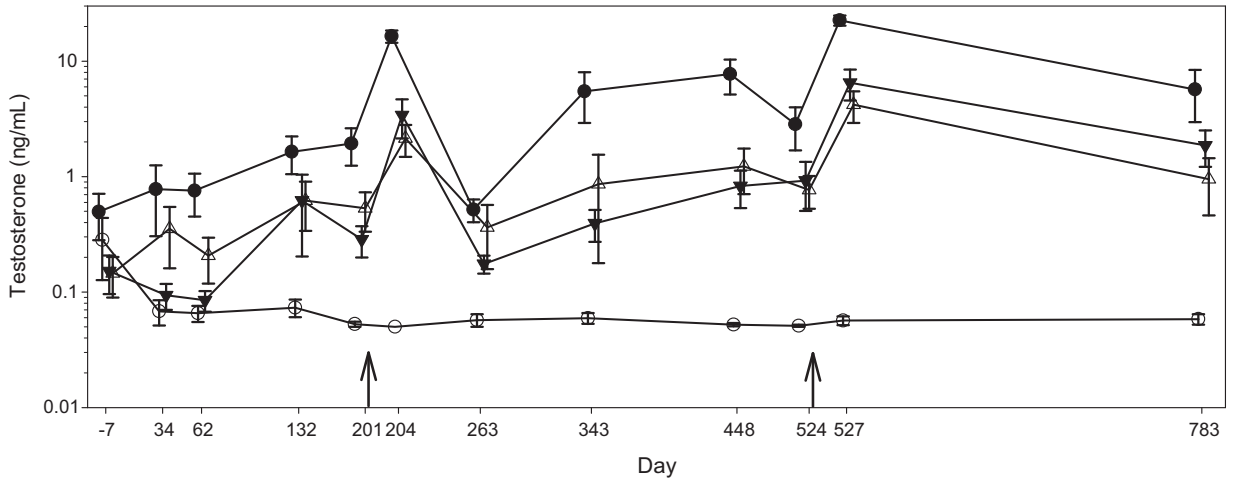

Fig. 2. Concentrations of testosterone in serum following intratesticular injection of saline (•), zinc acetate (ZA1, 57.75 mg, ▼ or ZA2, 71.75 mg, △) or surgical castration (○) on Day 0. Each animal was treated with 1500 IU of human chorionic gonadotrophin IM on Day 202 and 525 (↑).

castrated animals decreased after the time of castration on Day -7 to the time when the next sample was collected (Day 34) and remained at mean concentrations of <0.10 ng/mL throughout the rest of the study. A repeat of the analysis with castrated animals omitted from the analysis revealed that there was an effect of treatment ($P = 0.001$), and day ($P < 0.001$) but the interaction was not significant ($P = 0.345$). This indicated that the significant treatment by day interaction that was detected using the original model was due to the change in differences over time that occurred between the castrated animals and the other treatments.

Mean concentrations of testosterone in the Saline, castrated, ZA1 and ZA2 bulls were 5.58 ± 0.79, 0.08 ± 0.01, 1.28 ± 0.27 and 1.01 ± 0.17 ng/mL, respectively. Mean concentrations of testosterone were always less in the castrated animals compared with all of the other treatments after Day 201 except on Day 263 when mean concentrations did not differ significantly between the castrated and ZA1 treated animals and Day 343 when mean concentrations did not differ significantly between the castrated and ZA1 and ZA2 treated animals. Concentrations of testosterone in the bulls that were not surgically castrated were less in the ZA1- and ZA2-treated bulls compared with the Saline-treated bulls ($P < 0.05$) but did not differ significantly between the ZA1- and ZA2-treated bulls. At 48 h after each administration of hCG, mean concentrations of testosterone in the Saline-, ZA1- and ZA2-treated animals increased ($P < 0.05$) compared with concentrations measured 24 h before hCG was administered and were greater in the Saline-treated bulls compared to the bulls treated with ZA and the castrated bulls (Fig. 2). In the castrated bulls, the mean concentration of testosterone was also significantly less than the bulls treated with ZA at these times. The maximum concentration of testosterone recorded throughout the study in any castrated animal was always ≤ 0.15 ng/mL. In 33.3% (3/9) of the ZA1- and 30.0% (3/10) of the ZA2-treated animals the maximum concentration of testosterone that was recorded throughout the study was ≤ 0.21 ng/mL. The maximum concentrations of testosterone in the remaining animals in the Saline-, ZA1- and ZA2-treated animals were all >16.0 (16.6–33.4), 3.0 (3.3–16.2), and 1.3 (1.31–9.6) ng/mL, respectively.

