Effects of ring castration with local anesthesia and analgesia in Holstein calves at 3 months of age on welfare indicators¹

S. Marti,* A. Velarde,† J. L. de la Torre,‡ A. Bach,*§ A. Aris,* A. Serrano,* X. Manteca,‡ and M. Devant*²

*Department of Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), and Animal Nutrition, Management, and Welfare Research Group, Torre Marimon, 08140 Caldes de Montbui, Barcelona, Spain; †IRTA, Animal Welfare, Finca Camps i Armet, 17121 Monells, Girona, Spain; ‡Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, and Animal Nutrition, Management, and Welfare Research Group, 08193 Bellaterra, Barcelona, Spain; and §Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Barcelona, Spain

ABSTRACT: Forty-seven Holstein calves (130 ± 3.43 kg of BW and 95 ± 1.5 d of age) were randomly assigned to 2 treatments [intact (INT), n = 23; or castrated (CAS), n = 24] to evaluate the effect of ring castration at 3 mo of age on welfare indicators. Castration was performed with local anesthesia (2% lidocaine, 3 mL in each testis and 2 mL in the scrotum) and analgesia (flunixin meglumine, intramuscularly, 3 mg/kg of BW). No local anesthesia or analgesia was used with INT calves. Serum cortisol concentration was determined at −120, 0, 30, 60, 90, and 180 min with respect to castration. At d 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, serum haptoglobin concentration was determined, rectal body and scrotal temperatures were measured, lesions at the castration site were scored, and the activity and behavior of 18 calves (9 INT and 9 CAS) were recorded continuously for 24 h. Weekly BW and concentrate and straw DMI were recorded. To evaluate humoral immunity, at 14 d after castration, ovalbumin was injected subcutaneously and serum antibody titers against ovalbumin before the injection and at d 35 were determined. At d 49 after castration, calves were intravenously injected with ACTH, and at 0, 1, 2, and 4 h thereafter, serum cortisol and testosterone concentrations were determined. Average daily gain was greater (P < 0.001) in INT than CAS calves (1.36 vs. 1.16 ± 0.038 kg/d, respectively). Area under the curve of cortisol at castration day was reduced (P < 0.05) in CAS calves compared with INT calves (18 vs. 33 ± 5.2 nmol/L per hour, respectively). The main scrotal lesion score observed in CAS calves throughout the study was 0, corresponding to no visible swelling, inflammation, or infection. However, scrotal lesion scores classified as 1 (swelling) were greater (P < 0.01) at 21 and 28 d after castration than at 1, 3, 7, and 14 d. Abnormal standing occurred more frequently (P < 0.001) in CAS calves compared with INT calves (26 vs. 5 ± 0.03%, respectively) from 3 to 14 d after castration. Head turning tended (P = 0.06) to be greater at d 14 of the study in CAS than INT calves (3.0 vs. 2.6 ± 0.04%, respectively). At d 49, 100% of CAS calves had no testes and no serum testosterone was detected. In summary, ring castration of Holstein calves performed at 3 mo of age with local anesthesia and analgesia decreased ADG and affected some behavioral traits during the first 14 d after castration. However, intake, serum cortisol and haptoglobin concentrations, rectal temperature, and humoral immunity were not altered.

