



Effects on performance and carcass and meat quality attributes following immunocastration with the gonadotropin releasing factor vaccine Bopriva or surgical castration of *Bos indicus* bulls raised on pasture in Brazil[☆]

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ABSTRACT

Bos indicus bulls 20 months of age grazed on pasture in Minas Gerais, Brazil either received 2 doses of the GnRF vaccine Bopriva at d0 and d91 (group IC, n = 144) or were surgically castrated on d91 (group SC, n = 144). Slaughter on d280, was 27 weeks after castration. Adverse safety issues in 8% of group SC bulls following surgery contrasted with 0% in group IC bulls. At d105 testosterone levels were suppressed to similar levels in both groups. Importantly, group IC bulls had higher live weight, hot carcass weight, ADG ($P < 0.005$) and dressing percentage ($P < 0.0001$) compared to group SC animals. There were no negative effects on carcass or meat quality traits, thus immunocastration was concluded to offer a safe and effective method that provides production gains, and improves animal welfare in *Bos indicus* beef bulls without impacting meat and carcass quality.

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1. Introduction

Beef cattle production uses surgical castration globally to provide major advantages to the producer and consumer, including better meat and carcass quality through increased fat deposition, and modified behaviour resulting in safety of management and improved animal welfare (Bouissou, Boissy, Neindre, & Veissier, 2001; Stookey & Watts, 2004). However, raising castrated bulls has major disadvantages for the producer when compared to entire animals, including slower growth and less efficient feed utilization (Field, 1971; Seideman, Cross, Oltjen, & Schanbacher, 1982).

Brazil has a very large beef production industry, which grazes a range of cattle on pasture with breeds selected dependent upon region and climatic conditions (Ferraz & Felício, 2010). *Bos indicus* or *Bos*

indicus cross breeds are commonly raised for beef in the tropical areas of Brazil, with the common husbandry practice of growing entire bulls until late castration at approximately 18 to 24 months of age to capture the growth advantages of entire animals, and then slaughter at 30 to 36 months of age (Silva et al., 2003). This practice is also commonly conducted in other Latin American countries where *Bos indicus* cross breeds are raised provides improved meat and carcass quality. However, late neutering of bulls has several recognized problems. Set-back due to stress associated with late castration procedures has been recognized as important (Bretschneider, 2005), which has been further studied in Brazil (Carvalho, Silva, & Hoe, 2011; Silva et al., 2003). An additional complication following surgical castration in tropical regions is the high risk of infestation of the wound with the New World screw-worm fly, *Cochliomyia hominivorax*, which requires expensive preventative and therapeutic treatment (Muniz et al., 1995). Therefore the recent trend is to revert back to raising entire bulls to increase yield and eliminate productive cost associated with surgical castration complications at the expense of carcass and meat quality.

Gonadotropin releasing factor (GnRF) is released by the hypothalamus, and controls the release of LH and FSH and reproductive development in all mammals. Immunization against GnRF has been demonstrated as a simple approach for the immunocastration of

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livestock species, including cattle (Adams & Adams, 1992; Hoskinson et al., 1990; Robertson, Wilson, & Fraser, 1979) and has been proposed for the late neutering of bulls to capture the improved performance of the entire animal, while controlling unwanted behaviour by strategically timed vaccination and as a welfare-friendly, non-surgical method of castration (Bonneau & Enright, 1995; Jago, Bass, & Matthews, 1997; Robertson et al., 1979). Wider application of GnRF vaccines has been demonstrated in feedlot systems (Amatayakul-Chantler et al., 2012; Cook, Popp, Kastelic, Robbins, & Harland, 2000) and in pasture reared *Bos indicus* cattle (Hernandez et al., 2005; Ribeiro et al., 2004).

The commercialization of Improvac (Vivax in Brazil), a vaccine for use in male pigs (Dunshea et al., 2001) provided the scientific platform for the development and commercialization of Bopriva, a GnRF vaccine developed specifically for use in cattle. We report here a study with the vaccine Bopriva, designed to compare the effects of castration by either surgery or immunocastration on performance, carcass quality, and meat quality in *Bos indicus* bulls.

