Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone

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ABSTRACT: The purpose of this research was to compare the frequency of aggressive behaviors in beef bulls actively immunized against gonadotropin-releasing hormone relative to contemporary nonimmunized control bulls and surgically castrated steers. Eight males were assigned to each of these treatments in each of 4 yr. Immunized males were treated with a GnRH-keyhole-limpet hemocyanin (KLH) conjugate at approximately 4 mo of age. A secondary (booster) immunization was administered at 12 mo. Steers were castrated at 4 mo of age. Animals in each treatment in each year were housed as a single group prior to testing. At approximately 16 mo of age, each group of eight animals was placed in a 10- × 16-m enclosure for 20 min on five occasions at 2 to 3 d intervals. An observer recorded butts initiated by each animal as well as participation in bouts of sparring. Relative to control bulls, immunocastration reduced the frequency of butts initiated ($P < 0.05$) and participation in sparring bouts ($P < 0.05$) to levels typically observed in steers ($P > 0.05$). These observations indicate that active immunization against GnRH reduces the incidence of aggressive behavior in male beef cattle and are consistent with our postulate that immunoneutralization of GnRH is an effective alternative to surgical castration in the management of beef cattle.

Key Words: Aggression, Beef Cattle, Behavior, Castration, GnRH, Immunization

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Introduction

Surgical castration is used to eliminate testicular function in male cattle (Bos taurus), thus, improving carcass quality and reducing the frequency of undesirable sexual and aggressive behaviors. Castration may be stressful (Cohen et al., 1990) and is associated with reduced growth rate both in the short- and long-term (Huxsoll et al., 1998). Active immunization against GnRH provides an alternative to surgical castration. Immunocastration reduces or eliminates many of the disadvantages of surgical castration, such as reduced growth rate and feed efficiency (Adams et al., 1996) and the increased health risks associated with the surgery (Robertson et al., 1994). Immunocastration requires less labor and equipment than surgical castration and imposes less risk to the stockperson. Carcass quality of immunocastrated beef animals is comparable to that of steers and significantly higher than the carcass quality of nonimmunized bulls (Adams et al., 1996; Huxsoll et al., 1998; Cook et al., 2000). In addition, immunocastrated males are less prone to exhibit male-male sexual behaviors (Moore et al., 1989; Teague et al., 1992; Finnerty et al., 1996; Jago et al., 1997), thus reducing the risk of bodily injury and conserving energy for more efficient growth and development.

Reducing aggressive behavior increases the economic return to the cattle producer by reducing the incidence of injury to both animals and their handlers, reducing bruised and dark-cutting meat at the time of slaughter (Romans et al., 1994; Jones, 1995), reducing damage to pastures, fences, and to feeding and handling equipment (Seideman et al., 1982), while conserving energy to enhance growth. The purpose of this research was to compare the aggressive behavior of beef bulls actively immunized against GnRH with that of nonimmunized control bulls and surgically castrated steers.

Materials and Methods

In each of 4 yr, 24 male beef calves (Hereford, Angus and Hereford × Angus breeding) were assigned to three treatments of eight animals each. Bull calves actively immunized against GnRH were treated with a GnRH-keyhole-limpet hemocyanin (KLH) conjugate at approximately 4 mo of age. A secondary (booster) immuni-
zation was administered at 12 mo. Steers were castrated at 4 mo of age. Nonimmunized intact bulls were used as a control. Control bulls and steers were selected at random in each year. Immunized bulls were selected from groups of 10, 12, 14, and 19 individuals in years 1995, 1997, 1998, and 2000, respectively, based on responsiveness to treatment (serum T-levels).

Animals were reared at the University of California Sierra Foothill Research and Extension Center, Browns Valley, CA, until weaning at 6 to 7 mo of age. They were then transferred to the UC Davis campus where they were housed in treatment groups in 11.5- × 19-m pens with concrete floors. At Davis, they received a starter diet of 40% grain until they reached 318 kg and thereafter were fed a concentrate diet of 80% grain.

At primary immunization, 2 mL of an emulsion composed of equal volumes of Freund’s complete adjuvant, Freund’s incomplete adjuvant and saline containing 5 mEq of a GnRH-KLH conjugate was delivered to two subcutaneous sites on the dorsal aspect of the upper neck. Each milligram-equivalent of conjugate was estimated to contain 0.65 mg GnRH covalently linked to 1 mg KLH. The GnRH-KLH conjugate was prepared as described previously (Adams and Adams, 1990). Briefly, 20 mol of p-aminophenylacetic acid (0.1 M in 2 N HCl) and 20 mol sodium nitrite (0.1 M in H2O) were allowed to react for 5 min at 4°C. The reactants were neutralized by the addition of 1.4 mL of 0.5 M sodium bicarbonate (NaHCO3) and were combined immediately with 0.72 mL 40% dimethylformamide in H2O (vol/vol) containing 160 mol NaHCO3 and 20 mg GnRH. After incubation for 20 to 24 h at 4°C, the reaction was halted by acidification to pH 2.0 with 2 N HCl, and the mixture was repeatedly extracted (five times) with anhydrous diethyl ether (8 mL/extraction). After extraction the aqueous phase was adjusted to pH 5.0 with 0.5 M NaHCO3 and combined with 2 mL saline containing 20 mg KLH. The GnRH-KLH conjugate was separated from reactants of low molecular weight by dialysis against saline. Using this procedure, the efficiency of incorporation of radiolabeled GnRH into the GnRH-KLH conjugate was 65%. At secondary immunization, bulls received 2 mL of an emulsion containing 2 mEq of GnRH-KLH in saline and an equal volume of Freund’s incomplete adjuvant. The emulsion for secondary immunization was also injected at two subcutaneous sites in the upper neck.

