Space allowance influences individually housed Holstein bull calf innate immune measures and standing behaviors after castration at 3 weeks of age

M. S. Calvo-Lorenzo,*1 L. E. Hulbert,*2 M. A. Ballou,† A. L. Fowler,*3 Y. Luo,‡ K. C. Klasing,* and F. M. Mitloehner*4
*Department of Animal Science, University of California, Davis 95616
†Department of Animal and Food Sciences, Texas Tech University, Lubbock 79409
‡Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506

ABSTRACT

Dairy calves in the Southwest regions of the United States are typically raised individually in wooden hutches with 1.23 m² of space. The objective of the study was to determine if increased space allowance in wooden hutches influences measures of innate immunity and behaviors of Holstein bull calves pre- and postcastration. Calves were randomly assigned at 4 d of age to conventional (CONV; 1.23 m² of space; n = 18), moderate (MOD; 1.85 m² space; n = 17), or maximized space allowance (MAX; 3.71 m² space; n = 19) in hutches. Calves were surgically castrated at 24 d of age. Peripheral whole blood samples were collected at −1, +1, +5, and +12 d of castration. Accelerometer loggers (n = 16 calves per treatment) were used from −3 to +5 d of castration to assess standing behaviors. All calves decreased total standing duration the day of castration versus precastration. Overall, MAX spent the most time in the stand position postcastration versus CONV and MOD. Within treatments, MOD and MAX had increased plasma cortisol 1 d postcastration versus precastration. A treatment × time tendency was observed for cortisol at 12 d postcastration; MAX had the least circulating cortisol. A treatment × time tendency for circulating haptoglobin (Hp) was observed and Hp was greatest among CONV 1 d pre- and 12 d postcastration. Compared with precastration, CONV had increased Hp at 1, 5, and 12 d, whereas MOD had increased Hp at 5 d, and Hp remained similar within MAX. A treatment × time tendency for tumor necrosis factor-α (TNF-α) from lipopolysaccharide-stimulated whole blood was observed; at 1 d postcastration, MOD had the most TNF-α, whereas MAX had the least. Within MAX, calves had increased TNF-α from precastration to 5 d postcastration. A treatment × time interaction was observed for whole blood bactericidal activity against Escherichia coli (WB anti-E). The CONV tended to have the greatest WB anti-E at d −1, but at d 1 and 5 postcastration, CONV had the least WB anti-E. Overall, MAX had less intensity of neutrophil oxidative burst versus CONV and MOD. The lower response of neutrophil oxidative burst and slower Hp secretion after castration is indicative that the wound site likely had less microbial exposure. The findings of this study suggest that calves housed with more space are potentially at less risk of too much inflammation after castration, which may likely be due to the effects of increased space on hide cleanliness and increased standing time.

Key words: bovine, inflammation, castration, behavior

INTRODUCTION

In 2011, California’s 1.78 million milking cows produced more than 19% of the dairy calves in the United States (NASS, 2012). In California and much of the Southwest United States, wooden hutches are used to house calves up to approximately 90 d of age. Each hutch provides 1.23 m² of space allowance per calf and calves within one unit can make limited contact with each other (Love et al., 2016). Many producers choose to individually house calves for the first few weeks of life because it may reduce the risk of disease transmission, facilitate farm worker health assessment and treatment of calves, and eliminate competition for milk/milk replacer, grain, and water (NAHMS, 2007; Costa et al., 2016; Hulbert and Moisá, 2016; Love et al., 2016). The conventional wooden hutch (versus the plastic or fiberglass hutch) is likely favorable in the Southwest region because producers modify these hutches with slatted-flooring, thus providing calves a more sanitary, cooler floor surface than dirt or bedding, especially during the
summer months (Mogensen et al., 1997; Hulbert and Moisá, 2016; Love et al., 2016).

In recent years, the value of neonatal dairy bull calves tripled and in 2012 almost 1 in 5 steaks consumed in the United States were from dairy breeds (Warynski, 2012; Hulbert and Moisá, 2016). Thus, dairy beef is an important commodity for high-producing dairy states, especially after drought events reduced the number of cattle in the beef industry (Buriaga-Robles, 2015). Many dairy calves are sold to specialized calf raising operations, otherwise known as calf ranches (Schafer, 2005). Many calf ranches use the above described wooden hutch as their primary housing and management system.

Calf ranchers have a narrow timeframe to perform castration among preweaned calves. The American Veterinary Medical Association (AVMA) recommends that castration take place before weaning (Coetzee et al., 2010; AVMA, 2014). Logically, younger animals are easier to handle and have less tissue to excise; therefore, the confounding effects from damaged tissue and stress from handling are potentially reduced (Molony et al., 1995; Fisher et al., 1996; Stafford and Mellor, 2005). There are also incentives within the beef and dairy industries to castrate before weaning; uncastrated dairy-breed calves are typically discounted when sold at stockyards and feedlots when they reach 120 d of age (Webster et al., 2013; Hulbert and Moisá, 2016). However, the timeframe for calf-managers to castrate is further narrowed because dairy calves are at the greatest risk for enteric disease from age 0 to 3 wk and step-down weaning is often initiated at 6 wk of age (NAHMS, 2007; Hulbert and Moisá, 2016). Therefore, calf ranchers will choose around 3 wk of age to castrate (Hulbert and Moisá, 2016). At this age, maternal antibodies are at their lowest if calves had successful passive transfer of maternal antibodies from colostrum and the calf is just starting to produce its own antibodies (Godden, 2008; Hulbert and Moisá, 2016).