2. Materials and methods

2.1. Animals and study design

Nelore (*Bos indicus*) bulls raised for beef on pasture were studied to compare traditional surgical castration with immunocastration using a GnRF vaccine. The study site was located in Santa Vitória in Minas Gerais State (MG), southeast Brazil. A total of 288 Nelore bulls approximately 20 months of age were sourced at the property where the study was conducted. The clinical protocol was reviewed and approved by the Ethical Review Board (Zoetis) and the trial site was evaluated and approved for Third Party Risk Assessment (Zoetis) prior to study initiation. Written consent was obtained from the owner of the cattle prior to study initiation. A farm hand or cowboy was in attendance each day of the study, and the study veterinarian was on call during the study.

On study day -14, bulls were weighed and screened for general health and to confirm that they were entire with two descended testes. Animals were allocated by weight to one of 16 blocks each comprised of 18 bulls, and the 18 bulls from each live weight (LW) block were randomly and equally allocated to one of the two treatment groups. The treatment groups were surgical castration (**Group SC**) and vaccination with the immunocastration GnRF vaccine Bopriva (**Group IC**). For the duration of the study animals from the two study groups were kept mixed in equal proportions in one of two pasture enclosures of approximately 180 hectares each, with 144 bulls allocated per enclosure at the start of the study; with each bull provided with a minimum of 1.25 hectares space. The pasture was primarily mombaça grass (*Panicum maximum*). The study was conducted from late July to early May the following year, with high rainfall, pasture growth and nutrition from mid-September until the end of the study period, ensuring there was no need to move cattle or to provide supplementary feed during the critical post castration period. Water and mineral salt supplement were provided *ad libitum* in each enclosure. On d91 all animals were treated with Treo ACE at a 1 mL per kg body weight dose (3.5% doramectin solution, Zoetis). On d105 of the study rabies vaccine (Rai-Vet, Biovet) and foot and mouth disease vaccine (Bovicel, Valee) were given on the right side of the neck to all animals in the study.

The bulls were slaughtered on d280, with all animals slaughtered on the same day within 12 hours of arrival at a commercial abattoir. Slaughter thus was conducted 189 days (27 weeks) post the second dose of GnRF vaccine and post surgical castration. Both in-life and post slaughter phases of the study were blinded to operators, with animals and tissue samples identified by numbered tags and barcodes respectively, which prevented identification of treatment group or individual animal.

2.2. Castration

Castration of the Nelore bulls was achieved by one of two methods, either immunocastration or by surgical castration.

2.2.1. Immunocastration

The GnRF vaccine, Bopriva (Zoetis, Parkville, Australia), was formulated, tested and batch released before shipment to Brazil. Each 1 mL dose of vaccine contained 400 µg of modified GnRF peptide covalently conjugated to carrier protein, together with Advasure, an aqueous adjuvant. The GnRF vaccine was administered to cattle on the lateral aspect of the left side of the neck through a 12.5 mm 16 gauge needle inserted at a 45° angle to the surface of the neck with the needle directed cranially. A safety vaccinator (Sekurus 1 ml Fixed Dose Bopriva model, Simcro, New Zealand) was used to prevent inadvertent self-administration and to ensure subcutaneous injection, using the stippled safety shroud of the safety vaccinator to tent the skin of the animal, which also facilitated one handed administration. Injection sites were not disinfected but were selected from dry areas free of feces with new needles fitted after every ten vaccinations. The day the first dose of GnRF vaccine was administered was defined as study d0, with the second booster dose given on d91. Bulls in group IC were routinely inspected after each vaccination as part of general health checks, and on d105 at bleeding.

2.2.2. Surgical castration

Surgical castrated bulls were castrated on d91 followed standard practice in the Santa Vitória region of Brazil. Specifically, animals were restrained in a chute, a scalpel was used for scrotal incision, and the testicles were removed by traction. Neither antibiotic therapy nor analgesia was administered at the time of castration as per usual practice, and the scalpel was disinfected between animals with iodine scrub solution.

Following the standard practice at the farm to prevent screw worm infestation, all animals in this study were administered Topline topical spray (fipronil, Merial) and a subcutaneous injection of Treo ACE at 1 mL/50 kg body weight (3.5% doramectin solution, Zoetis).