Concentrations of testosterone in serum 48 h after administration of hCG in bulls treated with ZA were related to the total testicular mass recorded at the time of slaughter (Fig. 3; $P < 0.001$; Day 204, $R^2 = 0.94$; Day 527, $R^2 = 0.95$). For every 1% increase in total testicular mass at slaughter concentrations of testosterone 48 h after administration of hCG increased by 1.40% on Day 204 and 1.59% on Day 527, respectively.

Body weights of animals with mean concentrations of testosterone between Days -7 to 783 of <1.0 ng/mL were compared with those animals with concentrations of testosterone that were ≥ 1.0 ng/mL. A group by time interaction was found for the effects of the mean concentration of testosterone on body weights ($P = 0.009$). Following

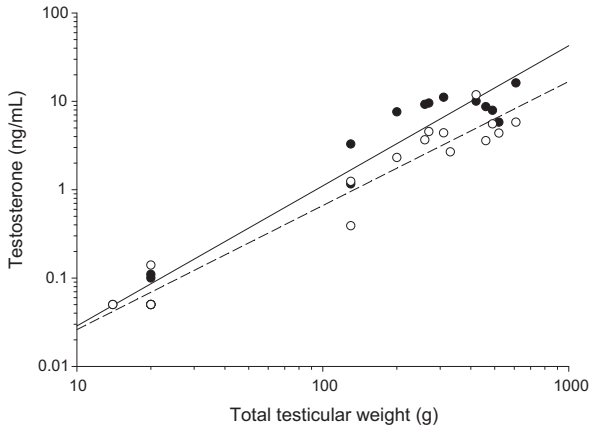

Fig. 3. Relationship between total testicular mass at the time of slaughter and concentration of testosterone recorded 48 h after administration of hCG in bulls treated intra-testicularly with zinc acetate. Equation from the linear regression: Day 204: Log_{10} [testosterone] = $1.40 \times \text{Log}_{10}$[total testicular mass] – 2.99, (—○—); Day 527: Log_{10} [testosterone] = $1.59 \times \text{Log}_{10}$[total testicular mass] – 3.14, (—•—).

