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 Moisés, 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

Received August 5, 2016.

Accepted November 16, 2016.

¹Present address: Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140.

²Present address: Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506.

³Present address: Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546.

⁴Corresponding author: fmmitloehner@ucdavis.edu

summer months (Mogensen et al., 1997; Hulbert and Moisés, 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 (Wardynski, 2012; Hulbert and Moisés, 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 (Burciaga-Robles, 2015). Many dairy calves are sold to specialized calf raising operations, otherwise known as calf ranches (Schaefer, 2005). Many calf ranches use the above described wooden hutches 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 Moisés, 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 Moisés, 2016). Therefore, calf ranchers will choose around 3 wk of age to castrate (Hulbert and Moisés, 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 Moisés, 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 Moisés, 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 Moisés, 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 ($\pm$ 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 m^2 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 m^2 of space per calf.

For the MAX hutch, both inter-barriers were removed, providing 1 calf 3.71 m^2 of space. Hutches were made of solid wooden panels ($2.44 \times 1.52 \times 1.37 \text{ m}$), 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; Hulbert 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 $17.01 \pm 4.80^\circ\text{C}$ and $72.0 \pm 21.3\%$ 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, Fairmont, 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

Table 1. The formulated nutrient content (as-fed basis) of the milk replacer and calf feed fed to calves

Item	Milk replacer ¹	Feed ²
DM, %	97	92
Ether extract (min ³), %	20	4.8
CP (min), %	20	18
Crude fiber (max ³), %	0.15	5.8
Calcium (max), %	0.75	0.8
Calcium (min), %	0.6	0.6
Phosphorus, %	0.5	0.3
Vitamin A (min), IU/kg	46,297	26,455
Vitamin D ₃ (min), IU/kg	9,259	—
Vitamin E (min), IU/kg	661	—
Monensin Na, %	—	0.441

¹Calves were bottle-fed a commercial milk replacer (Veal Tech Inc., Oswego, IL) at 1.2% of calf BW (259 g/2 L of water) twice daily at 0600 and 1500 h. Milk replacer was composed of dried whey protein concentrate, dried whey, dried skim milk, proprietary blend of milk and vegetable fats with coconut oil (butylated hydroxytoluene) and citric acid added, lecithin, calcium carbonate, L-lysine, DL-methionine, threonine, inulin (fructooligosaccharide), yeast autolysate dehydrate (mannan-oligosaccharide), dicalcium phosphate, vitamin A acetate, cholecalciferol (vitamin D₃), D-alpha tocopherol acetate (vitamin E), manadione sodium bisulfate complex (vitamin K), vitamin B₁₂ supplement, ethylenediamine dihydroiodide, thiamine mononitrate, niacin supplement, riboflavin, pyridoxine HCl, calcium pantothenate, D-biotin, ascorbic acid, folic acid, Mg sulfate, Cu proteinate, Cu sulfate, Zn proteinate, Zn sulfate, Co sulfate, Na molybdate, Na selenite, mono- and diglycerides.

²Calves were fed ad libitum a commercial calf feed [Best Start, 5506 ALG calf starter mix (2010–18)-M; A.L. Gilbert Company, Oakdale, CA] that provided macroingredients including grain products, processed grain by-products, plant and milk protein products, animal and plant fat products, molasses and roughage products with trace minerals, and a proprietary blend of yeast product.

³min = minimum; max = maximum.

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 $1,250 \times g$ 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-*E. coli***) was performed on heparinized whole blood. The blood was diluted (1:2) with RPMI 1640, which produced a final concentration of either 2,000 cfu/mL of *Escherichia coli* 8739 (Microbiology, St. Cloud, MN) or 0 cfu for quality control media (RPMI only). Samples were placed in a 38.5°C circulating water bath for 10 min. Then, 50 μ L was cultured in duplicate tryptic soy agar Petri dishes for 24 h in a humidified 5% CO₂ chamber at 38.5°C. The percentage of colony-forming units compared with control plates (~200 cfu) was calculated.

