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Immunocastration of Bos indicus × Brown Swiss bulls in feedlot with gonadotropin-releasing hormone vaccine Bopriva provides improved performance and meat quality

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ABSTRACT: The objective of this study was to determine the effects of a GnRH vaccine on feedlot performance and meat quality in Bos indicus Zebu × Brown Swiss bulls. The study was a 2 × 2 factorial arrangement of treatments with 1,600 bulls allocated by BW into 4 groups of ~400 animals. The GnRH vaccine (Bopriva) was injected on d 0 and 42, and anabolic implants given on d 0 (Component E-S) and d 84 (Synovex Choice). Group designations were: Con = placebo control; Imp = implants alone; Vac = GnRH vaccine alone; and Vac+Imp = GnRH vaccine together with implants. The second GnRH vaccination at d 42 resulted in elevated titers of IgG antibody and suppressed concentrations of testosterone in vaccinated groups (Vac and Vac+Imp) at d 56 (P < 0.001), with titers and suppressed testosterone persisting to d 147 (P < 0.001). Groups Vac and Vac+Imp had reduced testes weights at slaughter on d 147 (P < 0.001). Bulls in group Vac were not different in final BW, HCW, or ADG (d 42 to 147) relative to bulls in group Con. Bulls in group Vac+Imp had greater final BW than bulls in group Imp (P = 0.008) and greater BW than bulls in group Vac and group Con (P < 0.001). The HCW of Vac+Imp bulls was greater than the Vac or Con bulls (P < 0.001) but was not different to the Imp bulls (P = 0.294). Improved ADG was obtained by vaccination with the GnRH vaccine, in the presence of implants (group Vac+Imp compared with group Imp, P < 0.001) or absence of implants (group Vac compared with group Con, P = 0.028). Meat quality of bulls receiving the GnRH vaccine was improved irrespective of implant status, with a 1.6- to 2.6-fold increase in the proportion of bulls in groups Vac and Vac+Imp, respectively, grading as USDA Choice (P < 0.002) and with greater fat depth at the 12th rib (P < 0.001). Meat tenderness was improved in the vaccine groups (Vac and Vac+Imp) compared with groups Con and Imp (P < 0.004). Use of the GnRH vaccine Bopriva in Bos indicus × Brown Swiss bulls finishing in a feedlot under Mexican husbandry conditions can provide improved performance in combination with implants (increased BW and ADG) and improved meat quality, with or without implants, and in particular, better USDA carcass grading and loin fat cover.

Key words: bull, feedlot, gonadotropin-releasing hormone vaccine, immunocastration, meat quality, performance

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INTRODUCTION

Gonadotropin-releasing hormone is the key hypothalamic factor controlling sexual and reproductive function and development in animals. In bulls, GnRH vaccines induce neutralizing antibodies, resulting in immunocastration characterized by suppression of LH and testosterone (Robertson et al., 1979; Bonneau and Enright, 1995; Geary et al., 2011). Beef cattle production often uses castration as a key management tool to provide major advantages, including better carcass quality through increased fat deposition, reduced aggressive and sexual behaviors resulting in ease and safety in handling, less carcass damage, and improved animal welfare (Huxsoll et al., 1998; Bouissou et al.,...
Conversely, raising intact bulls offers major advantages, including better ADG and more efficient feed use (Field, 1971; Seideman et al., 1982; Lee et al., 1990). Vaccination against GnRH has been proposed for strategic late neutering of male animals, including bulls, and as a welfare-friendly, nonsurgical method of castration (Robertson et al., 1979; Adams and Adams, 1992; Bonneau and Enright, 1995). Thus, immunocastration could potentially realize production gains from raising entire male cattle, capture improved meat quality, and control unwanted behavior by strategically timed vaccination. The commercialization of Improvac, a vaccine for use in male pigs (Dunshea et al., 2001), provided the scientific platform for Bopriva, a GnRH vaccine developed specifically for use in cattle. Beef production systems in central and southern Mexico using Bos indicus breeds produces lower quality carcasses (Mendez et al., 2009). A product that could improve meat quality would benefit producers in those regions. The study described here was conducted in Bos indicus bulls, using the GnRH vaccine Bopriva, to determine the effect of immunocastration on performance and meat quality in a Mexican feedlot.

**MATERIALS AND METHODS**

The protocol for this field study was reviewed and approved by the appropriate animal use Ethical Review Board (Pfizer) before study commencement, and also underwent third-party risk assessment. Veterinary care was continuously available for the study’s duration.

**Vaccines and Vaccination**

The GnRH vaccine, Bopriva (Pfizer Animal Health, Parkville, Australia), was formulated and batch released before shipment to Mexico. Each 1-mL dose contained 400 µg of a conjugate of modified GnRH peptide covalently linked to carrier protein, together with Advasure, a low reactogenic aqueous adjuvant for use in cattle. The vaccine was administered to cattle on the lateral aspect of the left side of the neck through a 12.5-mm, 16-gauge needle, using a safety vaccinator (Sekurus, 1 mL fixed dose Bopriva model fitted with a BA11 safety shroud; Simcro, New Zealand) to prevent inadvertent self administration. The stippled safety shroud of this safety vaccinator tented the skin of the animal, facilitating administration with 1 hand and assured consistent delivery by subcutaneous injection. A solution of 5% dextrose in water (Baxter, Deerfield, IL), given as a 1-mL dose, was injected subcutaneously as a placebo vaccine.