Key words: beef, castration, welfare

INTRODUCTION

Castration of bulls has been proposed as a method to reduce meat quality problems of Holstein calves because it reduces sexual and aggressive behavior and improves carcass and meat quality (Mach et al., 2009). Ring castration at 3 mo of age requires less labor, and rates of failure are smaller compared with Burdizzo castration (Stafford, 2007). However, ring castration has been questioned from a welfare point of view (Molony...
et al., 1995; Thüer et al., 2007) based on an increased incidence of abnormal standing postures observed during short scanning periods in ring-castrated calves compared with intact calves. Stafford et al. (2002) evaluated the effect of local anesthesia or anesthesia plus a nonsteroidal anti-inflammatory drug (NSAID) on acute serum cortisol in calves castrated using different castration methods. They reported that the cortisol response was virtually eliminated when local anesthesia plus NSAID was administered before castration, and thus concluded that calves experienced little or no pain-induced distress during the 8-h period after castration. However, in that study no other welfare indicators were evaluated. Although animal welfare evaluation is complex and no standardized protocol exists, additional welfare indicators apart from behavior and plasma metabolites have been proposed (Broom, 1991). There is a need to establish objective variables to quantify the pain or stress caused by castration. The aim of the current study was to assess the impact of ring castration, with local anesthesia and analgesia, of Holstein calves at 3 mo of age on potential indicators of stress or pain. These indicators included performance (growth and intake), serum cortisol concentrations at castration day, serum haptoglobin concentration (tissue damage indicator), rectal and scrotal temperatures, testes lesion scoring, humoral immunity, cortisol response after a ACTH challenge, and behavioral postures and activity for 7 wk after castration.

MATERIALS AND METHODS

Animals were managed following the principles and guidelines of the IRTA Animal Care Committee.

Animals, Housing, and Diets

Forty-seven Holstein calves were used in a complete randomized design. Animals were distributed into 47 individual partially slatted pens (1.20 × 1.45 m) allowing visual, olfactory, and body contact with herd mates. Calves were weighed the day before castration (d −1) and stratified by BW. Beginning with the heaviest and moving down the strata, animals were randomly assigned to 1 of 2 treatments; 23 calves remained intact (INT) and 24 calves were allocated to the castration (CAS) treatment. Average initial BW and age of calves were 130 ± 3.4 kg and 95 ± 1.5 d (mean ± SE), respectively. The experiment was 7 wk in length. Ring castration was performed as described by Stafford et al. (2002). Calves assigned to the CAS group received a 3-mL injection of local anesthesia (2% lidocaine, Xilocaína Ovejero, Laboratorios Ovejero, Vilecha, Spain), 20 min before castration, through the distal pole of each testicle. The testicles were then pushed dorsally off the needle and an additional 2 mL of local anesthesia was injected into the distal end of the scrotum. The scrotum was massaged to help diffuse the local anesthetic. At the same time, 3 mg/kg of BW of an analgesic (flunixin meglumine, Flunixin Inyectable Norbrook, Laboratorios Karizoo S.A., Caldes de Montbui, Spain) was administered intramuscularly. Two rubber castration rings (Insvet, Huesca, Spain) were placed simultaneously on the neck of the scrotum just proximal to the testes, using an elastrator (Insvet). Two rings were used to ensure that castration would still occur if one broke. Calves assigned to the INT group were restrained during the same time as CAS calves to allow blood sample collection, but no local anesthesia or analgesia was applied. Calves were fed a concentrate (36.7% corn, 19.5% barley, 10.5% wheat middlings, 10.1% corn gluten feed, 6.4% soyhulls, 5.0% wheat, 3.5% soybean meal, 2.5% canola, 1.5% calcium soaps of palm oil, 1.5% calcium carbonate, 0.80% urea, 0.70% sodium bicarbonate, 0.60% premix, 0.40% palm oil, 0.30% salt; 16.8% CP, 4.9% ether extract, 21.1% NDF, 6.0% ash, 0.9% Ca, 0.3% Cl, 0.3% Mg, 0.5% P, 0.7% K, and 0.4% Na; DM basis) and barley straw (3.5% CP, 1.6% ether extract, 70.9% NDF, and 6.1% ash; DM basis) in 2 separate troughs (0.3 × 0.6 × 1.2 m) until d 49 of the experiment. Feeds were offered daily (feed weights were recorded) for ad libitum intake. Calves were housed at a commercial farm from the Cooperativa Agraria de Guissona (Guissona, Spain).