The inflammatory regulation of leukocytes was measured using ex vivo stimulation of LPS (*E. coli* O111:B4; Sigma-Aldrich, St. Louis, MO) or phytohemagglutinin (**PHA**; L 8754; Sigma-Aldrich, St. Louis, MO) and measuring cytokine secretion of TNF- α or IFN- γ , respectively. Heparinized whole blood was diluted 1:5 with RPMI 1640 (Invitrogen, Carlsbad, CA) containing 1% ANTI-antibiotics (GIBCO Antibiotic-Antimycotic, Invitrogen). Whole blood was stimulated at a final concentration of 1 μ g/mL of LPS or 25 μ g/mL of PHA. The LPS-stimulated and PHA-stimulated samples were incubated for 24 or 72 h, respectively, in a humidified 5% CO₂ chamber. After incubation, the cell culture plates were centrifuged for 5 min at 1,200 $\times g$ at 4°C. The supernatant fraction was collected and stored in -40°C freezer until analysis. The LPS or PHA samples were measured for TNF- α or IFN- γ , respectively, using commercially available ELISA kits (KingFisher Biotech, St. Paul, MN). Standards were diluted in RPMI with 10% pooled bovine plasma and 1% antibiotics. The TNF- α intra- and inter-assay coefficients of variation were 8.4 and 3.8%, respectively, and sensitivity of the assay was 78.1 pg/mL. The IFN- γ intra- and inter-assay coefficients of variation were 8.5 and 3.3%, respectively, and sensitivity of the assay was 93.8 pg/mL.

Neutrophil inflammatory responses were measured using the simultaneous phagocytic and oxidative burst capacities of peripheral blood neutrophils in response to *E. coli* 8739 exposure, and was analyzed as previously described by Hulbert et al. (2011). Briefly, heat-killed *E. coli* were labeled with propidium iodide and stored at 10⁹ cfu/mL in 1 $\times$ PBS at -80°C. Two hundred microliters of whole blood (sodium-heparin) from the 6 mL vacutainer per animal was incubated in an ice bath for 5 min. Forty microliters of a 100 μ M working concentration of dihydrorhodamine (Invitrogen, Carlsbad, CA) and the fluorescently labeled *E. coli* were added to each sample, vortexed thoroughly, and then incubated in a 38.5°C water bath for 10 min. After completion of incubation, the samples were immediately placed in an ice bath for 5 min. Erythrocytes were hypotonically lysed, washed, and the leukocytes analyzed by dual-

color flow cytometry (Becton Dickinson FACScan Flow Cytometer, Fullerton, CA). Using flow-cytometer analysis software (Flowjo 7.6.3, Treestar Inc., Ashland, OR), neutrophils were gated on the scatter-plot of electric volume $\times$ side scatter. Neutrophils that were positive for both oxidative burst (**OB+**) and phagocytosis (**PG+**) were then gated using the fluorochrome (**FL**)-1 by FL-3 scatter-plot. The OB+ PG+ cell percentage and geometric mean fluorescence intensity (**GMFI**) of the FL-1 and FL-3 were analyzed for oxidative burst and phagocytosis, respectively.

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 $\times$ day and block $\times$ treatment $\times$ day was fitted. The random effect was calf nested within treatment $\times$ 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 ($\pm$ 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 ($\pm$ 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 $\pm$ 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, 0.66, 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 × day effect was found for the stand bout duration measure, no treatment × 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 × 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 × 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. Least squares mean influence of housing under various space allowances on dairy calf standing and lying behaviors after castration at age 24 d¹