**Hormonal Implants**

Implants were those routinely used at the feedlot. Component E-S (Elanco, Indianapolis, IN) was administered to cattle on d 0 at the recommended dose, including 9 pellets that contained 200 mg of progesterone, 20 mg of estradiol benzoate, and 29 mg of tylosin tartrate. Reimplantation on d 84 with Synovex Choice (Pfizer Animal Health, Kalamazoo MI) included 4 pellets per dose, containing 100 mg of trenbolone acetate and 14 mg of estradiol.

**Animals and Study Design**

*Bos indicus* Zebu × Brown Swiss bulls, sourced from southern Mexico, were purchased in multiple lots over a 2-mo period and accumulated into 10 blocks of 160 animals at a commercial feedlot in the state of Sonora, located in northern Mexico, before allotment and entry to the study. A total of 1,600 cattle were blocked by BW and divided equally into 4 treatment groups (Table 1). Cattle were housed in outdoor feedlot pens, with 10 pens per treatment group, at a stocking density not less than 11 m² per animal. Bulls were only entered into the study if they had 2 descended testicles and had a maximum of 2 permanent central incisors, indicating they were younger than 18 mo of age. At the initiation of the study, cattle were assessed to be 13 to 14 mo of age, based on dentition and BW, with an initial mean BW of 256 kg (range of 200 to 329 kg). Treatments and animal identification were blinded for all observations, analyses, and assays. On arrival at the feedlot, all cattle were treated metaphylactically with Excede (Pfizer Animal Health, New York, NY) to prevent bovine respiratory disease, Bovi-vitraz for ear ticks (Bayer Animal Health, Shawnee, KS), and Dectomax (Pfizer Animal Health) as a dewormer. The following vaccines were administered for the protection against respiratory diseases: Bovi-Shield Gold FP5, One Shot, and TSV 2 (Pfizer Animal Health). After allowing

**Table 1.** Experimental groups of bulls and treatments used in the present study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>Placebo vaccine</td>
<td>0 and 42</td>
</tr>
<tr>
<td>Imp</td>
<td>Anabolic implants¹</td>
<td>0 and 84</td>
</tr>
<tr>
<td>Vac</td>
<td>GnRH immunization²</td>
<td>0 and 42</td>
</tr>
<tr>
<td>Vac+Imp</td>
<td>GnRH immunization²</td>
<td>0 and 42</td>
</tr>
<tr>
<td></td>
<td>Anabolic implants¹</td>
<td>0 and 84</td>
</tr>
</tbody>
</table>

¹Anabolic implants given on d 0 and 84. Implantation on d 0 was with Component E-S (Elanco, Indianapolis, IN), given as a dose of 9 pellets containing 200 mg progesterone, 20 mg estradiol benzoate, and 29 mg tylosin tartrate. Reimplantation on d 84 was with Synovex Choice (Pfizer Animal Health, Kalamazoo, MI), each bull receiving 4 pellets containing 100 mg trenbolone acetate and 14 mg estradiol.
²GnRH vaccine, Bopriva (Pfizer Animal Health, Parkville, Australia), was administered as a 1-mL dose via subcutaneous injection.
14 d to acclimatize, animals were weighed and randomly allotted to treatments (d –1). The day GnRH vaccine was administered was defined as study d 0. The treatments and group designations were: control bulls given placebo vaccine (Con); implanted-only bulls that received anabolic implants on d 0 and 84 (Imp); bulls vaccinated with 2 doses of Bopriva GnRH vaccine, the first dose given on d 0 and a second booster dose on d 42 (Vac); and bulls immunized with Bopriva GnRH vaccine on d 0 and 42, and anabolic implants on d 0 and 84 (Vac+Imp; Table 1). Ten representative bulls from each pen of 40 bulls (25% of all bulls) were randomly selected and identified, forming a subset of 100 bulls from each treatment group. These subset bulls were sequentially bled for serology and used for detailed meat quality analyses postslaughter.

Cattle were fed 2 to 3 times daily, with 25 cm of bunk-space per bull. Daily rations were identical across all pens within each allotment block. The diet was typical of feedlot formulations for northern Mexico and the formulated mix was introduced in 3 phases. The final ration contained steamed flaked corn (57%), dried distillers grains (11%), wheat and oat straw (10%), molasses (10%), liquid fat (4%), whole cottonseed (3%), cottonseed meal (2%), plus vitamins and minerals. The energy content of this ration was 2.28 NEm Mcal/kg and 1.60 NEg Mcal/kg. Water was provided ad libitum. The bulls were slaughtered on the same day. Slaughter occurred between 100 and 112 d post the second dose of GnRH vaccine.

**Blood Sampling**

Sera were obtained from 100 bulls from each treatment group by sequentially blood sampling the 10 subgroup animals from each pen on study d –1, 42, 56, 84, 112, and 147. Blood samples of 20 mL were collected from each animal via tail or jugular venipuncture into vacuum serum separation tubes, using 18- or 20-gauge needles. Blood samples were allowed to clot for 2 h at ambient temperature. After centrifugation (2000 × g, 10 min), serum was stored frozen (−18°C) until analyses for antibody titers to GnRH peptide and testosterone concentrations, as indicated below.