Measurements and Sample Collection

The day of castration was considered d 0 of the study; all subsequent references to the day of study are relative to d 0, when castration was conducted. On d 0, a 10-mL blood sample was collected (without anticoagulant additives; BD Vacutainer Nonadditive Tube, BD Vacutainer, Franklin Lakes, NJ) by jugular venipuncture at −120, 0, 30, 60, 90, and 180 min relative to castration from calves in the CAS and INT treatments and was subsequently analyzed for serum cortisol concentration. On d 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, a 10-mL blood sample was collected (BD Vacutainer Nonadditive Tube) by jugular venipuncture from all calves for subsequent serum haptoglobin analyses. All blood samples were centrifuged at 1,500 × g at 4°C for 15 min, and serum was decanted and stored at −20°C until further analysis. In addition, on d 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, rectal temperature was measured using a digital electronic thermometer (Omron Healthcare BW, Hoofddorp, the Netherlands) and scrotal temperature was measured using an infrared thermometer (Center 350, Center Technology Corporation, Shu-Lin, Taiwan) at a horizontal distance of 20 cm from the testes. On the same days, lesions at the castration site were scored on an 11-point scale, as described by Molony et al. (1995), with 0 indicating no visible swelling, inflammation, or infection; 0.5 to 2.0 depicting increasing degrees of swelling without obvious erythema; 2.5 and 3.0 corresponding to swelling with obvious erythema but without pus; and 3.5 to 5 indicating the presence of pus with an increasing inflammatory response. Time
(in days) elapsed between castration and the fall of the testes was also individually recorded.

The behavior of 9 calves within each treatment was filmed continuously for 24 h on d 3, 7, 14, 21, 28, 35, 42, and 49 using a digital video-recording device (VDVR-9, Circontrol S.A., Terrassa, Spain) and digital color/monochrome cameras (VCAM-420DNA, Circontrol S.A.) fitted with heater resistors and autoiris varifocal lenses (VLEN-2812VA, 2.8 to 11.5 mm, Circontrol S.A.), which were installed approximately 3 m above the ground. Each camera simultaneously filmed 2 pens. Videotapes were processed by scan sampling at every 10-min interval to represent behavior over an entire hour. Only 12 h of recordings (0800 to 2050 h) were used to create the scan sample data set because the quality of the night recordings was not always acceptable. Behavioral categories were recorded (Table 1) and classified according to the methods of Molony et al. (1995) and Thiéry et al. (2007) as active behaviors (no activity, eating, tail wagging, head turning, foot stamping, and sleeping) and postures (standing and lying). Animal BW and feed refusals were measured on d 7, 14, 21, 28, 35, 42, and 49.

On d 14, crystallized ovalbumin (grade VII, Sigma-Aldrich, St. Louis, MO) was dissolved in sterile PBS (2 mg/mL) and 4 mL of the final solution was injected subcutaneously in the midventral region of all animals. Immediately before the antigen injection at d 14 and 35 of the study, a 10-mL blood sample was collected (BD Vacutainer Nonadditive Tube) by jugular venipuncture to determine antibody titers against ovalbumin. Blood samples were kept refrigerated on ice until centrifugation. At d 49, calves were intravenously injected with 2 IU of porcine ACTH/kg of BW0.75 (Sigma-Aldrich). Immediately before and at 1, 2, and 4 h after the ACTH injection, a 10-mL blood sample was collected (BD Vacutainer Nonadditive Tube) by jugular venipuncture for serum cortisol concentration analyses. Also on d 49 of the study, an additional 10-mL blood sample was collected (BD Vacutainer Nonadditive Tube) by jugular venipuncture for determination of serum testosterone concentrations. All blood samples were centrifuged at 1,500 × g for 15 min, and serum was decanted and stored at −20°C until further analysis.

**Chemical Analyses**

Feed samples were analyzed for DM (24 h at 103°C), ash (4 h at 550°C), CP by the Kjeldahl method (AOAC, 1995), NDF according to the method of Van Soest et al. (1991) using sodium sulfite and α-amylase, and fat by Soxhlet extraction with a previous acid hydrolysis (AOAC, 1995).