There is a misconception that because older animals have a greater peak of cortisol release during castration, they are more stressed than young calves (Bretschneider, 2005; Stafford and Mellor, 2011). However, a lack of a cortisol response to painful procedures among calves may be a sign of an underdeveloped or dysfunctional hypothalamic-pituitary-adrenal (HPA) axis (Mitra et al., 2009; Hulbert and Moisá, 2016). Calves are born with an innate inflammatory response (Kampen et al., 2006) that is dependent on its regulation from the HPA axis. Glucocorticoids reduce inflammation, whereas factors from damaged tissue directly and indirectly activate the HPA axis (Boutzios and Kaltsas, 2000; Mitra et al., 2009; Hulbert and Moisá, 2016).

Castration induces an inflammatory response that can be measured by monitoring acute phase proteins, circulating leukocyte populations, and neutrophil function (Pang et al., 2009, 2011; Ballou et al., 2013). For example, castrated Holstein calves had increased circulating leukocytes and haptoglobin (Hp), decreased neutrophil oxidative burst responses to Escherichia coli, and decreased tumor necrosis factor-α (TNF-α) secretion from whole blood stimulated with LPS (Ballou et al., 2013). The inflammatory response to surgical castration may also be influenced by calf behaviors, such as how much time the calf rests after castration. In general, castrated calves (without pain relief) spend significantly greater time standing postcastration versus precastration (White et al., 2008), and display increased behavioral indicators of discomfort, such as abnormal postures and increased activity (Robertson et al., 1994). Researchers (Repenning et al., 2013; Sutherland et al., 2013; Webster et al., 2013) added pain relief treatments for castrated calves and observed that these calves returned to maintenance behaviors sooner (grooming, resting, feeding, and drinking) than calves provided no pain relief. In addition, pain relief administered to calves in the Sutherland et al. (2013) study had less of a pro-inflammatory response, including reduced total circulating leukocytes and peripheral neutrophil:leukocyte ratios, than control calves. Ballou et al. (2013) also reported increased plasma Hp concentrations in calves provided pain relief versus calves not provided pain relief. Therefore, both the return-to-normal behavior activities and attenuated pro-inflammatory measures may be important indicators that calves experienced less pain or distress.

The conventional wooden hutch is criticized because calves have one-third to two-thirds less space than other individualized or group-based calf housing systems. Because of this criticism, producers may choose to modify the conventional wooden hutch to increase space allowance. After castration, it is expected that the calf will attempt to reduce discomfort by shifting from a lying to standing position, and shifting lying position between the right and left side (Robertson et al., 1994; White et al., 2008). The amount of space may influence whether or not the calf can enter a consummatory state (changing position). In short, less space may be less motivating for the calf to seek comfort. In addition, the larger hutch size may provide more sanitary surface areas to rest on after castration. The sanitation may reduce the amount of exposure to microbes; therefore, one may expect a lessened inflammatory response.

The objectives of this study were to determine if increased space allowance for calves housed in wooden hutches influences standing behaviors and inflamma-
tory responses to castration. The authors hypothesized that all calves will decrease standing time on the day of castration, but calves with increased space allowance will be more motivated to stand the days following castration than conventional calves. The authors also hypothesized that this altered activity from space allowance may help reduce measures of inflammation among calves with more space.

**MATERIALS AND METHODS**

**General Animal Care, Housing, and Space Allowance**

The present study was conducted at the University of California (UC), Davis, Department of Animal Science’s Feedlot and Environmental Research Facility located in Davis, California, from April to July 2011. All calves were housed and managed in accordance to the Guide for the Care and Use of Agriculture Animals in Research and Teaching (FASS, 1999). All procedures were approved by the UC Davis Institutional Animal Care and Use Committee (protocol 16279). This manuscript is a portion of a larger study that evaluated calf performance and health from 0 to 12 wk of age, and those data were reported elsewhere (Calvo-Lorenzo et al., 2016).