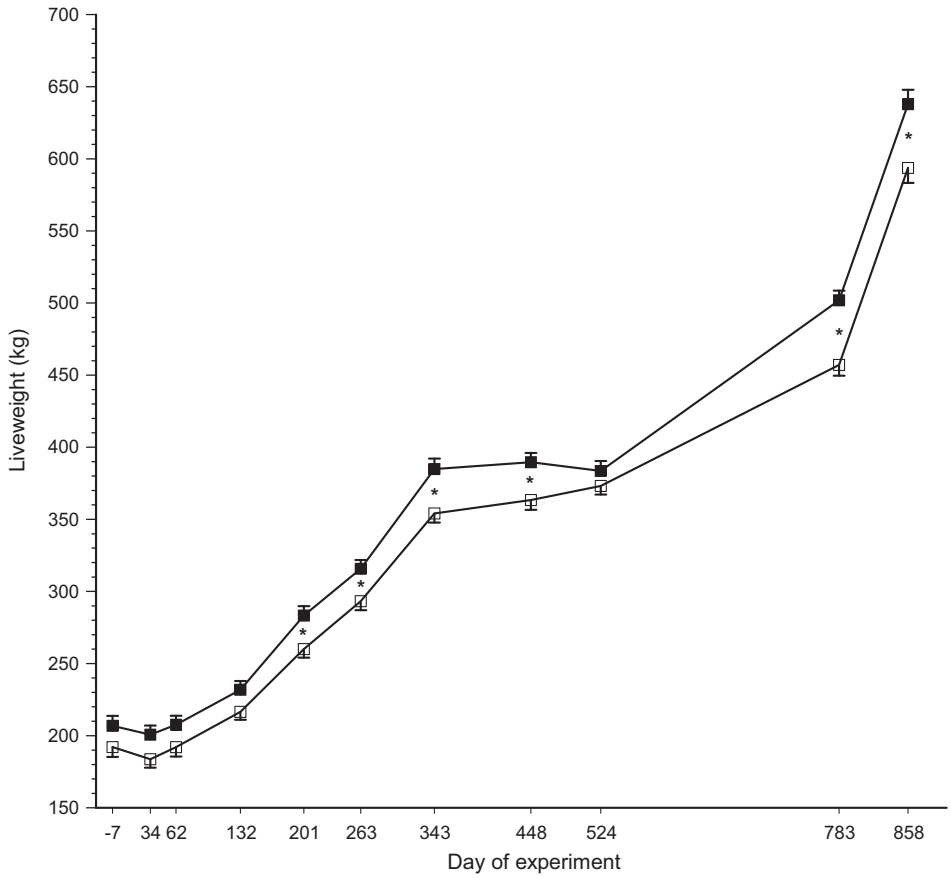

Fig. 4. Mean ± SEM of body weight in animals with mean concentrations of testosterone throughout the study of <1.0 ng/mL (□ or ≥ 1.0 ng/mL (■). * concentrations differ ($P < 0.05$).

castration the mean body weights of animals with mean plasma concentrations of testosterone ≥1.0 ng/mL were greater at six of the 11 times when body weights were recorded (Fig. 4).

3.5. Carcass characteristics

Mean carcass weight tended to differ between treatments with the greatest difference being evident between the Saline treated and the castrated bulls (Table 1). The gross value of the carcasses of the saline-, ZA1- and ZA2-treated bulls were 5.9%, 2.8%, and 6.1% greater, respectively, than the castrated animals but differences were not significant (Table 1). The monetary value received per kg differed between treatments (Table 1) but when multiple comparisons were taken into consideration using Tukey's HSD test means could not be statistically separated ($P > 0.05$). The monetary value received per kg was greatest for the castrated animals (Table 1). The average daily gain during the period when animals were maintained in a feedlot did not differ significantly between treatments (Table 1). For animals that had a mean concentration of testosterone throughout the sampling period ≥1.0 ng/mL compared to those that had a mean concentration <1.0 ng/mL carcass weight (340.9 ± 7.02 compared with 309.3 ± 6.17 kg, $P = 0.002$) and the gross average value of the carcasses ($1049.35 ± 13.10 compared with $991.69 ± 12.71, $P = 0.004$) were greater but the mean value per kg was lower (3.09 ± 0.03 versus $3.22 ± 0.04, P = 0.019) and the percentage of animals that were paid more than $1:10/kg carcass weight was less (23.5%, 4/17 compared with 60.0%, 9/15; $P = 0.036$)

4. Discussion

Reducing serum concentrations of testosterone and preventing sperm output in chemically sterilised but intact bull calves could offer a means of reducing male-like behaviour and preventing unwanted pregnancies in treated male calves. In the present study intra-testicular injection of *B. indicus* calves between 5 and 6 months of age with two doses of ZA had no adverse effects on production, growth and carcass characteristics compared with surgical castration. Both dose rates reduced serum concentrations of testosterone with some, but not all animals having circulating concentrations of testosterone that were similar to steers. The results indicate that chemical sterilisation of *B. indicus* bull calves with ZA can be used without affecting growth rates and can decrease serum concentrations of testosterone although the degree of suppression in concentrations of testosterone was variable. This could result in variable effects on behaviour and meat quality which

are affected by circulating concentrations of testosterone (Gortsema et al., 1974; Price et al., 2003).