Blood samples (10 mL) were collected by jugular venipuncture approximately 10 d prior to the initiation of behavioral testing. The blood was allowed to clot and serum was isolated by centrifugation. The serum concentration of testosterone was determined by radioimmunoassay using the procedure described by Adams et al. (1987). The intra- and interassay coefficients of variation for all assays were <10%. The serum concentration of testosterone in the pretest sample was used to estimate testicular activity and the effectiveness of the immunization protocol. The mean serum concentration of testosterone in the pretest sample and final body weight are summarized by treatment and year in Table 1.

At approximately 16 mo of age (late February or early March in each replicate year), each group of eight animals was moved from its home pen to an isolated 10 × 16 m-dirt-substrate enclosure for 20 min on five occasions at 2- to 3-d intervals. Exposure to such a novel area stimulates social interactions, particularly aggression (Price and Wallach, 1991; Finnerty et al., 1996). Tests were conducted between 1200 and 1600. One observer monitored and recorded all instances of butts initiated by each animal as well as participation in bouts of sparring following the “male only” testing procedure of Price and Wallach (1991). Individual animals were identified by large numbers painted or glued directly behind their shoulders on each side.

Because the aggressive behavior data for individuals within groups were not independent, statistical tests were conducted using groups as the experimental unit with the average score of the eight individuals in each group as the statistic used for each day of testing. Data for butts initiated and frequency of participation in sparring were analyzed by analysis of variance (SAS Inst. Inc., Cary, NC) for the effects of treatments, days, and years. Bonferroni t-tests were used to determine the significance (P < 0.05) of mean differences when a significant main effect was obtained. Day and day × treatment effects were not significant (P > 0.05).

Results and Discussion

Both the frequency of butts initiated (F = 12.77, df = 2, P < 0.007) and frequency of participation in sparring bouts differed across treatment groups (F = 11.07, df = 2, P < 0.01). Although immunocastrated bulls and steers did not differ with regard to butts initiated or sparring bouts, both immunized bulls and steers initiated fewer butts and participated in fewer sparring bouts than nonimmunized control bulls (Figures 1 and 2, respectively). Year differences in butting and sparring were not significant (F = 1.98, df = 3, P = 0.22 and F = 0.08, df = 3, P = 0.97, respectively).

Huxsoll et al. (1998) reported a reduction in aggressive behavior in beef cattle due to immuno-castration. This report was preliminary from the standpoint that the aggressive behaviors of only single cohorts of immuno-castrated males, nonimmunized control bulls, and surgically castrated steers were studied (the 1995 animals in this study). The present study extended and confirmed this preliminary work by including observations made over three additional replicates. In the present study, immuno-castration significantly reduced butting and sparring to levels observed in steers. Finnerty et al. (1996) reported lower levels of
aggressive behaviors in GnRH-immunized males than in nonimmunized control bulls but did not employ steers for comparison. Both Moore et al. (1989) and Teague et al. (1992) reported that the frequency of butting by bulls immunized against LHRH did not differ from butting frequency noted in nonimmunized bulls. Godfrey et al. (1996) reported intermediate levels of aggression in male goats immunized to GnRH relative to nonimmunized controls and surgically castrated males.

While choice of nonimmunized control bulls and steers was strictly random, animals representing the immunized treatment were selected based on their response to the immunization protocol. In 3 of 4 yr, none of the immunized bulls tested had a T-level higher than 0.2 ng/mL and most were below 0.1 ng/mL (Table 1). In 1997, 12 immunized bulls were available. Four had T-levels between 0.1 ng/mL, four had T-values ranging from 0.4 to 0.7 ng/mL, and the remaining four bulls ranged from 1.4 to 4.0 ng/mL. The eight individuals with the lowest T-values were chosen for testing. The reason for the lower effectiveness of the vaccination procedure in trial year 1997 is not known but may be due to year-to-year variation in conjugation efficiency. Preliminary data collected in 1994 showed that males with T-levels much higher than 0.2 ng/mL were often more aggressive than those with lower levels of T. One or two such males in a group of eight animals could incite the remaining subjects to exhibit higher than normal levels of butting and sparring. Finnerty et al. (1996) also noted that immunocastrated bulls often exhibited higher levels of aggressive behavior if housed with more aggressive nonimmunized males. It was for this same reason (i.e., social facilitation) that animals in the present study were tested only within treatment groups, and the data were analyzed using groups, rather than individuals, as the statistical unit. Preliminary observations in 1994 demonstrated that the pres-