Serum cortisol concentration was determined using an immunoassay technique (intra- and interassay CV of 5.6 and 7.1%, respectively; DRG-Cortisol ELISA EIA-1887, DRG Instruments GmbH, Marburg, Germany). Haptoglobin was determined by the hemoglobin binding method with the use of a commercial haptoglobin assay (intra- and interassay CV of 1.4 and 6.9%, respectively; assay phase range, Tridelta Development Limited, Maynooth, Ireland).

Serum was analyzed for antibodies specific for ovalbumin by indirect ELISA using Maxisorp 96-microwell plates (Nunc, Roskilde, Denmark) coated with 0.015 mg of ovalbumin per well. The plate was incubated for 18 h at 4°C to allow the ovalbumin to adhere to the wells. After the 18-h incubation, the plate was emptied and washed 3 times with 200 µL of PBS-0.05% Tween 20 (PBS-T) and further blocked with PBS-T for 2 h at 37°C. One hundred microliters of serum from samples obtained on d 14 and 35 was added to the plate at a dilution of 1:20 with PBS-T. This dilution was previously determined with a minimum of 6 different animals as the dilution giving the maximal signal. The plate with diluted serum was incubated for 1 h at 37°C and then washed 3 times with PBS-T. Horseradish peroxidase antiovinbo IgG (A3415, Sigma-Aldrich) was diluted 1:20,000 with PBS-T and 100 µL was added to the wells for 1 h at 37°C. After 3 PBS-T washes, the horseradish peroxidase reaction was developed with 100 µL of 3,3′,5,5′-tetramethylbenzidine substrate (Sigma-
Aldrich) and stopped with Stop Reagent for 3,3′,5,5′-tetramethylbenzidine substrate (Sigma-Aldrich). Finally, the ELISA plate was read at 450 nm with a microplate reader (model 680, Bio-Rad, Hercules, CA). All samples were analyzed in duplicate, and the nonspecific binding that occurred at d 14 was subtracted from the reading obtained at d 35 of the study. Variations in readings among different ELISA plates were corrected by normalizing the readings from each sample within a plate to a reference control sample included in each plate. Intra- and interassay CV were 5.5 and 21.1%, respectively.

Serum concentrations of testosterone were determined using solid-phase RIA (intra- and interassay CV of 4.1 and 6.3%, respectively) following the instructions of the manufacturer (Coat-A-Count Total Testosterone Kit, Diagnostic Products Corporation, Los Angeles, CA). The same technique was used in Mach et al. (2009). The kit was assayed in the laboratory, and testosterone recovery was 98.8% and intraassay CV was 4.1%.

Calculations and Statistical Analyses

Area under the curve (AUC) of serum cortisol concentration was calculated for the first hours after castration or ACTH injection by using the trapezoidal rule (Friend et al., 1977). Normality of the data before ANOVA analyses was evaluated by frequency histogram distribution and the Shapiro-Wilk test. Serum cortisol and haptoglobin data were transformed to a log scale to achieve a normal distribution before any statistical analysis. Scan samples were multiplied by 10 and duration (per hour) of each behavior was converted to a percentage of the total time observed; finally, these percentages were transformed to a log scale to achieve a normal distribution (Mitlöhner et al., 2001). The values presented herein correspond to nontransformed means; however, SEM and P-values correspond to the ANOVA analyses using log-transformed data.

Performance, serum haptoglobin concentration, serum cortisol concentration data on castration day or after ACTH injection on d 49, body and scrotal temperatures, and behavioral data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The mixed-effects model with repeated measures included time as a fixed effect and animal as a random effect. For all analyses, significance was declared at P ≤ 0.05 and tendencies were discussed at 0.05 < P ≤ 0.10.