While effects on circulating concentrations of testosterone were variable treatment with ZA reduced the steroidogenic capacity of the testes of treated animals. Synthesis of testosterone is coupled to the pulsatile release of luteinizing hormone from the anterior pituitary gland so isolated, individual samples from intact or treated bulls may not always reflect the true steroidogenic capacity of the testes (Katongole et al., 1971). After administration of hCG to bulls in the present study a two to three fold increase in concentrations of testosterone in serum was observed in most animals within 48 h which is consistent with the increases in testosterone that were observed following administration of hCG to bulls in other studies (Sundby et al., 1975; Cavalieri and Farin, 1999). Human chorionic gonadotrophin targets luteinizing hormone receptors on Leydig cells and stimulates an increase in testosterone synthesis (Pakarainen et al., 2007) thus providing an opportunity to test the steroidogenic capacity of the testes. In rats administration of hCG results in an increase in the number and volume of Leydig cells after treatment which in turn is likely to be due to an increase in the number and size of cellular organelles (Hodgson and De Kretser, 1984) resulting in the increase in steroidal synthesis of testosterone. In this study both the mean concentrations of testosterone and mean concentrations 48 h after administration of hCG were reduced in animals treated with ZA compared to saline treated controls which is consistent with a reduction in the number and/or function of Leydig cells following treatment. When testes from treated bulls in this study were examined at the time of slaughter a significant reduction in testicular mass and histological evidence of degeneration was found (Cavalieri et al., 2015) indicating that the suppression in concentrations of testosterone in serum observed was attributable to testicular degeneration and reduced testicular mass.

In 31.6% (6/19) of animals treated with ZA concentrations of testosterone were always <0.21 ng/mL suggesting that intra-testicular administration of ZA may result in animals that hormonally resemble surgical castrates. This indicates that treatment with ZA may, in some animals, destroy the steriodogenic capacity of testes and reduce concentrations of testosterone to concentrations that are similar to castrated animals. Testicular diameter at the time of treatment with both doses of ZA was found to positively affect the final testicular mass at slaughter and the probability that testes were >100 g at the time of slaughter (Cavalieri et al., 2015). This suggests that the ability of ZA to reduce serum concentrations of testosterone to those that are similar to steers depends on ensuring that the all of the endocrine and spermatogenic tissue within the testes are exposed to the chemical. Factors which could reduce variation in concentrations of testosterone in animals treated with ZA could include ensuring that animals are adequately restrained during treatment, adjusting the dose in relation to testicular size and improving dissemination of the chemical throughout the testes (Cavalieri et al., 2015).

The ability of zinc-containing compounds when used as a chemical sterilant to reduce circulating concentrations of testosterone appears to vary between studies and may vary with dose, species, formulation and the size of the testes at the time of administration (Fahim et al., 1982; USFDA, 1993; Brito et al., 2011; Fagundes et al., 2014). A positive relationship between testicular mass at slaughter and concentrations of testosterone 48 h after administration of hCG was observed in bulls treated with ZA. In animals that develop sizeable testes following treatment or maintain sufficient amounts of residual and functional testicular tissue it can be expected that circulating concentrations of testosterone will be elevated and treatment will be unlikely to prevent the development of secondary sexual characteristics and male-like behaviour that are attributed to the presence of testosterone. When Mach et al. (2009) castrated Holstein bulls at 8 months of age with a Burdizzo a reduction in average daily gain, bodyweight, hot carcass weight and adverse behaviours such as fighting and sexual behavior were reported compared to intact bulls presumably by reducing serum concentrations of testosterone. In bulls circulating concentrations of testosterone are known to affect mounting activity (Blockey and Galloway, 1978) and increase the frequency of sexual and agonistic behaviours (Jago et al., 1997). Thus if male-like behavior is to be prevented treatment will need to more consistently prevent the enlargement of testicular tissue after treatment to suppress concentrations of testosterone synthesis to basal.