**GnRH Antibody Assay**

Serum GnRH IgG antibody titers were determined by further development of a dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA; Bonin et al., 1999; Ankelo et al., 2007). Briefly, 384-well, streptavidin-coated plates (PerkinElmer Inc., Waltham, MA) were coated for 1 h at room temperature with 1μg/mL of biotinylated-modified GnRH peptide in DELFIA buffer (50mM Tris, 0.9% NaCl, 0.05% Tween 20, 20μM EDTA, 0.2% Ovalbumin). Plates were washed and then incubated for 1 h at room temperature with 50-μL of cattle serum in DELFIA buffer serially diluted starting from 1/800. After washing, 50-μL aliquots of europium-labeled protein G were added (Eu-W1024-Protein G, PerkinElmer Inc., Boston, MA) and after incubation at room temperature for 1 h, 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, PerkinElmer). Nonvaccinated cattle serum served as a negative control. Unknown samples were compared with serial dilutions of a standard positive reference immune cattle serum. Calculation of the standard curve and reading of unknown samples were performed using the WorkOut 2.5 software (Dazdaq Solutions Ltd, East Sussex, England). Intra-assay and interassay CV were 6.7% and 8.5%, respectively. Serum titers showed a range from 3,850 titer units for negative sera to 495,000 titer units for immune sera, with 11,500 relative light units being taken as the lower limit cutoff, differentiating positive from negative sera.

**Testosterone Assay**

Total serum testosterone in cattle sera was determined using a DIAsource Testo-Easia kit, following manufacturer instructions (Testo-EASIA kit, DIAsource Immunoassays S.A., Nivelles, Belgium). Intra-assay and interassay CV with cattle serum were determined to be 4.9% and 7.2%, respectively. The range of the assay was 0 to 19 ng/mL, with a detection limit of 0.05 ng/mL.

**Feedlot and Abattoir Data Collection**

Body weight of individual bulls was obtained on d –1 for allocation to groups and on d 42, 56, 84, 112, and 140, which was used to calculate ADG. The ADG was calculated for the period from d 42 to 147 to cover the period during which the GnRH vaccine was anticipated to have an effect. Feed consumption per pen was recorded daily (adjusted for orts) and used to calculate G:F by pen. Health observations were made daily by pen riders in the feedlot, with particular attention paid post-vaccination to assess safety of the GnRH vaccine under field conditions, with additional observations over the first 6 h postadministration. Injection site reactions were also evaluated during subsequent entry to the chute. After slaughter, the carcass data collected at the abattoir included HCW, dressing percentage, and paired testes.
weights after trimming of the epididymis. After overnight chilling, pH (Hanna pH meter HI99163, Hanna Instruments Inc., Woonsocket, RI) and color measurements at the 12th rib were obtained. For color, allowing 15 to 20 min bloom, 2 measurements were made by means of a Gretag-Macbeth ColorEye XTH (New Windsor, NY). The average lightness (L*), redness (a*), and yellowness (b*) of each muscle sample was recorded. A carcass with a pH measurement >6.0 was scored as DFD, consistent with Mexican grading. Measurements, including fat thickness and ribeye area at the 12th rib, were obtained to assist with the calculation of carcass USDA quality and yield grades. Steak samples (LM) of 2.5-cm thickness were excised at the 12th rib for Warner-Bratzler shear force (WBSF) analysis. These were sampled from the subset of carcasses from each treatment (~90 from original 100) and stored frozen. Samples were thawed for 24 to 30 h at 2 to 4°C before determining WBSF, according to American Meat Science Association Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). The steaks were broiled to an internal temperature of 70°C (Broiler Daweood, Mod DEG-22, 1500W, Imported by Gigante S.A de C.V., Mexico DF, Mexico), which were monitored with iron-constantan thermocouples (Omega Engineering Inc., Stamford, CT) and a portable recording thermometer. Upon reaching an internal temperature of 70°C, the steaks were removed from the broilers and allowed to cool to room temperature (20 to 25°C). 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 perpendicular to muscle fiber orientation, using a Warner-Bratzler machine (G-R Elec. Mfg. Co., Manhattan, KS).

**Statistical Analyses**

The study had a randomized complete block design within multiple purchased lots with the pen as the experimental unit. Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Categorical carcass data (DFD prevalence, USDA carcass grade, and USDA yield grade) were summarized using frequency distributions and analyzed with a generalized linear mixed model with a logit link function. The fixed effect in the model was treatment and random effects were purchased lots, block within purchased lots, treatment by block within purchased lots interaction, and residual. Continuous carcass data, as well as testes weights and feed efficiency, were analyzed with a general linear mixed model with the same fixed and random effects. Body weight, logarithm-transformed GnRH antibody values, and testosterone concentrations 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 purchased lots, block within purchased lots, treatment by block within purchased lots interaction, animal within purchased lots, block and treatment, and residual. For each analysis, treatment least squares means and 95% confidence intervals were calculated (at each time point for repeated measures analyses). If treatment effect or treatment by time interaction were significant, then all possible pairwise treatment comparisons were made at each time point for the repeated measures analyses. The ADG was calculated using functions of the least squares mean BW.

**RESULTS**

Immune responses to GnRH and hormone concentrations were assayed in sequential blood samples taken from the 100 subset bulls representative of the 400 animals in each group. The IgG antibody responses to GnRH peptide, assayed by DELFIA, showed that on d 42 after primary immunization on d 0, a small response (P < 0.001) was induced in bulls from the GnRH-vaccinated groups Vac and VAC+Imp (Figure 1). A strong response was detected 14 d after the second vaccination (d 56) in the Vac and VAC+Imp groups (P < 0.001), which was maintained at a greater titer than the base levels of antibody in the unvaccinated groups Con and Imp for the duration of the study. There were no differences between the mean GnRH antibody titers of the 2 vaccinated groups at any time point (Figure 1).