Sixty colostrum-fed bull calves [total plasma protein at age 4 d = 5.6 ± 1.1 g/dL (±SD); measured via Rhino Clinical handheld VET 360 Refractometer, Reichert Technologies, Depew, NY] were obtained from a commercial calf ranch in Tulare, California. The commercial calf ranch purchased the calves from 2 different commercial dairies. After the calves were bottle-trained, they were transported 365 km at 4 d of age to the UC Davis Department of Animal Science Feedlot and Environmental Research Facility. Upon arrival, they were randomly assigned to 1 of 3 space allowance treatments using the RAND function in Microsoft Excel (Microsoft Office Excel, 2007, Microsoft Corp., Redmond, WA). Wooden hutches were structurally modified to create the following 3 space allowance treatments: conventional (CONV; n = 20), moderate (MOD; n = 20), and maximized (MAX; n = 20) space allowance. Two CONV calves, 3 MOD calves, and 1 MAX calf had morbidity or mortality as described in Calvo-Lorenzo et al. (2016) and were excluded from this experiment. The CONV hutches had 2 inter-barriers that separated 3 calves within each structure (1.23 m² of space per calf; described in Calvo-Lorenzo et al., 2016) and the inter-barriers allowed for minimal contact between neighboring calves. The MOD hutches were modified to increase space allowance by placing only 1 inter-barrier in the center of a conventional hutch, which housed 2 calves individually with 1.85 m² of space per calf. For the MAX hutch, both inter-barriers were removed, providing 1 calf 3.71 m² of space. Hutches were made of solid wooden panels (2.44 × 1.52 × 1.37 m), elevated 0.2 m above a dirt floor, and had 0.06-m-wide wooden slatted flooring with 0.03-m gaps positioned across the width of the hutch.

Each hutch type was aligned into rows by treatment and each row was randomly placed inside 1 of 4 cattle pen enclosures (CPE; described in Calvo-Lorenzo et al., 2016) to reduce variation in measures caused by extreme weather conditions (Martin et al., 1975; Hultberg and Ballou, 2012). A 0.61-m separation between hutches was present. Within each CPE, each row was randomly assigned to the north, center, or south region of each CPE. The mean CPE temperature and relative humidity (HOBO H8 Pro Series, 2-channels, Onset Computer Corp., Bourne, MA) were 17.01 ± 4.80°C and 72.0 ± 21.3% SD, respectively. The minimum and maximum temperature across CPE was 5.5 and 29.7°C, respectively.

**Calf Feeding**

The Institutional Animal Care and Use Committee approved feeding strategies used in this experiment followed calf ranch standard feeding protocols, which was important to address industry needs pertaining to space allowance and castration. The feeding regimen for healthy calves included milk replacer (Table 1) twice daily (0600 and 1500 h), ad libitum feed (Table 1) and water, and if needed, an additional 2 L of electrolytes (BlueLite C, TechMix, LLC, Stewart, MN). All calves were examined daily for clinical signs of sickness and calves that appeared dehydrated or sick were treated by the collaborating veterinarian. Upon calf arrival to the UC–Davis research facility, the collaborating veterinarian determined that all calves had diarrhea and were dehydrated. Therefore, all calves had the milk replacer feeding reduced to the 0600 h feeding and were provided with 2 to 4 L of electrolytes in the a.m. and p.m. until d 3 of the trial. For this experiment, BW were recorded and analyzed for ages 21 and 45 d (Salter Brecknell PS-500 digital scale, Fairmount, MN).

**Castration**

After the morning milk replacer feeding, calves were castrated at 24 or 25 d of age (split into 2 blocks for sampling logistics), calves’ testicles were surgically removed by 2 well-trained calf ranch managers. Calves were restrained briefly, and an incision was made at the base of the scrotum to exteriorize the testes. The testicles were removed with gentle traction until the vas deferens was pulled apart from the body cavity.
A cleansing and disinfecting solution (Nolvasan Solution, Zoetis, Florham Park, NJ) was sprayed on the lesion to prevent infection. The authors anticipated that handling time may be different among treatments because calves with more space have more room to escape. Therefore, castration time was measured with a stopwatch. The stopwatch was started as soon as the handler entered the pen and stopped as soon as the calf was released. No anesthesia or analgesics were used for castration. Surgical castration without anesthesia is the predominant way that young calves are castrated in the United States and this method of castration is the standard operating procedure for many commercial calf ranches and dairies (Coetzee et al., 2010; AVMA, 2014). To address current industry needs and concerns related to space allowance and common management practices, this Institutional Animal Care and Use Committee approved castration procedure was used in the study.

**Behavior**

To assess the behaviors of calves with different hutch space allowances, daily stand position total duration, standing bout duration, frequency of standing bouts, and percentage of time a calf lay on its right or left side were measured on randomly selected calves (n = 16 calves per treatment) on d −3 to +5 relative to castration (d 0) using the Onset Pendant-G data-loggers (64k, Onset Computer Corporation, Bourne, MA). Forty-eight loggers were launched to collect the y- and z-axis position every 30 s. Loggers were attached to the calves’ hind leg 2 d before data collection as previously described (Calvo-Lorenzo et al., 2016) and loggers were retrieved 6 d after castration. The data were downloaded using the Onset HOBOware Software (Onset Computer Corporation, Bourne, MA) and were exported into Microsoft Excel to organize and summarize for statistical analysis as described by Ledgerwood et al. (2010) and Bonk et al. (2013) in SAS (v. 9.2, SAS Inst. Inc., Cary, NC).

**Blood Collection and Hematology Analysis**

Nine milliliters of peripheral blood was collected via jugular venipuncture at d −1, +1, 5, and 12 ± 0.5 relative to castration. Samples were collected in 2 blocks on consecutive days to accommodate logistics of conducting laboratory assays. Blood was collected into 2 vacutainers (6 mL of heparin and 3 mL of EDTA). Within 1 h after collection, the EDTA-vacutainer from each calf was analyzed for hematocrit, total leukocyte counts, and differential analyses of neutrophils, lymphocytes, and monocytes cells using a Procyte blood analyzer (Idexx Laboratories Inc., Sacramento, CA). The neutrophil:lymphocyte (N:L) ratio was calculated for each sample as an additional measure of inflammation.