On d105, bulls in group SC were closely inspected for abnormal appearance at the castration site, and recorded as purulent discharge, myiasis, hemorrhage, and spermatic cord funiculitis.

2.3. Assays

Blood was sampled by jugular venipuncture from a subset of animals ($n = 46$ for group SC and $n = 44$ for group IC) on study days 0, 91, 105 (2 weeks post second dose of vaccine) and d279, for analyses of immune responses and testosterone concentrations. On the days when Bopriva vaccine was administered (d0 and d91), blood was sampled prior to immunization. Serum was separated from clotted blood by centrifugation, aliquoted in duplicate, and frozen at -20°C until subsequent assay.

2.3.1. Anti-GnRF antibody assay

Serum anti-GnRF IgG antibody titers were determined using an optimized Dissociation Enhanced Lanthanide Fluorescence Immuno Assay (DELFA) (Ankelo et al., 2007; Bonin, Tiru, Hallander, & Bredberg-Raden, 1999) and results were expressed as relative light units (RLU). Briefly, 384-well streptavidin coated plates (Perkin Elmer Inc, Waltham, MA, USA) were incubated for 1 hour at room temperature (RT) with 1 µg/mL biotinylated modified GnRF peptide in DELFIA buffer (50 mM Tris, 0.9% NaCl, 0.05% Tween 20, 20 µM EDTA, 0.2% Ovalbumin). Plates were washed and then incubated for 1 hour at RT with 50 µL aliquots of cattle serum serially diluted to 1/800 in DELFIA buffer. After washing, 50 µL aliquots of europium labelled protein G were added (Eu-W1024-Protein G, Perkin Elmer Inc. Boston, MA, USA) and following incubation at RT for 1 hour, unbound europium labeled protein G was removed by washing. Bound lanthanide was then dissociated from the antigen

forming a highly fluorescent chelate by the addition of DELFIA Enhancement Solution (Wallac Oy, Turku, Finland). Intensity of fluorescence was measured using a time resolved fluorometer (EnVision 2102 Multilabel Reader, Perkin Elmer). Pooled non-vaccinated cattle serum served as a negative control. Unknown samples were compared to serial dilutions of a standard positive reference immune cattle serum. Calculation of a standard curve and reading of unknown samples were performed using the WorkOut2.5 software (Dazdaq Solutions Ltd, East Sussex, England). Intra- and inter-assay CV were 6.7% and 8.5%, respectively. Serum titers showed a 120 fold range from 3850 RLU titers units for negative sera to 495,000 titers units for immune sera, with 11,500 RLU being taken as the lower limit cut-off differentiating positive from negative sera.

2.3.2. Testosterone assay

Total serum testosterone concentrations in cattle sera were determined using a DIAsource Testo-EASIA kit following the instructions of the manufacturer (Testo-EASIA kit, DIAsource Immunoassays S.A., Nivelles, Belgium). Intra- and inter-assay CV with cattle sera were determined to be 4.85% and 7.15%, respectively. The range of the assay was 0 to 19 ng/mL with a detection limit of 0.05 ng/mL.

2.4. Preslaughter and abattoir measurements

LW of individual bulls in the study were obtained on d-14 for allocation to groups, and on d91 and d280, which were used to calculate ADG. ADG was calculated for the period from d91 to d280 specifically to cover the period during which surgical castration and the GnRF vaccine may have had a differing effect. In addition, the plane of nutrition was lower due to limited rainfall between d0 and d91.

Following slaughter, the following data was recorded of all carcasses: hot carcass weight (HCW), dressing percentage (HCW/LW as %), carcass pH, and dark firm and dry grading carcass (DFD). A carcass was defined as DFD if the pH of meat measured at 24 hours after slaughter was greater than or equal to 6.0.