Testosterone concentrations were within the range expected of normal postpubertal bulls in each group at d 0 (Figure 2) and BW were not different between the 4 groups on d 1 (Table 2). Bulls receiving GnRH vaccine (groups Vac and VAC+Imp) had slightly decreased concentrations of testosterone after primary vaccination at d 42 compared with placebo bulls (P < 0.001, Figure 2). Post the second dose of vaccine, both immunocastrated groups had markedly suppressed testosterone concentrations (P < 0.001) within 14 d of immunization and remained suppressed for the duration of the study, from d 56 to 147, irrespective of implant status (Figure 2). Compared with placebo bulls, animals that received anabolic implants alone had decreased testosterone at d 42 (group Con compared with group Imp, P < 0.001) and concentrations remained suppressed from d 56 to 112 (P < 0.001).

In this field study, efficacy of the GnRH vaccine was 97% across the groups of vaccinated bulls. In the subgroups bled for serology from groups Vac and VAC+Imp, 192 of 198 bulls had GnRH IgG antibody responses greater than the DELFIA assay cutoff at d 56 (i.e., 14 d after the second dose of GnRH vaccine). Testosterone
concentrations were suppressed at less than 5ng/mL in 192 of 198 bulls from the subgroups of Vac and Vac+Imp at d 56.

Safety of the GnRH vaccine was monitored by daily observation in the pens and by inspection in the chute after each vaccination. There were no clinical signs of systemic effects observed. During the observation period, visible swellings were observed at the injection site of groups Vac and Vac+Imp at a frequency of between 12% and 55%. Swellings had resolved to be unobservable within 28 d of injection and were not notable when passing through the chute on d 42, with 1 exception. One bull in the Vac group developed an abscess at the site of primary injection of vaccine that resolved without treatment by d 84.

Weights of paired testes were determined after removal of the epididymis (Table 3). The testes in groups Vac and Vac+Imp were smaller than in unvaccinated bulls, with lower weights, irrespective of implant administration (group Con compared with group Vac; \( P < 0.001 \)) and implant only group (group Imp compared with group Vac+Imp; \( P < 0.001 \)). Interestingly, implanted bulls had a reduction in testes weights compared with placebo control bulls (group Imp compared with group Con, \( P < 0.001 \); Table 3), demonstrating the suppressive effect of implants.

Performance Measurements

Without implants, the GnRH vaccine treated bulls showed no significant difference in growth performance to control bulls, with minor inconsistent variations between time points during the study and no significant difference in BW at completion on d 147 (group Vac compared with group Con, \( P = 0.618 \); Table 2). The BW recorded during the study showed bulls in the implanted groups were consistently heavier than nonimplanted

### Table 2. Body weight (kg; least squares mean ± SEM) of feedlot bulls

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>d 1</th>
<th>d 42</th>
<th>d 56</th>
<th>d 84</th>
<th>d 112</th>
<th>d 147</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>254.2 ( \pm ) 4.0</td>
<td>323.6 ( \pm ) 4.2</td>
<td>344.4 ( \pm ) 4.2</td>
<td>380.6 ( \pm ) 4.3</td>
<td>400.1 ( \pm ) 4.3</td>
<td>434.4 ( \pm ) 4.4</td>
</tr>
<tr>
<td>Imp</td>
<td>254.5 ( \pm ) 4.0</td>
<td>323.9 ( \pm ) 4.2</td>
<td>355.6 ( \pm ) 4.3</td>
<td>386.7 ( \pm ) 4.3</td>
<td>416.0 ( \pm ) 4.4</td>
<td>451.5 ( \pm ) 4.5</td>
</tr>
<tr>
<td>Vac</td>
<td>254.7 ( \pm ) 4.0</td>
<td>320.6 ( \pm ) 4.2</td>
<td>342.5 ( \pm ) 4.3</td>
<td>374.7 ( \pm ) 4.4</td>
<td>399.5 ( \pm ) 4.4</td>
<td>436.1 ( \pm ) 4.4</td>
</tr>
<tr>
<td>Vac+Imp</td>
<td>253.7 ( \pm ) 4.9</td>
<td>322.5 ( \pm ) 4.1</td>
<td>345.9 ( \pm ) 4.1</td>
<td>384.3 ( \pm ) 4.2</td>
<td>414.3 ( \pm ) 4.3</td>
<td>460.1 ( \pm ) 4.4</td>
</tr>
</tbody>
</table>

\( a \) Within a column, least squares means (LSM) ± SEM values without a common superscript differ (\( P < 0.05 \)).

\( 1 \) Con = control; placebo vaccine nonimplanted. Imp = implanted; placebo vaccine implanted. Vac = vaccinated, GnRH vaccine nonimplanted. Vac+Imp = vaccinated + implanted, GnRH vaccine implanted.

\( 2 \) At d147, Con group compared with Imp group, or compared with Vac+Imp group (\( P < 0.001 \)); Vac group compared with Imp or Vac+Imp group (\( P < 0.001 \)).
groups at d 112 and had greater carcass weights at each time point until the end of the study at d 147 (groups Imp and Vac+Imp compared with groups Con and Vac; \( P < 0.001 \)). Comparing final BW across groups showed 2 notable outcomes. First, bulls receiving GnRH vaccine together with anabolic implants had a greater mean final BW than that of the other 3 groups (Table 2), being heavier by 25.7 kg (least squares mean group mean) than placebo controls (group Vac+Imp compared with group Con, \( P < 0.001 \)), heavier by 8.6 kg than implants alone (group Vac+Imp compared with group Vac, \( P = 0.008 \)), and heavier by 24 kg than GnRH vaccine alone (group Vac+Imp compared with group Vac, \( P < 0.001 \)). The trends in group performance seen in BW were reflected in HCW, albeit with a reduced difference among groups (Table 3). Bulls receiving GnRH vaccine together with anabolic implants had greater mean HCW by 13.76 kg than placebo controls (group Vac+Imp compared with group Con, \( P < 0.001 \)), heavier by 17.48 kg than vaccine alone (group Vac+Imp compared with group Vac, \( P < 0.001 \)), and numerically heavier by 2.99 kg than implants alone (group Vac+Imp compared with group Vac, \( P = 0.294 \)). There was no difference (\( P > 0.05 \)) in the group mean HCW of placebo control bulls and GnRH-vaccinated animals (group Con compared with group Vac, \( P = 0.192 \)).