**Measures of Inflammation**

Plasma from the heparin vacutainer was collected after centrifugation at 1,250 × g for 15 min and stored at −20°C until analysis. Cortisol concentrations were measured by ELISA using a commercially available kit (Arbor assays, Ann Arbor, MI) and the intra- and inter-assay variations were 6.5 and 7.8%, respectively. Plasma glucose, urea nitrogen, Hp, and Zn concentrations were measured as described by Ballou et al. (2011). The intra-assay variations were 5.8, 3.8, 4.4, and 1.8% for glucose, urea nitrogen, Hp, and zinc concentrations, respectively. The inter-assay variations were 7.2, 4.0, 5.1, and 1.8% for glucose, urea nitrogen, Hp, and Zn concentrations, respectively.

For a humoral and cellular test of inflammation, a whole blood bactericidal activity test against *E. coli*
WB anti-

---

**Statistical Analysis**

All data from 2 calves each in the CONV and MAX treatments and 4 calves from the MOD treatment were removed from the data set because they were treated with antibiotics <4 d before or during castration. A linear, mixed model with the fixed effects of day, treatment, and the interactions of treatment × day and block × treatment × day was fitted. The random effect was calf nested within treatment × block. All data were analyzed by restricted maximum likelihood ANOVA using the MIXED procedure of SAS (v. 9.2, SAS Inst. Inc.). The ante-regressive (1) covariance structure for the within-subject measurement was used for all models. Repeated data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v. 9.2, SAS Inst. Inc.). Data that were not normally distributed were log- or arcsine square root-transformed before mixed model analysis. Variables that were transformed included cortisol, Hp, TNF-α, IFN-γ, N:L, neutrophil concentrations, and standing bout duration; the least squares means (±SEM) reported throughout were from the nontransformed data. Pair-wise comparisons were performed (1) among treatments at each time using a sliced-effect multiple comparison approach and (2) within each treatment across time using a Tukey-Kramer adjustment to control the family-wise type-1 error. Least squares means (±SEM) are reported throughout. A treatment difference of \( P < 0.05 \) was considered significant and \( P \leq 0.10 \) was considered a tendency.

**RESULTS**

Performance measures from 0 to 12 wk of age are reported elsewhere (Calvo-Lorenzo et al., 2016). For this portion of the experiment, BW were similar across treatments before castration (at age 21 d; \( P > 0.10 \)); however, BW differed across treatments by age 45 d [53, 59, and 58 ± 1.7 (SE) kg for CONV, MOD, and MAX respectively; \( P = 0.04 \)]. Increased space allow-
ance was associated with increased ADG from 21 to 45 d of age [0.56, 0.60, and 0.69 ± 0.05 (SE) kg for CONV, MOD, and MAX, respectively; \( P = 0.03 \)]. Also, a tendency was found for increased space allowance to increase total DMI during the 21- to 45-d period (1,088, 1,153, and 1,240 ± 61 g/d for CONV, MOD, and MAX, respectively; \( P = 0.07 \)).

Expert calf handlers from the calf ranch of origin spent between 40 to 184 s to restrain and castrate each calf, but it did take more time (\( P = 0.05 \)) to perform the castration on the MAX calves when compared with the CONV and MOD calves [88.2 vs. 64.2 and 63.7 ± 6.3 (SE) s, respectively]. Overall, MAX calves spent the most time in the stand position, followed by CONV calves, and MOD calves spent the least time in the stand position (\( P = 0.037 \); Table 2). On the day of castration (d 0), all calves decreased total standing duration and the standing bout duration compared with the days before castration, but they increased the frequency of standing bouts (day \( P < 0.001 \); Table 2). In addition, compared with the other treatments, MAX calves displayed the greatest standing bout duration 3 and 4 d after castration (\( P = 0.049 \); Table 2; Figure 1B). Although a treatment \( \times \) day effect was found for the stand bout duration measure, no treatment \( \times \) day effect was observed for the frequency at which calves changed position (frequency of standing bouts, \( P > 0.10 \); Table 2; Figure 1C).

All calves had increased circulating leukocytes, glucose, and N:L the day after castration compared with precastration (\( P < 0.001 \); Table 3). The CONV housed calves tended (\( P = 0.08 \); Table 3) to have lower plasma glucose concentrations than MAX housed calves. A tendency (\( P = 0.07 \); Table 3) was found for a treatment \( \times \) time interaction on plasma cortisol concentrations. At 12 d after castration, MAX calves had the least circulating cortisol 12 d after castration (Figure 2). Plasma cortisol was increased (\( P < 0.001 \)) the day after castration in both the MOD and MAX calves, but remained unchanged in the CONV calves (Figure 2). Within the MAX calf treatment, plasma cortisol at 12 d postcastration was not different than baseline (Figure 2). A tendency for a treatment \( \times \) time effect for circulating Hp (\( P = 0.09 \); Table 2) was observed and Hp concentrations were greatest among CONV calves 1 d before and 12 d following castration (Figure 3). The CONV calves had increased plasma Hp concentrations on 1, 5, and 12 d after castration compared with precastration (\( P < 0.01 \); Figure 3). In addition, the MOD calves had increased Hp on d 5 (\( P < 0.05 \); Figure 3), whereas Hp concentrations of MAX calves were similar after castration (Figure 3). All calves had increased plasma Zn concentrations 12 d after castration (\( P = 0.02 \); Table 3). In addition, the CONV calves