Item	Treatment (Trt)			SEM ²	Time relative to castration, d										SEM ²	Trt	P-value	
	CONV	MOD	MAX		-3	-2	-1	0	1	2	3	4	5	Day			Trt × Day	
Number of calves	16	16	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Stand position, h/d	6.7 ^{ab}	6.4 ^b	7.3 ^a	0.27	7.4 ^a	7.4 ^a	7.31 ^a	6.1 ^b	7.3 ^a	6.7 ^a	6.3 ^a	6.4 ^a	6.4 ^a	0.27	0.037	<0.001	0.135	
Standing bout, ³ min/d	31.5	29.0	36.3	2.92	32.3 ^a	34.4 ^a	33.4 ^a	22.7 ^b	43.5 ^c	35.7 ^a	31.7 ^a	28.4 ^d	28.6 ^d	4.19	0.226	<0.001	0.049	
Standing bouts, ⁴ no./d	14.0	13.9	13.5	0.74	14.6 ^a	13.8 ^a	13.9 ^a	17.2 ^b	11.4 ^c	11.9 ^c	12.6 ^{ac}	14.4 ^{ab}	14.4 ^{ab}	1.03	0.880	<0.001	0.282	
Lying, right side, ⁵ %	44.4	40.4	42.0	1.92	46.3	41.5	41.5	41.3	41.5	41.9	42.5	41.3	42.3	2.24	0.332	0.112	0.695	
Lying, left side, ⁵ %	55.6	59.7	58.0	1.92	53.7	58.5	58.5	58.7	58.5	58.1	57.5	58.7	57.7	2.20	0.332	0.112	0.695	

^{a-d}Least squares means differ (*P* < 0.05; Tukey-Kramer adjustment).
¹Calves were randomly assigned at age 4 d 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.
³Each bout of standing was averaged to calculate the mean bout length for each day; data were log-transformed to obtain *P*-values.
⁴The number of times the calf moved from the lie position to the stand position each day.
⁵While in the lying position, the percentage lying on the left or right side.

had reduced concentrations of plasma Zn throughout the observation period ($P = 0.004$; Table 3).

A tendency for a treatment $\times$ 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 $\times$ 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 1 and 5 d after castration 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 $\times$ 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 Moisé, 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

Figure 1. The daily (A) total stand position, (B) standing bout duration, and (C) number of standing bouts among calves housed in conventional (CONV; 1.24 m² of space/calf; n = 16), moderate (MOD; 1.85 m² of space/calf; n = 16), or maximum (MAX; 3.71 m² of space/calf; n = 16) space allowance after castration at 24 $\pm$ 0.50 (SD) d of age (d 0 relative to castration). *Means across treatments within a time point differ. †Means within MAX housing treatment are different from d -1 ($P < 0.05$). ‡Means within MOD housing treatment are different from d -1 ($P < 0.05$). A pound sign (#) represents a tendency ($P < 0.10$) for CONV > MAX calves.