Average daily gain was calculated for groups from d 42 to 147, with significant differences determined among groups (Table 3). Administration of GnRH vaccine resulted in a greater ADG when compared with the similarly treated control group, either in the absence of implants (group Vac compared with group Con, \( P = 0.028 \)) or given together with implants (group Vac+Imp compared with group Imp, \( P < 0.001 \)). The ADG of the bulls receiving GnRH vaccine together with anabolic implants was greater than in bulls receiving implants alone (group Vac compared with group Vac+Imp, \( P < 0.001 \)) and the combined treatment bulls had the greatest ADG of the 4 groups (Table 3). As expected, administration of anabolic implants alone increased ADG compared with bulls without implants (group Imp compared with group Con or group Vac; \( P < 0.001 \)).

\[ \text{Average daily gain was calculated for groups from d 42 to 147.} \]

**Table 3. Performance (least squares means ± SEM) of feedlot *Bos indicus* crossbred bulls.**

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>ADG(^3,4) d 42 to 147</th>
<th>Feed intake(^3,) kg/d</th>
<th>G:F ratio(^3) d 42 to 147</th>
<th>BW, kg d 147</th>
<th>HCW, kg</th>
<th>Dressing %(^4)</th>
<th>Combined tests weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>1.05(^a) ±0.02</td>
<td>8.07(^a) ±0.23</td>
<td>0.14 ±0.01</td>
<td>434.4(^a) ±4.3</td>
<td>261.7(^a) ±3.7</td>
<td>62.85 ±0.23</td>
<td>383(^a) ±11</td>
</tr>
<tr>
<td>n = 381</td>
<td>n = 10 pens</td>
<td>n = 10 pens</td>
<td>n = 381</td>
<td>n = 376</td>
<td>n = 376</td>
<td>n = 376</td>
<td>n = 382</td>
</tr>
<tr>
<td>Imp</td>
<td>1.22(^b) ±0.01</td>
<td>8.59(^b) ±0.23</td>
<td>0.14 ±0.01</td>
<td>451.5(^b) ±4.4</td>
<td>272.5(^b) ±3.7</td>
<td>63.06 ±0.34</td>
<td>280(^b) ±12</td>
</tr>
<tr>
<td>n = 377</td>
<td>n = 10 pens</td>
<td>n = 10 pens</td>
<td>n = 377</td>
<td>n = 368</td>
<td>n = 368</td>
<td>n = 368</td>
<td>n = 378</td>
</tr>
<tr>
<td>Vac</td>
<td>1.10(^c) ±0.01</td>
<td>8.22(^ab) ±0.23</td>
<td>0.14 ±0.01</td>
<td>436.1(^a) ±4.4</td>
<td>258.0(^a) ±3.7</td>
<td>62.92 ±0.33</td>
<td>155(^c) ±11</td>
</tr>
<tr>
<td>n = 389</td>
<td>n = 10 pens</td>
<td>n = 10 pens</td>
<td>n = 389</td>
<td>n = 386</td>
<td>n = 386</td>
<td>n = 386</td>
<td>n = 385</td>
</tr>
<tr>
<td>Vac+Imp</td>
<td>1.31(^d) ±0.01</td>
<td>9.11(^c) ±0.23</td>
<td>0.15 ±0.01</td>
<td>460.1(^c) ±4.3</td>
<td>275.4(^b) ±3.7</td>
<td>63.01 ±0.31</td>
<td>170(^c) ±11</td>
</tr>
<tr>
<td>n = 378</td>
<td>n = 10 pens</td>
<td>n = 10 pens</td>
<td>n = 378</td>
<td>n = 377</td>
<td>n = 377</td>
<td>n = 377</td>
<td>n = 377</td>
</tr>
</tbody>
</table>

\(^{a-d}\)Within a column, least squares means without a common superscript differ (\( P < 0.05 \)).

\(^{1}\)Slaughter was 100 to 112 d after the second dose of GnRH vaccine.

\(^{2}\)Con = control; placebo vaccine nonimplanted. Imp = implanted; placebo vaccine implanted. Vac = vaccinated, GnRH vaccine nonimplanted. Vac+Imp = vaccinated + implanted, GnRH vaccine implanted.

\(^{3}\)Calculated from feed consumption data accrued by pen at the feedlot.

\(^{4}\)No intergroup comparisons were made for dressing percentage or ADG, as the overall F-test did not show significance (\( P ≥ 0.05 \)).

\[ \text{Feed efficiency was determined as the ratio of gain in BW over feed consumed by group (Table 3) and the group average G:F ratio calculated for the period d 42 to d 147.} \]

**Meat Quality**

Table 4 shows that GnRH immunized bulls had a larger percentage (\( P < 0.002 \)) of carcasses that graded Choice (groups Vac and Vac+Imp) than nonvaccinated bulls (groups Con and Imp; i.e., vaccination increased the proportion of quality carcasses, whether receiving anabolic implants or not). Choice was defined as a combination of low-, average-, and high-choice graded animals. In nonimplanted bulls, the percentage grading as USDA Choice rose by 2.6-fold, from 16 to 42% in the GnRH-vaccinated animals (group Con compared with group Vac; \( P < 0.001 \)). With implants, the percentage grading as USDA Choice increased 1.6-fold, from 26% with implants only...
Table 4. Meat and carcass quality measurements (least squares means ± SEM) of feedlot Bos indicus crossbred bulls