### Table 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (Trt)</th>
<th>SEM(^2)</th>
<th>Time relative to castration, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of calves</td>
<td>16</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stand position, h/d</td>
<td>6.7 ± 0.3</td>
<td>—</td>
<td>−3 −2 −1 0 1 2 3 4 5 Trt × Day</td>
</tr>
<tr>
<td>Standing bout,3 min/d</td>
<td>31.5 ± 2.9</td>
<td>—</td>
<td>−3 −2 −1 0 1 2 3 4 5 Trt × Day</td>
</tr>
<tr>
<td>Standing bouts,4 no./d</td>
<td>14.0 ± 1.3</td>
<td>—</td>
<td>−3 −2 −1 0 1 2 3 4 5 Trt × Day</td>
</tr>
<tr>
<td>Lying, right side,5 %</td>
<td>44.4 ± 3.1</td>
<td>—</td>
<td>−3 −2 −1 0 1 2 3 4 5 Trt × Day</td>
</tr>
<tr>
<td>Lying, left side,5 %</td>
<td>55.6 ± 3.2</td>
<td>—</td>
<td>−3 −2 −1 0 1 2 3 4 5 Trt × Day</td>
</tr>
</tbody>
</table>

\(^1^\)Least squares means differ (\( P < 0.05 \); Tukey-Kramer adjustment).

\(^2^\)Calves were randomly assigned at age 4 d to conventional (CONV; 1.24 m\(^2\) of space/calf), moderate (MOD; 1.85 m\(^2\) of space/calf), or maximum (MAX; 3.71 m\(^2\) of space/calf).

---

**Note:** SEM = standard error of the mean; Trt = treatment (CONV, MOD, MAX); Day = day relative to castration; SEM\(^2\) = largest SEM; \( P \)-value = probability value.
had reduced concentrations of plasma Zn throughout the observation period ($P = 0.004$; Table 3).

A tendency for a treatment × time effect ($P = 0.10$; Table 2) for TNF-α secreted from LPS-stimulated whole blood was observed and MOD calves tended to have the greatest concentrations, whereas MAX calves had the least (Figure 4). Calves within the MAX calf treatment had elevated TNF-α 5 d after castration compared with baseline concentrations ($P < 0.05$; Figure 4). A treatment × time effect ($P = 0.016$; Table 2) was found for WB anti-\(E.\ coli\). Prior to castration, WB anti-\(E.\ coli\) tended to be greatest ($P < 0.10$; Figure 5) for the CONV calves. Surgical castration decreased ($P < 0.05$) the bactericidal activity of whole blood in the CONV calves at 1 and 5 d after castration. In contrast, WB anti-\(E.\ coli\) activity was not affected by castration among the MOD and MAX calves over time (Figure 5).

At 12 d after castration versus other time periods, all calves had the least percentage of neutrophils exhibiting both phagocytosis and oxidative burst (PG+OB+) and these PG+OB+ neutrophils had the least oxidative burst ($P < 0.05$; Table 3). Although the percentage of PG+OB+ neutrophils did not change from −1 d measures ($P > 0.10$; Table 3), the neutrophils had decreased OB+ GMFI 1 d after castration, which increased at 5 d, but not to the same intensity as −1 d ($P < 0.05$; Table 3). Overall, the MAX calves had neutrophils that expressed the least OB+ GMFI compared with CONV and MOD calves ($P < 0.05$; Table 3). No treatment, time, or treatment × time effects were observed for either neutrophil phagocytosis intensity (PG+) or IFN-γ secretion from PHA-stimulated whole blood throughout castration (Table 3).

**DISCUSSION**

Castration is a common management strategy that is primarily performed in cattle to prevent pregnancy when animals are matured and housed together. Castration may also improve animal temperament, worker safety, and meat quality, but the procedure is painful and stressful for calves (Ballou et al., 2013; Hulbert and Moisá, 2016). The authors hypothesized that on the day of castration, all calves will decrease standing time; however, calves with increased space allowance will have more motivation to stand postcastration and reduced measures of inflammation due to the altered activity from increased space allowance. Data from this study indicate that, indeed, the housing environment in wooden calf hutches affects calf standing behaviors and measures of immunity following surgical castration.