2.4.1. Meat quality measurements

Analyses of meat quality were made using samples from the subset of bulls from each group ($n = 47$ for group SC and $n = 48$ for group IC). After chilling of these carcasses for approximately 24 hours, *Longissimus dorsi* muscle was sampled from between the 11th and 12th rib. Samples were sealed and stored frozen, then thawed for 24 to 30 h at -4°C prior to analyses. The following quality analyses were made on the samples: fat thickness, rib-eye area, meat color, fat color, cooking loss and tenderness. Fat thickness was measured on the subcutaneous fat adjacent to the *Longissimus dorsi* muscle with a digital calliper. Rib eye area was measured by tracing and data was digitized (Digicom model MDD 1812). Digitized images were analyzed and evaluated using the SPLAN planimetry program (CINAG, UNESP, Botucatu). Meat and fat color measurements were made using standard methods (Honikel, 1998) with a colorimeter (CR-410, Minolta), and measurements were taken from the center of the meat sample. Briefly, thawed samples were unpacked and the surfaces of meat and fat exposed to air for 30 minutes to allow blooming. The color of meat and associated fat were each determined as the average of five readings. Cooking loss and tenderness as Warner–Bratzler shear force (WBSF): were determined on the same samples (*Longissimus dorsi*) according to standard procedures (Savell et al., 2009). The samples were grilled to an internal temperature of 71°C , monitored with iron-constantan thermocouple (Omega Engineering Inc., Stamford, CT, USA) and a portable recording thermometer. On reaching an internal temperature of 71°C , the steaks were cooled to RT ($20\text{--}25^{\circ}\text{C}$). Weight loss by cooking was determined by calculating differences in weight of these rib samples before and after cooking. For WBSF measurements, eight 2.5-cm long cores were removed from each steak, parallel to the longitudinal orientation of muscle fibers. Each core was sheared once at $2\text{--}5^{\circ}\text{C}$ perpendicular to the muscle

fiber orientation using a Warner–Bratzler machine (TA-XT 2i, Stable Micro System (UK)) equipped with a set of Warner–Bratzler blades (capacity 25 kg and sectioning speed of 20 cm/min). The WBSF was recorded as the average of data from eight cores.

2.5. Statistical analyses

The study had a generalized block design in two pastures with the animal as the experimental unit. Data were analyzed using SAS Version 9.2. Categorical carcass data (DFD, and fat color on a scale of 1 to 7 from white to dark yellow) were summarized using frequency distributions. The dichotomized DFD and fat color data were analysed with a generalized linear mixed model with a logit link function. The fixed effect in the model was treatment and the random effects were pasture and block within pasture. Body weight, logarithm transformed anti-GnRF values and testosterone were analyzed with a general linear model for repeated measures. The fixed effects in the model were treatment, time and treatment by time interaction and the random effects were pasture, block within pasture, treatment by block within pasture interaction, animal within pasture, block and treatment, and residual. ADG was calculated using functions of the least squares mean body weights. Continuous carcass data (HCW, dressing percentage, meat pH, WBSF, fat thickness at the 12th rib, rib eye area and meat color) were analyzed with a general linear mixed model. The fixed effect in the model was treatment. The random effects in the model were pasture, block within pasture and residual. For each analysis, treatment least squares means and 95% confidence intervals (back-transformed if appropriate) were calculated (at each time point, for repeated measures analyses). If the treatment effect (and/or the treatment by time interaction, where relevant) was significant, then all possible pairwise treatment comparisons were made (at each time point for the repeated measures analyses).

3. Results and discussion

3.1. Safety of castration procedures

Following surgical castration on d91, complications were scored 2 weeks later on d105. There was a total incidence rate of observed complications in 11 of the 135 group SC bulls (8.1%), comprised of purulent discharge (3.7%), myiasis (0.7%), spermatic cord funiculitis (3.0%) and hemorrhage (1.5%). Some animals had more than one concurrent complication. Following primary vaccination (d0) and the second booster vaccination (d91) with the GnRF vaccine, there were no systemic or localized adverse events recorded in group IC bulls. There was thus a marked difference noted in the incidence of adverse sequelae between the two methods of castration. The safety profile of the GnRF vaccine, as measured by systemic events post-vaccination or localized adverse events (injection site reactions), was considered as highly acceptable in this field application, an important outcome, as others have noted that poor safety of experimental GnRF vaccines has limited commercialization (Bonneau & Enright, 1995; Meeusen, Walker, Peters, Pastoret, & Jungersen, 2007). Administration of Bopriva to cattle via the safety vaccinator reported no human exposures to Bopriva during the study. In this study following surgical castration, there was an incidence of 8.1% of complications, which is a similar rate reported in a previous study in this region, where an incidence of myiasis of 10% and a mortality rate of 0.75% was found (Silva et al., 2003). An additional negative aspect of surgical castration is a setback in growth rate. Surgical castration has been reported in *Bos indicus* cross bulls in Brazil to result in a 50% reduction in ADG compared to intact bulls in the 2 months following castration (Porto et al., 2001) and in a recent Brazil study across 4 farms, between 11% and 34% of bulls lost LW in the 28 days following castration (Carvalho et al., 2011). Although this study did not monitor setback soon after surgical castration, the improved performance of the immunocastrates over surgical castrates by 8 kg at the end of the study ($P < 0.005$, Table 2) demonstrates that immunocastration has