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>Percentage USDA Choice</th>
<th>Fat thickness (12th rib), cm</th>
<th>Carcass pH at 24 h</th>
<th>Ribeye area, cm²</th>
<th>Tenderness WBSF, kg</th>
<th>Carcasses with pH &gt;6, %</th>
<th>Meat color luminosity</th>
<th>Meat color A</th>
<th>Meat color B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>16.0 ±0.3</td>
<td>0.3 ±0.0</td>
<td>5.76 ±0.0</td>
<td>74.0 ±1.3</td>
<td>8.2 ±0.7</td>
<td>13.8 ±0.7</td>
<td>29.9 ±1.3</td>
<td>8.9 ±0.7</td>
<td>8.3 ±0.7</td>
</tr>
<tr>
<td>Vac</td>
<td>42.0 ±0.5</td>
<td>0.5 ±0.0</td>
<td>5.73 ±0.0</td>
<td>68.9 ±1.3</td>
<td>7.4 ±0.7</td>
<td>16.5 ±0.7</td>
<td>29.6 ±1.3</td>
<td>9.4 ±0.7</td>
<td>8.4 ±0.7</td>
</tr>
<tr>
<td>Vac+Imp</td>
<td>41.5 ±0.5</td>
<td>0.5 ±0.0</td>
<td>5.74 ±0.0</td>
<td>73.5 ±1.3</td>
<td>7.8 ±0.7</td>
<td>24.2 ±0.7</td>
<td>30.6 ±1.3</td>
<td>9.5 ±0.7</td>
<td>8.9 ±0.7</td>
</tr>
</tbody>
</table>

a,bWithin a column, least squares means without a common superscript differ (P < 0.05).
1Slaughter was at 100 to 112 d after second dose of GnRH vaccine.
2Con = control; placebo vaccine nonimplanted. Imp = implanted; placebo vaccine implanted. Vac = vaccinated, GnRH vaccine nonimplanted. Vac+Imp = vaccinated + implanted, GnRH vaccine implanted.
3USDA Choice carcass grading is the sum of percent carcasses grading low choice, average choice, and high choice. Significant differences, Con compared with Imp group (P = 0.045); Con compared with Vac group (P < 0.001); Con compared with Vac+Imp group (P < 0.001); Imp compared with Vac group (P = 0.001), Imp compared with Vac+Imp group (P = 0.002); Vac compared with Vac+Imp group (P = 0.968). Number positive divided by number analyzed is shown.
4Fat thickness (12th rib): significant differences, Con compared with Imp group (P < 0.001); Con compared with Vac group (P < 0.001); Con compared with Vac+Imp group (P < 0.001), Imp compared with Vac+Imp group (P < 0.001); Vac compared with Vac+Imp group (P = 0.292).
5WBSF = Warner-Bratzler shear force; Con compared with Vac group (P = 0.003); Con compared with Vac+Imp group (P = 0.141); Imp compared with Vac group (P = 0.002); Imp compared with Vac+Imp group (P = 0.107).
6Comparison of the percentage of animals with carcass pH >6.0 among treatments was not able to be tested due to the lack of a significant F-test (P = 0.195).

Table 5. USDA yield grade frequency distributions among feedlot Bos indicus crossbred bulls

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>USDA yield grade 1</th>
<th>USDA yield grade 2</th>
<th>USDA yield grade 3</th>
<th>USDA yield grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of carcasses (%)</td>
<td>No. of carcasses (%)</td>
<td>No. of carcasses (%)</td>
<td>No. of carcasses (%)</td>
</tr>
<tr>
<td>Con, n = 375</td>
<td>273 (72.8)</td>
<td>101 (26.9)</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Imp, n = 366</td>
<td>206 (56.3)</td>
<td>145 (39.6)</td>
<td>14 (3.8)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Vac, n = 383</td>
<td>139 (36.3)</td>
<td>217 (56.7)</td>
<td>27 (7.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vac+Imp, n = 377</td>
<td>163 (43.2)</td>
<td>188 (49.9)</td>
<td>26 (6.9)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

1Yield grade is based on USDA beef yield grading standard expressed as number of carcasses and percentage per each yield grade per treatment. Yield grade takes into consideration external fat coverage, rib eye area, KPH, and HCW.
2Con = control; placebo vaccine nonimplanted. Imp = implanted; placebo vaccine implanted. Vac = vaccinated, GnRH vaccine nonimplanted. Vac+Imp = vaccinated + implanted, GnRH vaccine implanted.

Amatayakul-Chantler et al.

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(group Con; $P < 0.001$) but was thinner than in either of the GnRH-vaccinated groups (group Vac compared with group Imp; $P < 0.001$; group Vac+Imp compared with group Imp; $P < 0.001$). Shear force (Warner-Bratzler) results showed that bulls receiving GnRH vaccine alone had improved tenderness compared with unvaccinated bulls (group Con compared with group Vac; $P = 0.003$; group Imp compared with group Vac; $P = 0.002$) but were not different from group Vac+Imp bulls ($P = 0.154$). The muscle pH of ~90 carcasses from each group, selected at random and determined after overnight chilling, showed no differences among groups, with group means between pH 5.73 and 5.80 (Table 4). A combination of carcass pH values $>6.0$ and meat luminosity and color ($L^*$, $a^*$, and $b^*$) parameters are generally used for classification of carcasses as DFD (Scanga et al., 1988; Page et al., 2001; Bass et al., 2008). Analysis of pH data of the selected subgroup of 90 animals from each group showed greater numbers of carcasses with pH values $>6.0$ in the bulls receiving anabolic implants, irrespective of GnRH vaccination (Table 4). However, when analyzing for meat luminosity and values $a^*$ and $b^*$, all 4 groups showed similar values (Table 4); therefore, the incidence of DFD seen in this study was primarily due to high carcass pH values. Levels of DFD were 60% greater in implanted bulls, with a quarter classified as DFD. Comparison of DFD incidence among treatment groups was not conducted due to the lack of a significant $F$-test ($P = 0.195$). Vaccination with the GnRH vaccine was not associated with an increase of the percent DFD (Table 4).