As expected, castration caused all calves to decrease total standing duration, but increased the standing bout frequency compared with the days before castration. Sutherland et al. (2013) did not find significant differences in the frequency of standing and lying.
Table 3. Least squares mean influence of housing under various space allowances on dairy calf peripheral blood and immune measures after castration at age 24 d1

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (Trt)</th>
<th>Time relative to castration, d</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONV</td>
<td>MOD</td>
<td>MAX</td>
</tr>
<tr>
<td>Number of calves</td>
<td>18</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>35.8</td>
<td>31.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Total leukocytes, ×10^6</td>
<td>9.10</td>
<td>9.93</td>
<td>9.96</td>
</tr>
<tr>
<td>Neutrophil, %</td>
<td>37.2</td>
<td>38.2</td>
<td>38.6</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td>54.5</td>
<td>54.2</td>
<td>55.1</td>
</tr>
<tr>
<td>Monocyte, %</td>
<td>7.06</td>
<td>7.13</td>
<td>5.63</td>
</tr>
<tr>
<td>Neutrophil:lymphocyte3</td>
<td>0.758</td>
<td>0.754</td>
<td>0.774</td>
</tr>
<tr>
<td>Plasma analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>25.8</td>
<td>25.7</td>
<td>24.2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>70.8</td>
<td>72.6</td>
<td>74.5</td>
</tr>
<tr>
<td>Haptoglobin, o.d. × 100</td>
<td>1.79a</td>
<td>1.43b</td>
<td>1.30b</td>
</tr>
<tr>
<td>Urea nitrogen, mg/dL</td>
<td>6.60</td>
<td>6.50</td>
<td>6.00</td>
</tr>
<tr>
<td>Zinc, mg/L</td>
<td>1.05a</td>
<td>1.29b</td>
<td>1.21b</td>
</tr>
<tr>
<td>Whole blood culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS TNF-α, pg/mL</td>
<td>180.0</td>
<td>185.1</td>
<td>172.7</td>
</tr>
<tr>
<td>PHA IFN-γ, pg/mL</td>
<td>1,232.0</td>
<td>1,155.5</td>
<td>870.4</td>
</tr>
<tr>
<td>E. coli, % cfu killed</td>
<td>24.8</td>
<td>20.8</td>
<td>18.9</td>
</tr>
<tr>
<td>Neutrophil function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG+OB+, %</td>
<td>55.8</td>
<td>51.7</td>
<td>50.7</td>
</tr>
<tr>
<td>OB+, GMFI</td>
<td>495.6</td>
<td>455.8</td>
<td>365.0b</td>
</tr>
<tr>
<td>PG+, GMFI</td>
<td>441.6</td>
<td>408.2</td>
<td>420.0</td>
</tr>
</tbody>
</table>

a–dLeast squares means differ, P < 0.05.

1Calves were randomly assigned at 4 d of age to conventional (CONV; 1.24 m² of space/calf), moderate (MOD; 1.85 m² of space/calf), or maximum (MAX; 3.71 m² of space/calf) space allowance.

2Largest SEM.

3P-values were derived from log-transformed data.

4P-values were derived from arc sin square-root-transformed data.

5Haptoglobin measured in optical density (o.d.).

6Tumor necrosis factor-α (TNF-α) was measured from the supernatant fraction of LPS stimulated whole blood.

7IFN-γ was measured from the supernatant fraction of phytohemagglutinin (PHA) stimulated whole blood.

8Percent Escherichia coli colony-forming units killed by whole blood.

9Percent of neutrophils that exhibited both phagocytosis (PG+) and oxidative burst (OB+) response.

10Phagocytosis (PG+) and oxidative burst (OB+) flow-cytometric geometric mean fluorescent intensity (GMFI).
postures observed across control and castrated calves treated with or without pain relief 3 h postcastration. Similarly, Molony et al. (1995) observed that castration did not influence 1-wk-old calf total standing duration after the first 3 h of castration. However, White et al. (2008) noted that mixed-breed beef calves spent more time standing postcastration when compared with precastration measurements. Standing bout duration was greater among MAX compared with CONV and MOD calves on d 3 and 4, after castration. In addition, overall time periods, MAX calves spend more

Figure 2. Plasma cortisol concentrations (mean ± SE) for calves housed in conventional (CONV; 1.24 m² of space/calf; n = 18), moderate (MOD; 1.85 m² of space/calf; n = 17), or maximum (MAX; 3.71 m² of space/calf; n = 19) space allowance after castration at 24 ± 0.50 (SD) d of age (d 0 relative to castration). †Means within a housing treatment are different from d −1 (root-transformed data; P < 0.05). #Tendency (P < 0.10).

Figure 3. Plasma haptoglobin concentration [optical density (OD) × 100; mean ± SE] for calves housed in conventional (CONV; 1.24 m² of space/calf; n = 18), moderate (MOD; 1.85 m² of space/calf; n = 17), or maximum (MAX; 3.71 m² of space/calf; n = 19) space allowance after castration at 24 ± 0.50 (SD) d of age (d 0 relative to castration). †Means within a housing treatment are different from d −1 (root-transformed data; P < 0.05). #Tendency (P < 0.10).

Figure 4. Tumor necrosis factor-α (TNF-α) secretion from LPS-stimulated whole blood from calves housed in conventional (CONV; 1.24 m² of space/calf; n = 18), moderate (MOD; 1.85 m² of space/calf; n = 17), or maximum (MAX; 3.71 m² of space/calf; n = 19) space allowance after castration at 24 ± 0.50 (SD) d of age (d 0 relative to castration). †Means within a housing treatment are different from d −1 (log-transformed data; P < 0.05). #Tendency (P < 0.10).