RESULTS AND DISCUSSION

Intake and Animal Performance

Final BW (at d 49) and ADG (Table 2) were greater (P < 0.001) in INT (198 ± 1.6 kg, and 1.36 ± 0.038 kg/d, respectively) than in CAS calves (188 ± 1.6 kg, and 1.16 ± 0.038 kg/d, respectively). Body weight was affected (P < 0.001) by an interaction between castration and time. Up to d 7, no differences in BW between treatments were observed, but thereafter, CAS calves weighed less (P < 0.001) than INT calves. Average daily concentrate DMI (P = 0.10) and total DMI (P = 0.09) tended to be greater in INT calves than in CAS calves; however, when total DMI was expressed as a percentage of BW, no differences were found. Gain-to-feed ratio was reduced (P < 0.05) in CAS calves compared with INT calves.

In the present study, differences in ADG between CAS and INT calves were observed from the first week after castration and were maintained throughout the study. This result was unexpected, because in most studies (Fisher et al., 1996; Stafford et al., 2002; Ting et al., 2003; Pang et al., 2006), independent of age at castration, method of castration, or local anesthesia or analgesia procedure used, ADG decreased within the first days after castration, as occurred in the current study, but then ADG over the rest of the study lengths was unaffected. These differences could be attributed to the fact that in the cited studies, calves were fed grass silage supplemented with concentrate, and mean ADG was less than that observed in the present study. In agreement with the results of the current study, Fisher et al. (2001), who reported ADG close to those obtained in the current study, observed that band- or surgically castrated calves at 9 or 14 mo of age grew slower than intact calves. Morgan et al. (1993) observed that bulls had decreased muscle fractional degradation rates, in contrast to castrated calves, which could have contributed to their greater growth availability. Fisher et al. (2001) administrated exogenous testosterone to
castrated animals to investigate the roles of testosterone and castration in animal growth, but the exogenous testosterone administered was insufficient to increase plasma testosterone to the concentrations of intact calves and its effect on growth was minimal. Replacement of testosterone (using exogenous treatment) in castrated animals to concentrations equivalent to those of intact calves would help in elucidating the effects of the lack of testosterone in castrated animals on growth.

**Serum Cortisol Concentration at Castration Day**

Mean serum cortisol pretreatment values (−120 and 0 min before castration) were 16.1 ± 3.9 and 18.2 ± 3.7 nmol/L for INT and CAS calves, respectively. As depicted in Figure 1, mean serum cortisol from 30 to 180 min after castration was reduced (P < 0.001) in CAS (13.2 ± 1.56 nmol/L) compared with INT calves (13.2 ± 1.56 nmol/L). In addition, AUC of serum cortisol concentration from 0 to 180 min relative to castration time tended (P = 0.06) to be greater in INT than in CAS calves (32 vs. 19 ± 4.6 nmol/L per hour, respectively). Serum cortisol concentrations observed in the present study were close to those reported in other studies involving ring-castrated calves younger than 3 mo (Stafford et al., 2002; Thier et al., 2007). Serum cortisol concentration at castration day has been proposed as an indicator of acute pain and stress. Local anesthesia locally inhibits the action potential in nerve cells by inhibiting sodium influx through the nerve cell membrane. Systemic administration of an NSAID has been shown to act both centrally and peripherally, with central actions being related to supraspinal effects causing inhibition of spinal transmission of nociceptive inputs (McCormack, 1994). In the current study, serum cortisol in INT calves was greater than in CAS calves. These results disagree with previous reports (Stafford et al., 2002; Thier et al., 2007). The reason for this discrepancy is not clearly understood, but the small increase in serum cortisol in CAS calves could have been due to the administration of local anesthesia and analgesia. In addition, the castration method and the pain relief protocol (use of anesthesia or analgesia or both, and administration route and type of drug) affect the efficacy of acute pain alleviation (evaluated in the current study through serum cortisol concentrations). Stafford et al. (2002) concluded that injecting lidocaine into the testicles and into the distal end of the scrotum 20 min before the ring application successfully suppressed the acute pain and distress caused by ring castration, as indicated by the elimination of an increase in serum cortisol. Furthermore, these authors considered that giving ketoprofen in addition to a local anesthetic would be not necessary. Similarly, carprofen administered intravenously has been reported to only tend to reduce serum cortisol concentrations after band castration (Pang et al., 2006), but subcutaneous administration of carprofen in combination with an epidural injection of lidocaine in surgically castrated calves reduced serum cortisol concentrations more successfully than epidural-flunixin and an epidural alone (Stilwell et al., 2008). In contrast, Earley and Crowe (2002) and Ting et al. (2003) observed that systemic analgesia (ketoprofen intravenously) was more effective than local anesthesia or caudal epidural anesthesia at reducing the cortisol response after castration in surgically or Burdizzo-castrated calves, respectively.