Investigation of effects of treatment on animal welfare were not assessed in this study but should be investigated in future studies. There was evidence that some animals experienced a degree of pain and swelling following treatment but behavioural and physiological measures of animal welfare were not quantified. No breaches of scrotal integrity were found and any effect on gait appeared to subside within 48 h of treatment. Adverse effects of treatment with zinc gluconate have been reported in a small percentage of dogs in some (Tepsumethanon et al., 2005; LaCroix, 2006; Oliveira et al., 2007; Levy 2008; Forzán et al., 2014; Massei and Miller, 2013) but not all studies (Oliveira et al., 2012). Adverse reactions reported include transient testicular swelling mainly for the first 24 to 48 h, scrotal pain (6.3%, $n = 270$), necrotising orchitis and scrotal ulceration in 1 to 6% of dogs (US FDA 2003; LaCroix, 2006; Levy et al., 2008; Forzán et al., 2014). Relatively infrequent symptoms associated with treatment in dogs have included vomiting (4.4%), anorexia (4.0%), lethargy (2.2%) and leukocytosis and abnormalities of gait (USFDA, 1993). The pH of solutions of ZA used in the present study was potentially less than solutions containing zinc gluconate in which pH has been neutralised with arginine. No adjustments in dose for differences in testicular size were also undertaken. Thus differences in pH and dose in the present study may have contributed to apparent pain responses in some animals following administration of ZA as excess ZA may have leaked out of the testes and made contact with sensory nerve endings in some animals. In dogs, it is recommended that to avoid leakage into the scrotum which may increase the risk of scrotal lesions and pain responses that a new, small diameter needle for each testis be used and that care should be exercised to administer the drug entirely within the testis. It is also recommended that the dose be

adjusted according to testicular size (USFDA, 1993). In the present study, no scrotal lesions were observed in any of the bulls treated with saline or ZA throughout the course of the study. Similarly Brito et al. (2011) did not observe any scrotal lesions in adult bears treated with zinc gluconate. Use of a small diameter needle and restricting the volume of administration to 1 mL in the present study may have reduced any potential sequestration into the scrotum. Thicker scrotal skin in bulls compared to dogs and the ability of some dogs to chew an irritated scrotum may also have reduced the probability of scrotal lesions developing in bulls. Reasons, however, for apparent pain responses in some calves treated with ZA remain to be determined.

No significant differences in live or carcass weights were obtained between treatments in this study but statistical power was relatively low. Most studies have demonstrated that intact bulls have greater liveweight gains and feed efficiency than castrated animals (Field, 1971; Seideman et al., 1982; Earley and Crowe, 2002) due to the anabolic effects of testosterone (Katz, 2007; Mach et al., 2009) although meat quality can be reduced. Greater liveweights and carcass weights were evident in this study in animals that had mean circulating concentrations of testosterone $\geq$1.0 ng/mL. Numerically greater liveweights were also observed in the saline and zinc-treated bulls compared to the castrated animals which was most likely due to the anabolic effect of greater concentrations of testosterone in these animals compared to the castrated animals. While differences in liveweights and carcass weights between treatments were not significant the magnitude of the differences observed if maintained in larger treated populations are likely to be economically significant for producers in northern Australia if meat payment schemes for bulls and steers are the same. Producing beef from bulls compared to steers in northern Australia was found to be more profitable only when bulls received the same price as steers (Wainewright et al., 2011). If discounts are applied to meat derived from bulls then the profitability of bulls treated with ZA may be less compared with steers. In the present study animals with lesser mean concentrations of testosterone were paid a greater price per kilogram for the meat. This suggests that meat graders were awarding more favourable carcass grades and, therefore, greater prices to animals with lesser concentrations of testosterone. This is consistent with previous reports of greater meat quality in cattle with lesser concentrations of testosterone (Gortsema et al., 1974) Further investigations of carcass yields and meat quality derived from bulls treated with ZA will be needed to determine if bulls treated with ZA can be more profitable than steers. However, more consistency in the effects of treatment with ZA on serum concentrations of testosterone will be needed to produce more uniform lines of animals at the point of sale.