Figure 5. Whole blood bactericidal activity against Escherichia coli (WB anti-E. coli) in colony-forming units of conventional (CONV; 1.24 m² of space/calf; n = 18), moderate (MOD; 1.85 m² of space/calf; n = 17), or maximum (MAX; 3.71 m² of space/calf; n = 19) space allowance after castration at 24 ± 0.50 (SD) d of age (d 0 relative to castration). *Means across treatments within a time point differ (P < 0.01). †●Means within CONV housing treatment are different from d −1 (P < 0.05). #Tendency (P < 0.10).
the stand position than the other calves. These findings support the logic that if more space is available, calves may be more motivated to change position and stay in a more active position. In addition, more space may allow the calf to have more perceived control over its environment. For example, although handling times for castration were limited to no more than 3 min, calves in the MAX treatment took longer to castrate simply because it was more work to catch and restrain the animal in the larger space allowance. Perceived control over the animal’s environment is important for the animal to manage stressors (Moberg and Mench, 2000). The MAX calves may have more perceived control over their environment because unlike CONV and MOD calves, they have greater potential to escape the handler.

Increased space allowance may also be more motivating for an uncomfortable calf to stand and perform maintenance behaviors, such as eating feed and drinking. In the current experiment, calves with more space allowance may have resumed normal maintenance behaviors (feeding and drinking) at a faster rate than CONV calves because the CONV calves gained the least weight and tended to consume the least amount of feed (Calvo-Lorenzo et al., 2016). This motivation provided by space allowance may be similar to the use of analgesics in calves undergoing both castration and dehorning, where inappetence and decreased ADG were remedied with analgesics (Ballou et al., 2013; Sutherland et al., 2013).

When an animal cannot cope with a perceived threat through escape, the HPA axis can become exhausted. An acute increase, within minutes, in plasma cortisol concentrations and neutrophilia are common after surgical castration; however, others reported that concentrations return to baseline within 24 to 72 h (Cohen et al., 1990; Ballou et al., 2013; Mosher et al., 2013). Prolonged pain, inflammation, or psychological chronic stress may cause persistently elevated plasma cortisol concentrations, followed by adrenal-exhaustion (Hulbert and Moisá, 2016); therefore, the current research sought to determine if calves experienced chronic cortisol secretion across different space allowance treatments. Cortisol concentrations in the MOD and MAX calves were elevated from baseline the day after castration, which were similar to concentrations observed within 12 min to 6 h after castration in previous studies (King et al., 1991; Molony et al., 1995; Fisher et al., 1996). In contrast, the CONV calves had similar cortisol concentrations 1 d after castration as their baseline levels, which suggests that CONV calves had (1) a faster HPA axis response to the acute stress of surgical castration than MOD and MAX calves, or (2) they did not have a response at all, due to adrenal-exhaustion. The first suggests that CONV calves are more resilient to acute stress than the other calves, but the second speculation indicates the opposite where CONV calves potentially experienced adrenal-exhaustion before castration. Neither of these explanations can be confirmed without further research, where blood samples are collected serially before and after castration. Furthermore, plasma cortisol concentrations were expected to return to baseline concentrations in all calves 12 d after castration. However, only the MAX calves had values similar to their baseline concentrations. This suggests that the CONV and MOD calves were either chronically stressed or had greater inflammation after surgical castration.

The HPA axis regulates inflammation, and likewise, tissue damage can activate the HPA axis (Matthews, 2002; Blalock and Smith, 2007; Hulbert and Moisá, 2016). The WB anti- \textit{E. coli} activity in the present study was reduced among the CONV calves only at 1 and 5 d after castration, which suggests that CONV calves may not have had an acute cortisol response to castration. Acute secretion of glucocorticoids play an important role in modulating innate immunity, and inflammatory factors also can stimulate the HPA axis (Sorrells and Sapolsky, 2007; Hulbert and Moisá, 2016); therefore, increased cortisol concentrations may reduce whole blood bactericidal capacity. However, as explained by Hulbert and Moisá (2016), circulating cortisol concentrations do not explain all the immunomodulatory effects of stressors on calves. In contrast to the WB anti- \textit{E. coli}, the additional leukocyte responses evaluated in the current study did not indicate that the CONV calves had a more suppressed immune response after surgical castration. In fact, neutrophil oxidative burst intensity was decreased in all calves after castration compared with baseline, which was decreased further 12 d after castration. The further decrease in neutrophil oxidative burst may have been associated with increasing age of the calf and not related to castration because neutrophil oxidative burst is commonly reported to decrease over the preweaned period in dairy calves (Cobb et al., 2014).