the potential to avoid such impacts on production resulting from surgery.

The incidence of infestation of the castration wound in group SC bulls with screwworm fly (myiasis) was 0.7% in this study, which is low for the state of Minas Gerais in southeast Brazil, possibly due to the dual treatments with fipronil and doramectin given at castration. Others have reported an incidence of myiasis of 70% without treatment and 5% with fipronil pour on in this region (Lima, Malacco, Bordin, & Oliveira, 2004). Protection of cattle following castration against natural screwworm strikes has been recognized as a very significant improvement for livestock health and management in the tropical and subtropical areas of Latin America (Muniz et al., 1995). Immunocastration may be concluded to provide potential benefits for beef producers by both avoiding myiasis and removing the need to apply costly preventative treatments.

3.2. Immune response and testosterone suppression

At d0, there was a small difference in the mean anti-GnRF titers noted, which was attributed to natural variation in low background antibody levels (Table 1). Following primary vaccination with the GnRF vaccine at day 0 there was a low but appreciable antibody response detected in group IC at d91 (Table 1). However, this immune response did not have any detectable effect on testosterone concentrations in group IC bulls at d91, with no differences noted between group IC and group SC animals, at either d0 or d91, prior to surgical castration (Table 1). Following the second dose of GnRF vaccine on d91, antibody responses were rapid with high levels detected by d105 in group IC bulls (Table 1) and were associated with suppressed low testosterone concentrations fourteen days after the booster dose at d105 (Table 1). Notably, the mean testosterone concentration in group IC bulls at d105 was not different to that in the group SC bulls, indicating that immunocastration achieved similar levels of suppression of testosterone as obtained via surgical castration. By d279 prior to slaughter, the group IC mean testosterone concentration had risen to 6 ng/mL (Table 1), similar to pre-vaccination levels, although 65% of animals assayed at that time point in group IC still had testosterone levels below 5 ng/mL. Thus vaccination of *Bos indicus* bulls with two doses of the GnRF vaccine Bopriva at an interval of 13 weeks was shown here to provide a duration of effect of 188 days (approximately 27 weeks) in approximately 65% of animals. A recent study in *Bos indicus* cross bulls with the same GnRF vaccine given at a shorter 7 week interval between prime and boost, was shown to suppress testosterone for at least 105 days (Amatayakul-Chantler et al., 2012). As anticipated the group SC bulls had only residual levels of testosterone detected at d280, the very low concentration detected is attributed to a minor contribution from the adrenal glands in the castrated animals.

In this field study with a commercially available GnRF vaccine, efficacy (i.e. proportion of vaccinated animals with suppressed testosterone) was considered as high. Of the 43 group IC bulls analyzed at d105, one bull showed only background levels of anti-GnRF antibody by DELFIA but had levels of testosterone <0.1 ng/mL, and one further bull had a moderate immune response with serum levels of testosterone >5 ng/mL across the study, characteristic of entire bulls. It was not able to be determined whether these data were the result of failure of the vaccine to induce an effective immune response, a lack of compliance to administer 2 doses of vaccine to each animal, or with sample logistics in this 288 cattle field trial.

The efficacy and duration of testosterone suppression following immunization of cattle with experimental GnRF vaccines has been reported as variable. Immunization of *Bos taurus* bulls with 2 doses of an oil adjuvanted vaccine gave poor suppression of testosterone, with efficacy depending on genetic background and careful selection of calves (Price, Adams, Huxsoll, & Borgwardt, 2003), while vaccination of *Bos indicus* cross bulls did not achieve castration levels of testosterone, despite the use of 3 or 4 doses of an experimental GnRF vaccine with a water in oil adjuvant (Hernandez et al., 2005). The differences in outcomes to vaccination against GnRF is likely due to variations in formulation, specifically the active component of the vaccine, differences in the type of adjuvant used, and number of and interval between, doses.