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

Item	Treatment (Trt)			SEM ²	Time relative to castration, d				P-value		
	CONV	MOD	MAX		−1	1	5	12	Trt	Day	Trt × Day
Number of calves	18	17	19	—	—	—	—	—	—	—	—
Peripheral blood measures											
Hematocrit, %	35.8	31.0	33.3	1.4	34.5 ^a	33.7 ^b	33.4 ^b	32.9 ^b	0.13	0.006	0.55
Total leukocytes, ×10 ⁶	9.10	9.93	9.96	0.48	8.84 ^a	10.53 ^b	9.67 ^{ab}	9.64 ^{ab}	0.47	0.002	0.45
Neutrophil, %	37.2	38.2	38.6	2.2	38.7 ^a	41.8 ^a	36.0 ^b	35.6 ^b	0.89	<0.001	0.71
Lymphocyte, %	54.5	54.2	55.1	1.7	56.0 ^a	51.8 ^b	56.2 ^a	54.4 ^{ab}	0.91	0.002	0.87
Monocyte, %	7.06	7.13	5.63	0.96	4.75 ^a	5.96 ^a	7.19 ^b	8.54 ^b	0.40	<0.001	0.58
Neutrophil:lymphocyte ³	0.758	0.754	0.774	0.069	0.758 ^a	0.890 ^b	0.690 ^a	0.711 ^a	0.97	<0.001	0.75
Plasma analysis											
Cortisol, ⁴ ng/mL	25.8	25.7	24.2	1.2	18.1 ^a	27.0 ^b	23.7 ^b	32.3 ^c	0.84	<0.001	0.07
Glucose, mg/dL	70.8	72.6	74.5	1.2	67.6 ^a	71.9 ^b	70.9 ^{ab}	80.2 ^c	0.08	<0.001	0.52
Haptoglobin, ^{3,5} o.d. × 100	1.79 ^a	1.43 ^b	1.30 ^b	0.145	1.17 ^a	1.74 ^b	1.74 ^b	1.39 ^{ab}	0.21	0.009	0.09
Urea nitrogen, mg/dL	6.60	6.50	6.00	0.60	6.40 ^a	7.70 ^a	4.60 ^b	6.70 ^a	0.36	<0.001	0.62
Zinc, mg/L	1.05 ^a	1.29 ^b	1.21 ^b	0.051	1.12 ^a	1.11 ^a	1.15 ^{ab}	1.35 ^b	0.004	0.024	0.11
Whole blood culture											
LPS TNF-α, ^{3,6} pg/mL	180.0	185.1	172.7	22.9	140.6 ^a	143.4 ^a	233.4 ^b	199.6 ^b	0.21	<0.001	0.10
PHA IFN-γ, ^{3,7} pg/mL	1,232.0	1,155.5	870.4	295.1	912.5	1,152.2	1,331.3	947.9	0.98	0.51	0.98
<i>E. coli</i> , ⁸ % cfu killed	24.8	20.8	18.9	3.3	27.6 ^a	15.0 ^b	17.3 ^b	26.2 ^a	0.30	<0.001	0.016
Neutrophil function											
PG+OB+, ⁹ %	55.8	51.7	50.7	6.3	56.6 ^a	53.6 ^a	53.3 ^a	47.3 ^b	0.67	0.020	0.15
OB+, ¹⁰ GMFI	495.6 ^a	455.8 ^a	365.0 ^b	40.8	666.4 ^a	393.3 ^b	459.9 ^c	235.8 ^d	0.027	0.001	0.99
PG+, ¹⁰ GMFI	441.6	408.2	420.0	32.3	423.8	458.9	419.8	390.7	0.74	0.12	0.71

^{a-d}Least 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).

Figure 2. Plasma cortisol concentrations (mean $\pm$ 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 $\pm$ 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 $\pm$ 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 ($P < 0.10$).

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 time in

Figure 3. Plasma haptoglobin concentration [optical density (OD) $\times$ 100; mean $\pm$ 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 $\pm$ 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 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 $\pm$ 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 Moisé, 2016); therefore, the current research question 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 Moisé, 2016). The WB anti-*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 have not 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 Moisé, 2016); therefore, increased cortisol concentrations may reduce whole blood bactericidal capacity. However, as explained by Hulbert and Moisé (2016), circulating cortisol concentrations do not explain all the immunomodulatory effects of stressors on calves. In contrast to the WB anti-*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-*E. coli* capacity than either than MOD or MAX calves. In addition, the CONV and MOD calves had greater neutrophil oxidative burst activity when stimulated with *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 Moisés, 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 Moisés, 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 Stackhouse-Lawson, Qian Wang, Vanessa Arias, Jamie Sherman, Monica VanKlompberg, 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-Scrase, 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 Villaseñor, 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 Dairy-men (Modesto, CA), and an anonymous Tulare County, California, bull calf ranch.