Prior to castration, the CONV calves tended to have a greater WB anti- \textit{E. coli} capacity than either MOD or MAX calves. In addition, the CONV and MOD calves had greater neutrophil oxidative burst activity when stimulated with \textit{E. coli} when compared with the MAX calves. Together, these data suggest that the MAX calves had less active leukocyte responses or innate immune responses before castration. The exact reason for this is unknown, but it may be associated with decreased microbial exposure and less inflammation. A paradox exists with neutrophil function because excessive oxidative burst capacity may exacerbate the...
inflammatory response to tissue damage if neutrophils become necrotic (Paape et al., 2003; Hulbert and Moisá, 2016). Under sterile conditions, leukocytes are responsive to danger-associated molecular patterns produced by damaged tissues, and the animal will tend to have a more localized inflammatory response than in less sanitary conditions. However, under less sanitary conditions, leukocytes respond to both danger-associated molecular patterns and microbial-associated molecular patterns; the inflammatory response is more peripheral under these conditions and more markers of inflammation can be observed (Chang et al., 2004; Buckham Sporer et al., 2007; Hulbert and Moisá, 2016).

The Hp data may help further justify the explanation on immune responses across treatments. Typically, calves have an increase in plasma Hp concentrations after surgical castration (Faulkner et al., 1992; Ting et al., 2003; Pang et al., 2006). Fisher et al. (1997) and Earley and Crowe (2002) reported increased Hp concentrations at 1, 3, and 7 d postcastration of 5-month-old calves. Plasma Hp concentrations are related to the magnitude of an inflammatory stimuli (Conner et al., 1988); therefore, the CONV and MOD calves had greater inflammatory responses than the MAX calves. Furthermore, the CONV calves had more persistent inflammation than the MOD calves because plasma Hp concentrations remained elevated 12 d postcastration in the CONV calves. The lower concentrations of plasma Zn in CONV calves further suggest that those calves had more inflammation because reduced plasma Zn is associated with systemic inflammation and possibly more microbial exposure (Heegaard et al., 2000; Ballou et al., 2011, 2013).

As reported by Calvo-Lorenzo et al. (2016), the CONV and MOD calves in the current study were dirtier than MAX calves for the 3 wk before castration. These data are consistent with the conclusion that a dirtier environment and more lying time after castration influence the immune response to castration (Calvo-Lorenzo et al., 2016). Over one-half of the calf ranches in California often place wooden hutches on slatted flooring so that fecal matter falls below and away from the calf, which should aid with calf cleanliness (Love et al., 2016). The MAX calves may have had better control over their environment and potentially reduced their risk of chronic stress, but when the hutch is designed to provide more space, it provides a more sanitary environment for the newly castrated calf. The CONV calves may have had more microbial exposure from their hutch environment, resulting in their immune systems being “primed” for tissue damage from the castration procedure. Cobb et al. (2014) reported that group-housed calves had more active neutrophil responses when compared with individually housed calves, and they suggested that increased social interaction among the calves exposed them to a greater degree of microbial exposure. More research with calf housing needs to determine how the microbial environment is altered and if that has an effect on the leukocyte responses of calves.

**CONCLUSIONS**

As public concerns regarding the rearing and confinement of livestock species continues to rise, interest is growing in the methods in which calves are raised (USDA, 2011). These data suggest that increasing the space allowance among calves raised in wooden calf hutches may reduce the risk of excessive inflammation following surgical castration. From this study, it appears that because space allowance influenced the sanitation of the calf environment and standing behaviors, a reduced inflammatory response occurred. Too much inflammation can exacerbate infectious disease; therefore, it is possible that these data also indicate that a reduced risk of infectious disease may be associated with space allowance. However, an epidemiological approach will be the next step to testing this hypothesis. In addition, future research is needed to determine if CONV calves may benefit from a nonsurgical castration method or anti-inflammatory medication, rather than a surgical method that causes an open wound.

**ACKNOWLEDGMENTS**

The authors appreciate the assistance of University of California (Davis) students, researchers, and support staff, Roberta Franco, Clayton Neumeier, Sara E. Place, Kimberly Stockhouse-Lawson, Qian Wang, Vanessa Arias, Jamie Sherman, Monica VanKloppenberg, Veronica Arteaga, Jessica Botelho, Joseph Dorsch, Ashley Fowler, Connor Hoff, Benjamin Kasl, Lauren LeFort, Jacob Murphy, Nasario Ramos, Jessica Sampson, Alex Taylor, Tara Urbano, Jessica Varaitch-Srarse, Samantha Werth, Bruce Hoar, Alyssa Louie, Amanda Plunkett, Michael Tobias, Kevin Bauer, Jennifer Arnall, Cody Wohlman, Josh Ettlin, Joshua Krumheuer, Jessica Collier, Cameron Thompson, Gerry Johnson, James Moller, Dan Sehnert, Mark Rubio, Jose Villasenor, Frank Sauers, David Gall, Yongjing Zhao, and Yuee Pan for help associated with the trial, data collection, and analysis. The authors also thank Cassandra Tucker (University of California, Davis) for kindly lending her behavior data loggers and her invaluable advice, as well as her PhD student, Eranda Rajapaksha, who provided insight and suggestions on logger leg attachment and data output. All funding and support for this project was provided by the California Cattlemen's
Association (Sacramento, CA), Western United Dairymen (Modesto, CA), and an anonymous Tulare County, California, bull calf ranch.

REFERENCES