3.3. Growth and yield

There were no differences observed in the group mean LW at either d-14 or at d91 (Table 2). The similar growth rates of the group SC and group IC bulls during this period indicate that the primary dose of GnRF vaccine given at d0 to group IC bulls had no effect on growth rate. This is further supported by the data showing that at d91, there were no differences between groups in mean testosterone levels and only very low levels of antibody to GnRF in the group IC bulls post the primary dose of Bopriva (Table 1). The bulls were weighed prior to slaughter on d280, 27 weeks following castration. The group IC bulls had a mean LW 8 kg higher than the group SC bulls ($P < 0.005$, Table 2). This higher final LW was associated with a significantly higher ADG for the group IC bulls when compared to the group SC bulls ($P < 0.005$, Table 2). The ADG was calculated for the period d91 to d280 (Table 2), being the period that both surgical castration and immunocastration would have been anticipated to have had an effect. After slaughter and evisceration the higher LW of the group IC bulls translated to a higher mean HCW compared to group SC bulls of 8.4 kg ($P < 0.0001$, Table 2). In addition, the group IC bulls had a higher dressing percentage when compared to the group SC bulls ($P < 0.005$, Table 2). These growth improvements in immunocastrated bulls compared to surgically castrated bulls for LW, HCW, ADG and dressing percentage are counter to the majority of previously published results. Previous comparisons of the performance

Table 1
Serum anti-GnRF titers and serum testosterone concentrations.

Group	Anti-GnRF Titer (DELFA - relative light units)				Testosterone (ng/mL)				% of animals with < 5ng/mL Testosterone value
	Day 0	Day 91	Day 105	Day 279	Day 0	Day 91	Day 105	Day 279	
SC	3855 ^a	5864 ^a	5574 ^a	5446 ^a	5.3 ^a ± 0.9	9.1 ^a ± 1.0	0.1 ^a ± 0.2	0.6 ^a ± 0.9	98%
Surgically Castrated Bulls	± 175 n = 41	± 658 n = 46	± 624 n = 43	± 607 n = 42	n = 41	n = 46	n = 43	n = 42	(41/42)
IC	4347 ^b	9909 ^b	64,449 ^b	6787 ^a	3.6 ^a ± 0.9	7.0 ^a ± 0.9	0.6 ^a ± 0.2	6.0 ^c ± 0.9	65%
Bopriva Immunocastrated Bulls	± 197 n = 41	± 1164 n = 42	± 7400 n = 42	± 785 n = 39	n = 41	n = 42	n = 42	n = 40	(26/40)

Values are for surgically castrated bulls (group SC) and GnRF vaccinated bulls (group IC), least square means ± SEM with number assayed (n).

Vaccination with the GnRF vaccine Bopriva, (Pfizer Animal Health, Australia) was on d0 and d91.

Unlike letters at each time point denotes significant differences between groups: b < 0.05, c ≤ 0.002.

Note that although at day -14 there were 46 animals from SC group and 44 from IC group designated as the subset for serology and meat quality assessment, data presented here from day 0 onwards does not represent the whole subset due to one of the following reasons; data were not collected; animal missing on the day or the sample was lost.

Table 2
Weight, growth and yield data.

Group	LW, kg d -14	LW, kg d 91	LW, kg d 280	ADG, kg/d	HCW, kg	Dressing percentage
SC	356 ^a ± 3.3	359 ^a ± 3.8	502 ^a ± 3.8	0.75 ^a ± 0.01	264.4 ^a ± 5.4	52.8 ^a ± 0.6
Surgically Castrated Bulls	n = 138	n = 134	n = 133	n = 133	n = 130	n = 130
IC	356 ^a ± 3.3	361 ^a ± 3.8	510 ^b ± 3.9	0.79 ^b ± 0.01	272.8 ^c ± 5.4	53.5 ^b ± 0.6
Bopriva Immunocastrated Bulls	n = 132	n = 131	n = 124	n = 124	n = 117	n = 117

Live Weight (LW), Hot Carcass Weight (HCW), Dressing Percentage (HCW/LW expressed as %) and ADG from d91 to d280.