REFERENCES

- AVMA. 2014. Literature review on the welfare implications of castration of cattle. American Veterinary Medical Association. Accessed Jan. 26, 2016. <https://www.avma.org/KB/Resources/LiteratureReviews/Pages/castration-cattle-bgnd.aspx>.
- Ballou, M. A., C. J. Cobb, L. E. Hulbert, and J. A. Carroll. 2011. Effects of intravenous *Escherichia coli* dose on the pathophysiological response of colostrum-fed Jersey calves. *Vet. Immunol. Immunopathol.* 141:76–83.
- Ballou, M. A., M. A. Sutherland, T. A. Brooks, L. E. Hulbert, B. L. Davis, and C. J. Cobb. 2013. Administration of anesthetic and analgesic prevent the suppression of many leukocyte responses following surgical castration and physical dehorning. *Vet. Immunol. Immunopathol.* 151:285–293.
- Blalock, J. E., and E. M. Smith. 2007. Conceptual development of the immune system as a sixth sense. *Brain Behav. Immun.* 21:23–33.
- Bonk, S., O. Burfeind, V. S. Suthar, and W. Heuwieser. 2013. Technical note: Evaluation of data loggers for measuring lying behavior in dairy calves. *J. Dairy Sci.* 96:3265–3271.
- Boutzios, G., and G. Kaltsas. 2000. Immune System Effects on the Endocrine System. <http://www.ncbi.nlm.nih.gov/books/NBK279139/>. Endocrinology, South Dartmouth, MA.
- Bretschneider, G. 2005. Effects of age and method of castration on performance and stress response of beef male cattle: A review. *Livest. Prod. Sci.* 97:89–100.
- Buckham Sporer, K. R., J. L. Burton, B. Earley, and M. A. Crowe. 2007. Transportation stress in young bulls alters expression of neutrophil genes important for the regulation of apoptosis, tissue remodeling, margination, and anti-bacterial function. *Vet. Immunol. Immunopathol.* 118:19–29.
- Burciaga-Robles, L. O. 2015. Neonatal and young (< 205 kg) feeding programs in calf-fed Holsteins. Abstract 516 in Proc. ADSA-ASAS Joint Annual Meeting 2015, Orlando, FL. <http://m.jtmtg.org/abs/t/65556>.
- Calvo-Lorenzo, M. S., L. E. Hulbert, A. L. Fowler, A. Louie, L. J. Gershwin, K. E. Pinkerton, M. A. Ballou, K. C. Klasing, and F. M. Mitloehner. 2016. Wooden hutch space allowance influences male Holstein calf health, performance, daily lying time, and respiratory immunity. *J. Dairy Sci.* 99:4678–4692.
- Chang, L. C., S. A. Madsen, T. Toelboell, P. S. Weber, and J. L. Burton. 2004. Effects of glucocorticoids on Fas gene expression in bovine blood neutrophils. *J. Endocrinol.* 183:569–583.
- Cobb, C. J., B. S. Obeidat, M. D. Sellers, A. R. Pepper-Yowell, D. L. Hanson, and M. A. Ballou. 2014. Improved performance and heightened neutrophil responses during the neonatal and weaning periods among outdoor group-housed Holstein calves. *J. Dairy Sci.* 97:930–939.
- Coetzee, J. F., A. L. Nutsch, L. A. Barbur, and R. M. Bradburn. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. *BMC Vet. Res.* 6:12 <https://doi.org/10.1186/1746-6148-6-12>.
- Cohen, R. D. H., B. D. King, L. R. Thomas, and E. D. Janzen. 1990. Efficacy and stress of chemical versus surgical castration of cattle. *Can. J. Anim. Sci.* 70:1063–1072.
- Conner, J. G., P. D. Eckersall, A. Wiseman, T. C. Aitchison, and T. A. Douglas. 1988. Bovine acute phase response following turpentine injection. *Res. Vet. Sci.* 44:82–88.
- Costa, J. H. C., M. A. G. von Keyserlingk, and D. M. Weary. 2016. Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. *J. Dairy Sci.* 99:2453–2467.
- Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *J. Anim. Sci.* 80:1044–1052.
- FASS. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching, 1st ed. Federation of Animal Science Societies, Champaign, IL.
