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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