5. Conclusions

Intra-testicular administration of ZA to *B. indicus* bulls between 5 and 6 months of age did not significantly affect growth but did significantly reduce mean concentrations of testosterone and mean concentrations of testosterone 48 h after treatment with hCG. Concentrations of testosterone in bulls following treatment with ZA were variable but in 31.6% (6/19) of bulls treated with ZA circulating concentrations of testosterone were similar to steers. Carcass weights tended to be higher in saline treated control and ZA treated bulls compared to surgically castrated steers presumably due to the anabolic effects of testosterone. These results indicate that intra-testicular administration of both doses of ZA that were used in the present study did not significantly affect growth and carcass weights and variably reduced the steriodogenic capacity of the testes. Determining ways of yielding more consistent responses to treatment would be helpful in evaluating this method as a potential alternative to surgical castration in *B. indicus* bulls. Further work will also be needed to evaluate effects on animal welfare.

Conflicts of interest

M Wang is employed in part by Ark Sciences who manufacture Zeuterin™ a commercial product containing zinc gluconate which is used for chemically sterilising dogs. His role was limited to the supply and administration of zinc acetate in this study and he was not involved in data collection or analysis.

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References

Blockey, M.a.D., Galloway, D.B., 1978. Hormonal control of serving capacity in bulls. Theriogenology 9, 143–151.

Brito, L.F., Sertich, P.L., Rives, W., Knobbe, M., Piero, F.D., Stull, G.B., 2011. Effects of intratesticular zinc gluconate treatment on testicular dimensions, echodensity, histology, sperm production, and testosterone secretion in American black bears (*Ursus americanus*). Theriogenology 75, 1444–1452.

Canpolat, I., Gur, S., Gunay, C., Bulut, S., Eroksuz, H., 2006. An evaluation of the outcome of bull castration by intra-testicular injection of ethanol and calcium chloride. Rev. Med. Vet. 157, 420–425.

Cavalieri, J., Farin, P.W., 1999. Birth of a Holstein freemartin calf co-twinned to a schistosomus reflexus fetus. Theriogenology 52, 815–826.

Cavalieri, J., Wang, M.W., Johnson, L., 2015. Chemical sterilisation of *Bos indicus* bull calves following intratesticular injection of zinc acetate: Effects on semen quality and testicular changes. Anim. Reprod. Sci. 156, 23–33.

Earley, B., Crowe, M.A., 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80, 1044–1052.

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

Fahim, M., Fahim, Z., Harman, J., 1982. Chemical sterilization in the male part I: rats. Syst. Biol. Reprod. Med. 9, 261–265.

Field, R.A., 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32, 849–858.