Values are least square means ± SEM. n = number of animals by group observed at each time point.

Bulls were surgically castrated at d91 or vaccinated with GnRF vaccine at d0 and d91 and slaughtered 27 weeks later at d280.

Means in the same column with unlike superscripts differ significantly. b < 0.005, c < 0.0001.

Note the variation in number of animals on d91 and d280 are due to one of the following reasons; data were not collected; animal missing on the day or the sample was lost.

of immunocastrates with surgically castrated bulls have predominantly shown no differences in growth rates, whether in *Bos indicus* bulls on pasture (Hernandez et al., 2005; Ribeiro et al., 2004), or in *Bos taurus* bulls in a feedlot (Adams, Daley, Adams, & Sakurai, 1993; Cook et al., 2000). In the study reported here, the return of low to moderate levels of testosterone may have been sufficient to have beneficial effects on growth rate and final LW and HCW.

The method used to castrate bulls has been shown to induce varying degrees of stress and affect average daily food intake and ADG (Fisher, Crowe, Alonso de la Varga, & Enright, 2006; Knight, Cosgrove, Lambert, & Death, 1999). In this study the improved performance of the immunocastrates may have been the result of less set-back due to reduced stress, more consistent food intake and a higher ADG when compared to surgically castrated animals. With the data available in this study, it is not possible to determine whether return of low levels of testosterone or reduced setback or the combination of both were responsible for the improved performance.

3.4. Carcass quality

Following slaughter, measurements of muscle pH and the percentage classified as DFD (pH > 6.0) were determined on the carcasses of all animals. Further carcass quality measurements were made on samples from the subset of animals from each group (group SC, n = 47, group IC, n = 48) of rib eye area and fat depth at the 12th rib. The data presented in Table 3, show there were no differences between the 2 groups of bulls for each of these measurements of carcass quality. The lack of any differences between groups in the carcass quality data is to a degree predictable, since both groups had been castrated at approximately the same time (d91), with a slight delay in generation of an effective immune response in group IC bulls. These carcass quality findings are considered important, as the higher growth rate found in group IC bulls may have impacted certain quality characteristics; a higher growth rate may have removed any improvements in carcass quality obtained by castration, such as greater fat cover, tenderness and cooking loss. While previous studies in *Bos indicus* bulls on pasture in Brazil showed no differences in carcass quality between immunocastrated and surgically castrated groups (Hernandez et al., 2005; Ribeiro et al.,

2004), it should be noted that in those previous studies there were no associated improvements in performance.

Furthermore the use of Bopriva improved certain carcass and meat qualities such as 12th rib fat coverage, meat tenderness and USDA grading in *Bos indicus* cross bulls under Mexican feedlot conditions when compared to non-castrates in the presence and absence of implants (Amatayakul-Chantler et al., 2012). In addition, that previous study also demonstrated improved performance in Bopriva treated bulls when compared to non-castrated bulls in the presence of implants.

3.5. Meat quality

Quality measurements were made on samples from the subset of animals from each group (group SC, n = 47, group IC, n = 48). Samples taken from the region of the 12th rib were analyzed for meat color, fat color, cooking loss and WBSF tenderness. The data showed that for the majority of these meat quality characteristics, there were no differences between the treatment groups, irrespective of the method of castration (Tables 3 and 4). One significant difference found was a lower meat color b* (yellow to blue) in the group IC bulls (Table 4), while it was notable the fat color b* was not different (Table 4). This effect on meat color b* is considered minor and irrelevant to general meat quality (Roca, R.O. personal communications). Note that although no difference was seen in the WBSF between the two groups, these numbers are high due to no aging process, as the carcass was chilled for 24 hours, deboned, packed and frozen to transport to meat lab for further analysis.

An important conclusion from these data is that there were no negative effects of GnRF vaccination, on meat quality despite immunocastration providing growth advantages. Recovery of testosterone concentrations in the group IC animals towards the end of the study may have adversely affected temperament, and excitable temperament has been shown previously in *Bos indicus* bulls cattle to be correlated with meat quality (Voisinet, Grandin, O'Connor, Tatum, & Deesing, 1997). In the study by Voisinet et al., animals with a more excitable temperament had an increased incidence of dark cutters and higher WBSF shear force measurements.