**Serum Haptoglobin Concentration**

Increased production of acute phase proteins, such as haptoglobin, aids in the regulation of inflammation after tissue damage (Baumann and Gauldie, 1994). Therefore, after castration, which causes local tissue trauma, an increase in serum haptoglobin could be expected. However, in the present study, castration did not result in increased serum haptoglobin concentration, with only a numerical increase in CAS animals being observed at d 3 and 7 after castration. Ting et al. (2005) studied the effect of Burdizzo castration performed at different ages on animal welfare and reported an increase in serum haptoglobin concentration on the third day after castration in bulls castrated at 2.5 or

### Table 2. Dry matter intake and performance of intact Holstein calves (INT) or 3-mo-old ring-castrated Holstein calves administered local anesthesia and analgesia (CAS)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>INT</td>
<td>CAS</td>
</tr>
<tr>
<td>Initial and castration age, d</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Final BW (d 49 of study), kg</td>
<td>198</td>
<td>188</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.36</td>
<td>1.16</td>
</tr>
<tr>
<td>Concentrate DMI, kg of DM/d</td>
<td>4.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Straw DMI, kg of DM/d</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Total DMI, kg/d</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Total DMI as percentage of BW, kg of total DMI/kg of BW</td>
<td>2.87</td>
<td>2.85</td>
</tr>
<tr>
<td>G:F</td>
<td>0.27</td>
<td>0.24</td>
</tr>
</tbody>
</table>

$^1$Treatment = treatment effect; time = time effect (wk); treatment × time = treatment × time interaction effect.
3.5 mo, in contrast to bulls castrated at 1.5 mo of age. Pang et al. (2006) also observed an increase in serum haptoglobin concentration on the third day of castration in band-castrated bulls at 5.5 mo of age. However, as in the present study, when castration was performed with analgesia and carprofen (an NSAID), serum haptoglobin concentration was not different from that of intact bulls (Pang et al., 2006). In addition, results from Ting et al. (2003) support the observation that NSAID administration (ketoprofen) on the castration day mitigates the increase in serum haptoglobin after castration. As mentioned before, systemic administration of an NSAID is pain relieving and has anti-inflammatory effects.