Compared with placebo control bulls, treatment with the GnRH vaccine alone decreased ribeye area (group Con compared with group Vac; $P < 0.001$), but this decrease was not observed in vaccinated bulls that also received implants (group Imp compared with group Vac+Imp; $P = 0.676$). There were no differences in the dressing percentages among groups (data not shown). The majority of the carcasses graded as yield grade 1 and 2 (i.e., $>90\%$) on the USDA yield grade system. Nonvaccinated groups (Con and Imp) delivered more animals with yield 1 grades, whereas in the GnRH-vaccinated groups (Vac and Vac+Imp), more animals were classified as yield grade 2 (Table 5). Only 1 bull in group Imp was classified as yield grade 4.

**DISCUSSION**

This study reports for the first time the use of the GnRH vaccine Bopriva in *Bos indicus* cross bulls in a feedlot, with and without anabolic implants. Bulls immunized with the vaccine on d 0 and 42 with a 6-wk interval between immunizations showed strong IgG immune responses to GnRH peptide, which were sustained at elevated titers for 15 wk after the second immunization, up until slaughter on d 147. Increased antibody titers to GnRH in immunized groups Vac and Vac+Imp were associated with suppressed testosterone concentrations, and suppression of testosterone to concentrations $<5\text{ng/mL}$ was achieved within 2 wk of the second dose of GnRH. More importantly, concentrations remained suppressed to the end of the study at 15 wk, with minimal intervention or handling. Other studies in feedlot *Bos taurus* bulls have reported that to obtain a 15-wk duration of testosterone suppression required either 3 doses of a GnRH vaccine (Cook et al., 2000) or use of Freund’s oil adjuvant given as 2 doses (Adams and Adams, 1992; Adams et al., 1993). Studies with a GnRH vaccine in *Bos indicus* bulls on pasture used Freund’s type oil adjuvant with 3 (Ribeiro et al., 2004) or 4 doses (Hernandez et al., 2005) to achieve extended duration of an immunocastration effect. Interestingly, after the first vaccination of the GnRH vaccine on d 0, there was a detectable, but small, serum IgG response to GnRH peptide in immunized groups Vac and Vac+Imp, together with a slight suppression of testosterone. The minor effect from primary immunization on testosterone concentrations may be important as it allows the continued natural anabolic effect of testosterone until the time of the second immunization. The safety profile of this vaccine formulated with the aqueous adjuvant Advasure was judged highly acceptable in a commercial feedlot setting, as the few injection site swellings rapidly resolved and did not progress to abscess formation. A high level of safety has been noted as important, because unacceptable safety caused by adjuvants used in earlier experimental GnRH vaccines have hindered further development and commercialization (Bonneau and Enright, 1995; Meeusen et al., 2007).

In the study reported here, immunization of *Bos indicus* × Brown Swiss bulls with the GnRH vaccine had no effect on final BW or HCW in the absence of anabolic implants, but there was a significant effect when the vaccine and implants were used together. Immunization against GnRH increased ADG only to a small degree when comparing the Vac group with the Con group. Earlier studies without implants variably reported immunocastration in *Bos taurus* bulls to have either no effect on performance (Adams and Adams, 1992; Huxsoll et al., 1998) or result in reduced growth and ADG compared with entire bulls (Adams et al., 1993; Cook et al., 2000). The study reported here, with 1,600 animals and 400 bulls per group, was designed to provide a high degree of statistical confidence in growth, feed efficiency, and meat quality data. The findings here in GnRH-vaccinated cattle without implants showed no negative effect on growth. In these bulls, other factors, such as modified sexual or aggressive behavior (Jago et al., 1997; Price et al., 2003), may assist in maintaining feed intake and growth, and thus compensate for the reduced conen-
trations of the natural anabolic hormone testosterone. Bulls receiving GnRH vaccine together with anabolic implants (group Vac+Imp) had a heavier final BW and greater ADG than the other 3 groups, with improvements over implants alone. This is the first time that a cumulative and interactive effect has been described between GnRH vaccination and anabolic implants. This combined effect of the GnRH vaccine together with anabolic implant treatments on BW in Bos indicus cross bulls at 13 to 14 mo of age is in contrast to similar studies in Bos taurus bulls of 12 mo of age, where an experimental GnRH vaccine given with implants was reported to have no effect on final BW compared with implants alone (Adams et al., 1993). The improvement in performance obtained by combined treatment with the GnRH vaccine with implants was smaller when considering HCW. This may reflect increased levels of abdominal fat in vaccinated bulls, which would be lost during evisceration and, hence, reduce expected HCW. However, there were no significant differences in dressing percentage. Previous studies with experimental GnRH vaccines without implants in Bos taurus bulls found either a negative impact on HCW (Cook et al., 2000) or no difference (Adams and Adams, 1992; Adams et al., 1993).