Table 3
Carcass quality measurements.

Group	Muscle pH	DFD % Carcasses	Ribeye area cm ²	Fat depth 12th rib, mm.	WB shear force kg	Cooking loss %
SC	5.73 ^a	18.5 ^a	72.1 ^a	3.71 ^a	9.07 ^a	23.23 ^a
Surgically Castrated Bulls	± 0.02	n = 130	± 1.5	± 0.23	± 0.29	± 0.87
IC	5.75 ^a	22.0 ^a	72.3 ^a	4.08 ^a	9.02 ^a	23.88 ^a
Bopriva Immunocastrated Bulls	± 0.02	n = 118	± 1.5	± 0.23	± 0.28	± 0.86
	n = 118		n = 48	n = 48	n = 48	n = 48

Values are least square means ± SEM. Means in the same column with unlike superscripts differ significantly.

Note the variation in number of animals are due to one of the following reasons; data were not collected; animal missing on the day or the data/sample was lost.

Table 4
Meat and Fat Color Characteristics: Luminosity, Color A and Color B.^a

Group	Meat color L*	Meat color a* (Red)	Meat color b* (Yellow)	Fat color L*	Fat color a* (Red)	Fat color b* (Yellow)
SC	39.05 ^a	19.05 ^a	7.21 ^a	64.55 ^a	9.70 ^a	11.97 ^a
Surgically Castrated Bulls	± 0.29	± 0.22	± 0.13	± 0.49	± 0.40	± 0.39
IC	38.64 ^a	19.13 ^a	6.84 ^b	65.14 ^a	9.00 ^a	12.08 ^a
Bopriva Immunocastrated Bulls	± 0.23	± 0.21	± 0.13	± 0.48	± 0.39	± 0.38

Values are least square means ± SEM.

Means in the same column with unlike superscripts differ significantly. $b < 0.05$.

^a Meat and fat color measurements were determined on a subset of animals from each group: group SC, $n = 47$, group IC, $n = 48$.

Previous studies with an experimental GnRF vaccine in *Bos indicus* bulls also showed no difference in color, cooking loss, or tenderness (Ribeiro et al., 2004), however, as noted above there was no performance advantage from immunocastration in that previous study. A further study analyzing meat from immunocastrated *Bos indicus* bulls showed only minor differences in fatty acid profiles between surgical and immunocastrates (Ruiz et al., 2005).

4. Conclusions

Immunization of 20 month old *Bos indicus* bulls with the GnRF vaccine Bopriva provides a consistent immune response to GnRF peptide and is efficacious at suppressing testosterone to levels indistinguishable from castrates within 14 days of the second vaccination. Immunocastration provides marked production improvements for LW, HCW, ADG and dressing percentage, without detrimental effects on carcass quality parameters (carcass pH, incidence of DFD, rib eye area, and fat depth) or on meat quality characteristics (meat color, fat color, cooking loss and tenderness). Our concluding hypothesis is that improved performance in immunocastrated bulls is a result of either low levels of testosterone towards the end of the growth period of 27 weeks exerting a natural anabolic effect or from an absence of a post surgery setback, or a combination of both mechanisms. Importantly, irrespective of the underlying mechanism(s), the improved performance while maintaining equivalent carcass or meat quality makes this an attractive approach for producers that are raising *Bos indicus*.

In addition to production gains, there are two further important potential benefits provided by the GnRF vaccine Bopriva: (a) improved animal welfare as immunocastration would replace conventional surgical intervention usually conducted without analgesia and (b) immunocastration avoids the creation of a wound, thus providing a direct approach to the control of screw worm strike and so would reduce the costly preventative treatment of castration wounds.

Immunocastration offers an attractive alternative for Latin American countries where *Bos indicus* breeds are used. The trend to raise entire bulls emphasizes the need for improvement in meat quality for export purposes in addition to in country consumption. Bopriva may provide a welfare friendly alternative to surgical castration and compared to raising entire bulls, improves carcass and meat quality while maintaining performance.

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