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**Table 1.** Mean (± SE) testosterone concentrations and body weights by treatments and years (eight animals per treatment in each year)*

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<thead>
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<tr>
<td><strong>Testosterone, ng/mL</strong></td>
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<tr>
<td>Control bulls</td>
<td>2.95 ± 0.41</td>
<td>4.06 ± 0.56</td>
<td>6.29 ± 1.93</td>
<td>12.29 ± 1.46</td>
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<td>Immunocastrated bulls</td>
<td>0.09 ± 0.02</td>
<td>0.32 ± 0.10</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.01</td>
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<tr>
<td>Steers</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.05 ± 0.01</td>
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<tr>
<td><strong>Body weight, kg</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Control bulls</td>
<td>529.8 ± 19.5</td>
<td>537.5 ± 17.0</td>
<td>634.1 ± 19.9</td>
<td>515.1 ± 11.7</td>
</tr>
<tr>
<td>Immunocastrated bulls</td>
<td>473.1 ± 17.5</td>
<td>505.7 ± 11.1</td>
<td>506.6 ± 13.0</td>
<td>476.9 ± 12.4</td>
</tr>
<tr>
<td>Steers</td>
<td>468.1 ± 14.2</td>
<td>546.1 ± 17.9</td>
<td>533.8 ± 19.4</td>
<td>481.1 ± 18.8</td>
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*Control bulls and steers were selected at random in each year. Immunized bulls were selected from groups of 10, 12, 14, and 19 individuals in years 1995, 1997, 1998, and 2000, respectively, based on responsiveness to treatment (serum T-levels).
ence of intact bulls in mixed-treatment groupings would consistently result in higher levels of aggressive behaviors for the immunized bulls and steers than they would exhibit in within-treatment tests. Selecting immunized bulls with relatively low T-levels for the immunized treatment precluded the random choice of individuals for these samples but yielded results which, we believe, more accurately reflect the nature of animals responsive to this treatment.

It is noted that Freund’s complete adjuvant has not been approved for general use in livestock. Alternative immunologic adjuvants examined in our laboratory have not been as effective as Freund’s complete adjuvant. Hence, only animals treated with Freund’s complete adjuvant were used in this study. Efforts are currently ongoing in a number of research laboratories to develop improved conjugation methods and immunization techniques. Such technological advances will likely increase the efficiency of immunocastration and reduce the proportion of animals that only partially respond to GnRH immunization in terms of their testicular activity.

All butts and sparring bouts were tallied regardless of intensity. Both butts and sparring bouts varied greatly in intensity from slight to highly forceful physical interactions. Because the intensity of both butts and sparring occurred on a continuum and a permanent visual record of the contests was not obtained, no attempt was made to quantify the intensity of interactions. Casual observation suggested that the intensity of aggressive interactions was often greater in control bulls than in immunocastrated males or steers, an observation also noted by Teague et al. (1992).

The animals were approximately 16 mo of age at testing. This is an active period for aggressive behaviors in male cattle (Price and Wallach, 1991). Hinch et al. (1983) reported that sparring in bulls became more serious and intense after 14 mo of age. Similarly, Tennessen et al. (1985) found an increasingly greater disparity in the aggressive behavior of intact bulls and steers as they matured from 9 to 15 mo of age.

The decision to test eight animals per treatment in each year was based on both the availability of animals and preliminary observations suggesting that eight individuals was the maximum number of individuals in the nonimmunized bull group (treatment exhibiting the most aggression) that could be accurately monitored under the test conditions employed. Butting and sparring were particularly frequent when the animals were first introduced to the test arena on each test day.

Incidence of male-male mounting was infrequent under the test conditions used in these studies. For example, only eight male-male mounts were observed in all 5 d of testing in 1998 (300 min total). Moreover, all episodes of male-male mounting involved four nonimmunized control bulls. In 2000, eight male-male mounts were again observed (300 min), three by control bulls and five by steers. Two males in each treatment initiated all mounts. Moore et al. (1989) and Teague et al. (1992) noted that immunocastration significantly reduced the incidence of male-male mounting. These reports failed to indicate whether testing had been conducted with between-treatment or within-treatment groups of animals, a variable which could have influenced the disparity of the treatment differences obtained (Finnerty et al., 1996).

It was concluded that active immunization against GnRH is an effective alternative to surgical castration for reducing the incidence of aggressive behavior in male beef cattle. Immunization techniques must be improved to consistently reduce testicular function in bulls to levels needed to suppress aggressive behavior.

Implications

Reducing aggressive behavior increases the economic return to the cattle producer by reducing the incidence of injury to both animals and their handlers and reducing bruised and dark-cutting meat at the time of slaughter, while conserving energy to enhance growth. Surgical castration of male cattle reduces aggressive behaviors relative to intact bulls, but the associated surgical intervention can jeopardize the animals’ welfare. Immunization of beef bulls against GnRH provides an alternative to surgical castration and affords many of the same advantages to meat production offered by both steers and intact bulls. The present study demonstrates that the frequency of aggressive behavior in immunocastrated bulls is more like that of steers than intact, nonimmunized bulls.

Literature Cited