**Rectal Temperature, Scrotal Temperature, Scrotal Lesion Scoring**

Castration did not affect rectal temperature (39.1 ± 0.032°C) throughout the study. Pang et al. (2006) observed an increase in rectal temperature only at 2 d after castration in band-castrated animals. In the present study, at d 35 relative to castration, testes began to slough off, and at d 49, all testes had completely sloughed off (55, 92, and 100% of CAS calves had no testes at 35, 42, and 49 d, respectively). Thus, scrotal temperature data were analyzed only from 0 to 28 d relative to castration. Mean scrotal temperature in CAS calves (28.8 ± 0.15°C) was reduced (P < 0.001) compared with that of INT calves (33.7 ± 0.15°C). Ting et al. (2005) observed a decrease in the difference between rectal and scrotal skin temperature in Burdizzo-castrated bulls, in contrast to that of intact bulls, because Burdizzo castration causes important tissue damage and inflammation, which raise scrotal temperature. In contrast, in the present study, the difference between rectal and scrotal temperatures in CAS animals (10.6 ± 0.21°C) was greater (P < 0.001) than that in INT calves (5.7 ± 0.22°C) because ring castration ceases blood flow to the testes. In the present study, a lesion score classified as 0, corresponding to no visible swelling, inflammation, or infection, was the main recorded lesion. However, the prevalence of castration lesion scores corresponding to an inflammation score of 1 increased (P < 0.05) from values around 0 to 8%, recorded from d 0 to 14 relative to castration, to 33% on d 21 and 28. The reduced wound incidence was in agreement with serum haptoglobin concentration data observed in the present study because castration did not affect serum haptoglobin. In contrast to the present study, Molony et al. (1995) reported that ring castration of calves at 1 wk of age resulted in a mean lesion score of around 4 at 28 d after castration. In agreement with the results of Molony et al. (1995), Thièr et al. (2007) observed an increased response to local palpation that persisted over 7 wk after castration in 1-mo-old ring-castrated animals, independent of the use of local anesthesia (although no analgesia was used). In agreement with the current study, Stafford et al. (2002) reported that, independent of anesthesia or analgesia use, several days after castration, the scrota of calves castrated at 3 mo of age were dry, with no swelling. In addition, these authors observed that at d 29, most animals had scrota that were shriveled and detached, and at d 38, most animals had small wounds.

**Behavior**

Normal standing, normal lying, and abnormal lying postures did not differ between treatments (Table 3). Abnormal standing postures were greater from d 3 to 14 after castration (P < 0.01) in CAS than INT calves (Figure 2). Foot stamping was not observed in INT or CAS calves. Head turning tended (P = 0.06) to be greater 14 d after castration in CAS than INT calves (Figure 3). Postures and active behavior 49 d after castration did not differ between CAS and INT calves (Table 3). The application of analgesia, such as an NSAID, seems to be essential in avoiding abnormal behaviors after castration. In the 2 reference studies (Molony et al., 1995; Thièr et al., 2007) in which the researchers observed abnormal behavior after ring castration attributed to chronic pain, no analgesia was used. In support of the results from the current study, Ting et al. (2003) reported that in 13-mo-old Holstein bulls, the use of an NSAID was more effective in reducing abnormal postures after Burdizzo castration than the application of local anesthesia.

**Ovalbumin Antibody Titers and Response to ACTH Injection**

Immunological assessment is a useful indicator of cattle welfare (Amadori et al., 1997). Castration did not affect the humoral response against ovalbumin. The difference in ovalbumin immunoglobulins between ovalbumin vaccination (14 d) and at d 35 of the study was 0.62 ± 0.09 and 0.71 ± 0.09 antibody titers for CAS and INT calves, respectively. Supporting the results presented herein, Pang et al. (2006) did not observe a detrimental effect of castration on cell-mediated immunity in the days after castration when evaluating different methods of castration and analgesia in 5-mo-old bulls. In contrast, Ting et al. (2003) observed a decrease...
in cell-mediated immunity 1 and 3 d after castration of 13-mo-old bulls when using the Burdizzo method.

Castration did not affect serum cortisol response to ACTH injection. Serum cortisol AUC between 0 and 4 h after ACTH injection was 517 ± 32.9 and 486 ± 32.2 nmol/L per hour for INT and CAS calves, respectively. The increase in plasma cortisol concentration, as a consequence of activation of the hypothalamic-pituitary-adrenal axis, is one of the best known and consistent neuroendocrine responses to stress (Sevi et al., 2002). In a welfare assessment of farm animals, the aim of administering exogenous ACTH is to stimulate adrenal secretion of cortisol, whose release may be strengthened by the existence of a concurrent stressful event, and ACTH administration has been used to study the consequences of long-term stressors. Indeed, there is evidence that graded cortisol responses to stress can be attributed to both the relative stressfulness and the cumulative action of each stressor (Mears and Brown, 1997). Hence, in contrast to the results observed in the present study, it was expected that if castration had a long-term stressful effect, CAS calves would have a greater serum cortisol response to ACTH injection than INT calves. To our knowledge, no published studies have evaluated the effect of castration on cortisol response after ACTH injection.