- Faulkner, D. B. T., T. Eurell, W. J. Tranquilli, R. S. Ott, M. W. Ohl, G. F. Cmarik, and G. Zinn. 1992. Performance and health of weanling bulls after butorphanol and xylazine administration at castration. *J. Anim. Sci.* 70:2970–2974.
- Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *J. Anim. Sci.* 74:2336–2343.
- Fisher, A. D., M. A. Crowe, M. L. O’Nuallain, D. J. Prendiville, P. O’Kiley, and W. J. Enright. 1997. Effects of suppressing cortisol following castration of bull calves on adrenocorticotrophic hormone, in vitro interferon-gamma production, leukocytes, acute-phase proteins, growth, and feed intake. *J. Anim. Sci.* 75:1899–1908.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* 24:19–39.
- Heegaard, P. M. H., D. L. Godson, M. J. M. Toussaint, K. Tjørnehøj, L. E. Larsen, B. Viuff, and L. Rønsholt. 2000. The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Vet. Immunol. Immunopathol.* 77:151–159.
- Hulbert, L. E., and M. A. Ballou. 2012. Innate immune responses and health of individually reared Holstein calves after placement into transition-pens 23 d after weaning. *J. Dairy Res.* 79:333–340.
- Hulbert, L. E., C. J. Cobb, J. A. Carroll, and M. A. Ballou. 2011. The effects of early weaning on innate immune responses of Holstein calves. *J. Dairy Sci.* 94:2545–2556.
- Hulbert, L. E., and S. J. Moisé. 2016. Stress, immunity and the management of calves. *J. Dairy Sci.* 99:3199–3216.
- Kampen, A. H., I. Olsen, T. Tollersrud, A. K. Storset, and A. Lund. 2006. Lymphocyte subpopulations and neutrophil function in calves during the first 6 months of life. *Vet. Immunol. Immunopathol.* 113:53–63.
- King, B. D., R. D. H. Cohen, C. L. Guenther, and E. D. Janzen. 1991. The effect of age and method of castration on plasma cortisol in beef calves. *Can. J. Anim. Sci.* 71:257–263.
- Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. *J. Dairy Sci.* 93:5129–5139.
- Love, W. J., T. W. Lehenbauer, B. M. Karle, L. E. Hulbert, R. Anderson, A. L. Van Eenennaam, T. B. Farver, and S. S. Aly. 2016. Survey of dairy practices associated with respiratory health of pre-weaned calves on California dairies. *J. Dairy Sci.* 99:1483–1494.
- Martin, S. W., C. W. Schwabe, and C. E. Franti. 1975. Dairy calf mortality rate: The association of daily meteorological factors and calf mortality. *Can. J. Comp. Med.* 39:377–388.
- Matthews, S. G. 2002. Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol. Metab.* 13:373–380.
- Mitra, R., R. Adamec, and R. Sapolsky. 2009. Resilience against predator stress and dendritic morphology of amygdala neurons. *Behav. Brain Res.* 205:535–543.
- Moberg, G. P., and J. A. Mench, ed. 2000. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. CABI, Wallingford, UK.
- Mogensen, L., C. C. Krohn, J. T. Sorensen, J. Hindhede, and L. H. Nielsen. 1997. Association between resting behaviour and live weight gain in dairy heifers housed in pens with different space allowance and floor type. *Appl. Anim. Behav. Sci.* 55:11–19.
- Molony, V., J. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after different methods of castration of calves. *Appl. Anim. Behav. Sci.* 46:33–48.
- Mosher, R. A., C. Wang, P. S. Allen, and J. F. Coetzee. 2013. Comparative effects of castration and dehorning in series or concurrent castration and dehorning procedures on stress responses and production in Holstein calves. *J. Anim. Sci.* 91:4133–4145.
- NAHMS (National Animal Health Monitoring System). 2007. Dairy 2007: Heifer calf health and management practices on U.S. dairy operations. USDA:APHIS:VS, ed. USDA, Ft. Collins, CO. <https://>