As expected, growth performance was improved in group Imp bulls by anabolic implants for BW and HCW, although testosterone concentrations in this group were lowered by implants from d 42 to 112. It is concluded that the anabolic steroids provided by the implants more than compensate for the reduction in the natural anabolic effect of testosterone. Others have found that implantation with progesterone and estradiol had no effect on the growth of entire bulls (Adams and Adams, 1992), whereas trenbolone acetate was reported to have a positive effect on growth and HCW (Jones et al., 1991). Further studies may be required to determine whether Bos indicus cross bulls respond differently than Bos taurus bulls to implantation with anabolic steroids. Feed efficiency, as the G:F ratio, was not found to be different among groups. This may relate to the greater level of fat deposition in the GnRH-immunized bulls with deposition of less dense fat in the carcass and viscera, rather than more dense muscle, resulting in reduced BW.

An important conclusion from this study is that meat quality can be improved by using the GnRH vaccine, irrespective of the use of implants. Immunization with the GnRH vaccine appreciably increased the proportion of carcasses grading USDA Choice, increased loin fat cover at the 12th rib, and showed a trend toward improved tenderness. The increase in the percentage of bulls grading USDA Choice was most striking, with an increase in nonimplanted bulls from 16% in group Con animals to 42% in group Vac bulls, and with implants from 26% in group Imp bulls to 42% in group Vac+Imp animals. Similarly, the loin subcutaneous fat cover at the 12th rib increased from a mean of 0.35 cm in group Con animals to 0.52 cm in group Vac bulls, and from 0.44 cm in group Imp animals to 0.54 cm in group Vac+Imp bulls. These are concluded to be highly significant improvements in carcass USDA quality grading score and fat coverage. Reflecting the same trend, the USDA yield grade distribution in the 2 vaccinated groups (Vac and Vac+Imp) resulted in an increased proportion of carcasses with elevated USDA yield grade scores compared with those from the nonvaccinated groups (Con and Imp). Taking the Imp group as the comparator, vaccination with the GnRH vaccine raised the proportion of yield grade 2 grading carcasses from 39.6 to 56.7% (group Vac) and to 49.9% (group Vac+Imp), and increased the proportion of yield grade 3 carcasses from 3.8% in group Imp to 7.0% in group Vac and 6.9% in group Vac+Imp. In addition, there was a trend for lower WBSF measurements in the GnRH vaccine only bulls, which showed a significant improvement in tenderness compared with placebo. Surveys of cattle carcass attributes and beef quality in Mexico have shown that of the 3 production regions in Mexico, the central and southern regions, where Bos indicus are primarily raised for Mexican consumption, not only is fat content inconsistent, but the overall desirability, tenderness score, and meat color are low compared with meat production in the northern region (Delgado et al., 2005; Mendez et al., 2009). This new study has demonstrated that the GnRH vaccine can be used effectively in the common Bos indicus × Bos taurus (Zebu × Brown Swiss) to improve meat quality. The GnRH vaccine provides a positive solution to quality issues associated with raising Bos indicus cross beef in the central and southern regions of Mexico.

The use of implants in Bos indicus steers has been reported to reduce marbling and muscle tenderness (Watson et al., 2008). The GnRH vaccine may thus provide a ready method to improve meat quality when bulls are administered anabolic implants. The incidence of DFD meat in this study, defined as muscle having a pH >6.0, was greater in groups receiving implants and was not correlated with vaccination against GnRH. It was also noted that only carcasses with pH values >6.0 contributed to DFD grading, as the difference among groups of luminosity and meat color measurements were not different. Previous research (Scanga et al., 1988) found that steers treated with androgen and estrogen implants when entering a feedlot and then reimplemented produced greater mean percentages of DFD per pen when compared with other more moderate growth-promoting implant strategies. In addition to implant selection and dosing regimen, the study by Scanga et al. (1988) also found that if cattle were kept on feed for 100 d or more after reimplantation, the incidence of DFD was reduced by an
average of 69%.

Although behavior was not monitored in the large study described here, in other beef production settings, behavior has been shown to have relevance to productivity and quality gains. For example, Bos taurus feedlot animals individually rated as less aggressive and calmer were found to have improved growth rates and reduced levels of DFD (Voisinet et al., 1997b), and reduction in stress levels in feedlot cattle has been associated with improved meat quality (Ferguson and Warner, 2008; Hall et al., 2011). Conversely, highly excitable Bos indicus cross cattle were shown to have reduced meat quality scores (Voisinet et al., 1997a). The ability to control unwanted sexual behavior in bulls with a GnRH vaccine (Jago et al., 1997; Huxsoll et al., 1998; Price et al., 2003) has encouraged the use of such vaccines to promote animal welfare. A reduction in sexual and aggressive activities in feedlot bulls may result in improved growth and meat quality, as well as improving animal welfare. Further studies are required to determine whether the GnRH vaccine Bopriva could be used to modify bull behavior sufficiently to yield production and welfare gains. Others (Janett et al., 2012) have demonstrated that Bopriva modified behavior as decreased activity scores in vaccinated postpubertal bulls when compared with entire bulls, using an ALPRO DeLaval meter (DeLaval AG, Sursee, Switzerland). This new approach used a wireless ALPRO activity meter designed for dairy herd management, combined with activity scoring.

In summary, the use of the GnRH vaccine Bopriva in feedlot-grown, intact Bos indicus × Brown Swiss bulls provided striking increases in meat quality, including USDA grading and 12th rib fat depth. Combining vaccination against GnRH with anabolic implants increased BW, HCW, and ADG, and retained marked improvements in meat quality parameters.

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