**Testosterone**

Mean serum testosterone concentration at 49 d of the study in INT calves was 269 ± 32 ng/dL, whereas in CAS calves, no serum testosterone could be detected. Knight et al. (2000) observed that testosterone concentration decreased immediately at d 0 after band or surgical castration. Amann and Walker (1983) observed that serum testosterone concentrations decreased below

<table>
<thead>
<tr>
<th>Item1</th>
<th>Treatment</th>
<th>SEM2</th>
<th>P-value3</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posture, %</td>
<td>INT</td>
<td>CAS</td>
<td>SEM2</td>
<td>Treatment</td>
<td>Time</td>
<td>Treatment × time</td>
</tr>
<tr>
<td>Normal standing</td>
<td>40.0</td>
<td>38.5</td>
<td>0.02</td>
<td>0.60</td>
<td>0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>Abnormal standing</td>
<td>0.5</td>
<td>2.8</td>
<td>0.03</td>
<td>0.001</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Normal lying</td>
<td>15.2</td>
<td>11.5</td>
<td>0.07</td>
<td>0.15</td>
<td>0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Abnormal lying</td>
<td>44.3</td>
<td>47.2</td>
<td>0.02</td>
<td>0.64</td>
<td>0.001</td>
<td>0.53</td>
</tr>
<tr>
<td>Active behavior, %</td>
<td>Idle</td>
<td>67.1</td>
<td>65.6</td>
<td>0.01</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Eating</td>
<td>16.3</td>
<td>15.0</td>
<td>0.02</td>
<td>0.32</td>
<td>0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Foot stamping</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>6.8</td>
<td>7.0</td>
<td>0.05</td>
<td>0.80</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>Head turning</td>
<td>2.6</td>
<td>3.0</td>
<td>0.04</td>
<td>0.24</td>
<td>0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>Sleeping</td>
<td>7.2</td>
<td>9.4</td>
<td>0.04</td>
<td>0.14</td>
<td>0.001</td>
<td>0.72</td>
</tr>
</tbody>
</table>

1Only data corresponding to 12 h (0800 to 2050 h) were used to create the scan sample data set. Behavior was analyzed at scan intervals of 10 min. To represent behavior over an entire hour, scan samples were multiplied by 10. Duration (per hour) of each behavior was converted to a percentage of the total time.
2The values presented herein correspond to nontransformed means; however, SEM and P-values correspond to the ANOVA analyses using log-transformed data.
3Treatment = treatment effect; time = time effect (wk); treatment × time = treatment × time interaction effect.
20 ng/dL at 1 h after castration, whereas testosterone serum concentration of intact Holstein bulls around 22 wk of age was 267 ng/dL.

In summary, 3-mo-old ring-castrated calves administered analgesia and anesthesia had reduced growth, and during the first 14 d after castration, they showed increased abnormally standing. The calves tended to increase head turnings, indicating distress, during this period. Whether these transient alterations in behavior and reduced growth are sufficient to recommend avoiding castration at 3 mo of age and administration of analgesia and anesthesia for welfare reasons should be further evaluated because no clear definition exists regarding how long these behavioral traits would need to be altered to consider that castrated animals were suffering chronic pain. On the other hand, the reduced growth observed in CAS bulls could be attributed to the absence of serum testosterone because this hormone has anabolic effects.

Conclusions

Welfare indicators such as DMI, serum cortisol, serum haptoglobin concentration, and wound healing were unaffected after ring castration performed with local anesthesia and analgesia at 3 mo of age. Despite growth being reduced and some behavior traits being altered during the first 2 wk after castration, ring castration of calves at 3 mo with administration of analgesia and anesthesia could be considered a method that controls pain and does not greatly compromise animal welfare.

LITERATURE CITED