- www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_ir_CalfHealth.pdf.
- NASS. 2012. Agricultural statistics board; United States department of agriculture. Cattle. National Agricultural Statistics Service. Accessed Mar. 8, 2012. https://www.nass.usda.gov/Publications/Ag_Statistics/2012/chapter07.pdf.
- Paape, M. J., D. D. Bannerman, X. Zhao, and J. W. Lee. 2003. The bovine neutrophil: Structure and function in blood and milk. *Vet. Res.* 34:597–627.
- Pang, W. Y., B. Earley, M. Murray, T. Sweeney, V. Gath, and M. A. Crowe. 2011. Banding or burdizzo castration and carprofen administration on peripheral leukocyte inflammatory cytokine transcripts. *Res. Vet. Sci.* 90:127–132.
- Pang, W. Y., B. Earley, T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon- γ production, acute-phase proteins, feed intake, and growth. *J. Anim. Sci.* 84:351–359.
- Pang, W. Y., B. Earley, T. Sweeney, S. Pirani, V. Gath, and M. A. Crowe. 2009. Effects of banding or burdizzo castration of bulls on neutrophil phagocytosis and respiratory burst, cd62-l expression, and serum interleukin-8 concentration. *J. Anim. Sci.* 87:3187–3195.
- Repenning, P. E., J. K. Ahola, R. J. Callan, J. T. Fox, J. T. French, R. L. Giles, R. K. Peel, J. C. Whittier, and T. E. Engle. 2013. Effects of pain mitigation and method of castration on behavior and feedlot performance in cull beef bulls. *J. Anim. Sci.* 91:4975–4983.
- Robertson, I. S., J. E. Kent, and V. Molony. 1994. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. *Res. Vet. Sci.* 56:8–17.
- Schaefer, D. M. 2005. Yield and quality of Holstein beef. Pages 323–333 in *Proc. Managing and Marketing Quality Holstein Steers*. R. Tigner and J. Lehmkuhler, ed. Wisconsin Agric. Service Assoc., Madison, WI.
- Sorrells, S. F., and R. M. Sapolsky. 2007. An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav. Immun.* 21:259–272.
- Stafford, K. J., and D. J. Mellor. 2005. The welfare significance of the castration of cattle: A review. *N. Z. Vet. J.* 53:271–278.
- Stafford, K. J., and D. J. Mellor. 2011. Addressing the pain associated with disbudding and dehorning in cattle. *Appl. Anim. Behav. Sci.* 135:226–231.
- Sutherland, M. A., M. A. Ballou, B. L. Davis, and T. A. Brooks. 2013. Effect of castration and dehorning singularly or combined on the behavior and physiology of Holstein calves. *J. Anim. Sci.* 91:935–942.
- Ting, S. T. L., B. Earley, J. M. L. Hughes, and M. A. Crowe. 2003. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. *J. Anim. Sci.* 81:1281–1293.
- USDA. 2011. U.S. Department of Agriculture: Animal welfare information center. States' farm animal welfare statutes. Accessed Aug. 27, 2011. <https://www.nal.usda.gov/awic/state-and-local>.
- Wardynski, F. 2012. Raising dairy steer calves for profitable beef production. Michigan State University Extension. Accessed Jun. 1, 2016. http://msue.anr.msu.edu/news/raising_dairy_steer_calves_for_profitable_beef_production.
- Webster, H. B., D. Morin, V. Jarrell, C. Shipley, L. Brown, A. Green, R. Wallace, and P. D. Constable. 2013. Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. *J. Dairy Sci.* 96:6285–6300.
- White, B. J., J. F. Coetzee, D. G. Renter, A. H. Babcock, D. U. Thomson, and D. Andresen. 2008. Evaluation of two-dimensional accelerometers to monitor behavior of beef calves after castration. *Am. J. Vet. Res.* 69:1005–1012.