Fordyce, G., Hodge, P.B., Beaman, N.J., Laing, A.R., Campero, C., Shepherd, R.K., 1989. An evaluation of calf castration by intra-testicular injection of a lactic acid solution. Aust. Vet. J. 66, 272–276.
Forzán, M.J., Garde, E., Perez, G.E., Vanderstichel, R.V., 2014. Necrosuppurative orchitis and scrotal necrotizing dermatitis following intratesticular administration of zinc gluconate neutralized with arginine (EsterilSol) in 2 mixed-breed dogs. Vet. Path. 51, 820–823.
Gortsema, S., Jacobs, J., Sasser, R., Gregory, T., Bull, R., 1974. Effects of endogenous testosterone on production and carcass traits in beef cattle. J. Anim. Sci. 39, 680–686.
Hodgson, Y.M., De Kretser, D., 1984. Acute responses of Leydig cells to hCG: evidence for early hypertrophy of Leydig cells. Mol. Cell. Endocrinol. 35, 75–82.
Jago, J.G., Cox, N.R., Bass, J.J., Matthews, L.R., 1997. The effect of prepubertal immunization against gonadotropin-releasing hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. 75, 2609–2619.
Katongole, C.B., Naftolin, F., Short, R.V., 1971. Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. J. Endocrinol. 50, 457–466.
Katz, L.S., 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52, 56–63.
Kutzler, M., Wood, A., 2006. Non-surgical methods of contraception and sterilization. Theriogenology 66, 514–525.
LaCroix, C.E., 2006. Evaluation of a single intratesticular injection of zinc gluconate neutralized by Arginine (Neutersol®) as a chemical sterilant in sexually mature, male dogs. In: Proc Third Int Symp Nonsurg Methods Pet Populations Control, Alexandria, VA.
Levy, J.K., Crawford, P.C., Appel, L.D., Clifford, E.L., 2008. Comparison of intratesticular injection of zinc gluconate versus surgical castration to sterilize male dogs. Am. J. Vet. Res. 69, 140–143.
Mach, N., Bach, A., Realini, C.E., Font i Furnols, M., Velarde, A., Devant, M., 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.
Massei, G., Miller, L.A., 2013. Nonsurgical fertility control for managing free-roaming dog populations: A review of products and criteria for field applications. Theriogenology 80, 829–838.
Newman, R., 2007. A guide to best practice husbandry in beef cattle, branding, castrating and dehorning. Meat and Livestock Australia Ltd, Sydney, Australia. In: Meat & Livestock Australia Limited.
Oliveira, E.C., Moura, M.R., De Sa, M.J., Silva Jr., V.A., Kastelic, J.P., Douglas, R.H., Marques Jr., A.P., 2012. Permanent contraception of dogs induced with intratesticular injection of a zinc gluconate-based solution. Theriogenology 77, 1056–1063.
Oliveira, E.C.S., Moura, M.R., Silva, V.A., Peixoto, C.A., Saraiva, K.L.A., De Sa, M.J.C., Douglas, R.H., Marques, A.D., 2007. Intratesticular injection of a zinc-based solution as a contraceptive for dogs. Theriogenology 68, 137–145.
Pakarainen, T., Ahtiainen, P., Zhang, F.-P., Rulli, S., Poutanen, M., Huhtaniemi, I., 2007. Extragonadal LH/hCG action—not yet time to rewrite textbooks. Mol. Cell. Endocrinol. 269, 9–16.
Petherick, J.C., 2006. Animal welfare provision for land-based livestock industries in Australia. Aust. Vet. J. 84, 379–383.
Price, E.O., Adams, T.E., Huxsoll, C.C., Borgwardt, R.E., 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 81, 411–415.
Rosales Nieto, C.A., 2013. Advancing puberty in female sheep: It's all about fat and muscle. PhD thesis, School of Animal Biology. The University of Western Australia., http://research-repository.uwa.edu.au/files/3358445/Rosales_Nieto_Cesar_2013.pdf (viewed 08.03.2015).
Seideman, S.C., Cross, H.R., Oltjen, R.R., Schanbacher, B.D., 1982. Utilization of the intact male for red meat production - A review. J. Anim. Sci. 55, 826–840.
Soto, F., Viana, W., Sousa, A., Pinheiro, S., Mucciolo, G., Hosomi, F., Azevedo, S., Dias, R., 2007. Evaluation of zinc gluconate, either associated or not to dimethyl sulfoxide, as contraceptive method for male dogs. J. Anim. Reprod. 4, 119–124.
Soto, F.R., Viana, W.G., Mucciolo, G.C., Hosomi, F.Y., Vannucchi, C.I., Mazzei, C.P., Eyherabide, A.R., De Fatima Lucio, C., Dias, R.A., De Azevedo, S.S., 2009. Evaluation of efficacy and safety of zinc gluconate associated with dimethyl sulphoxide for sexually mature canine males chemical neutering. Reprod. Domest. Anim. 44, 927–931.
Stafford, K.J., Mellor, D.J., 2005. The welfare significance of the castration of cattle: a review. N. Z. Vet. J. 53, 271–278.
Sundby, A., Tollman, R., Velle, W., 1975. Long-term effect of HCG on plasma testosterone in bulls. J. Reprod. Fertil. 45, 249–254.
Tepsumethanon, V., Wilde, H., Hemachudha, T., 2005. Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. J. Med. Assoc. Thai. 88, 686–689.
Wainewright, S., Parker, A., Holmes, W., Zerby, H., Fitzpatrick, L., 2011. An economic case study of entire male grain-fed beef from a north-western Queensland production system. Anim. Prod. Sci. 51, 570–574.
United States Food Drug Administration, 1993. Freedom of Information summary. In: Neutersol® injectable solution for dogs (zinc gluconate neutralized by arginine), New Animal Drug Application (NADA), pp. 141–217, http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm118024.pdf (viewed 27.01.15).