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Wooden hutch space allowance influences male Holstein calf health, performance, daily lying time, and respiratory immunity

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ABSTRACT

Dairy calves in the western United States are commonly raised individually in wooden hutches with a space allowance of 1.23 m^2/calf. Recent legislative initiatives in California and across the United States were passed regarding concern over space allowance for farm animals. The objective of this study was to determine if rearing male Holstein calves in wooden hutches modified to increase space allowance would influence measures of performance, lying time per day, health, and respiratory immunocompetence. At 4 d of age, 60 calves were randomly assigned to 1 of 3 housing treatments: (1) conventional housing (CONV; 1.23 m^2/calf), (2) 1.5 × CONV (MOD; 1.85 m^2/calf), or (3) 3 × CONV (MAX; 3.71 m^2/calf). Intakes of milk and solid feed were recorded daily and body weight was measured at 0, 3, 6, 10, and 12 wk of age. For the first 3 wk of the trial, calves were scored daily for fecal consistency, hydration, and hide cleanliness. In addition, calves were scored for respiratory health (i.e., nasal and eye discharge, ear position) until 7 wk of age. The total lying duration per day was recorded using data loggers at 3, 6, and 10 wk of age. Eight clinically healthy calves from each treatment were sensitized with subcutaneous ovalbumin (OVA) and then challenged with aerosolized OVA to assess calf respiratory immunity at 11 wk of age. Bronchoalveolar lavage fluid (BALF) was collected 4 d after the OVA challenge and analyzed for leukocyte differentials and OVA-specific IgG, IgG_1, IgA, and IgE. Calf average daily gain and body weight were positively associated with space allowance at approximately 3 wk before weaning and throughout postweaning, respectively. A greater space allowance decreased lying time after 46 d. Space allowance did not influence fecal consistency, but there was a tendency for MAX calves to take 1 d longer to recover from loose feces than MOD calves. The MAX calves had the fewest (%) observations with feces on their body compared with CONV or MOD. At 3 wk of age, peripheral eosinophil concentrations decreased with increased space allowance. However, observations (%) of eye discharge increased with greater space allowance. Among calves challenged with OVA, MOD calves had the least BALF OVA-IgE, and the percent of BALF eosinophils decreased with increased space allowance. Increased space allowance for calves raised in wooden hutches may improve some measures of calf performance, health, and respiratory immunocompetence.
Key words: space allowance, behavior, health, performance

INTRODUCTION

California (**CA**) has more than 1,800 dairies and approximately 1.8 million milking cows (NASS, 2012); therefore, the CA dairy industry produces a large number of calves per year (1.75 million milk cows calved in 2011; NASS, 2012). Since the 1990s, there has been an upsurge in specialized calf raising operations (**CRO**); CRO producers raise replacement heifers for dairy producers and purchase dairy bull calves for beef (Wolf, 2003; Walker et al., 2012). These facilities represent over 11.5% of the US dairy heifer population (Walker et al., 2012) and the majority of dairy-beef calves (Bur-

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ciaga-Robles, 2015). Several factors may be considered for a successful CRO, including economics, morbidity and mortality, antibiotic use, and biosecurity practices (Hulbert and Moisá, 2016). A recent survey of CRO reported that the average and median calf mortality rates were 6.9 and 3.6%, respectively (Walker et al., 2012). This mortality average is less than the national average (9.6% of preweaned and postweaned dairy heifer calf mortality in 2006; USDA, 2010). This reduced mortality rate may be partially due to the type of CRO housing systems, as individual housing is important for biosecurity and biocontainment (Quigley et al., 1995; Anderson, 1998). Housing systems for neonatal or preweaned heifers in the United States have traditionally comprised individual hutches or pens, with approximately 42 and 37% of US preweaned heifers raised in outdoor individual hutches or individualized pens inside enclosed barns in 2010, respectively (USDA, 2012). In California, 9 out of 10 calves are raised in individual housing, and one of the most common housing structures is the conventional wooden hutch (Love et al., 2016). Each hutch houses 3 calves individually with 1.23 m^2/head of space until just after weaning, and over half of CA calf raisers place the hutches on wooden slatted flooring to improve sanitation and abate heat stress, especially during the summer months (Love et al., 2016). There has been growing public concern about housing of farm animals; California passed legislation that prohibits laying hens, gestation sows, and veal calves from being reared in housing systems that inhibited animals from "turning around freely" (i.e., California's Prop 2; California Attorney General, 2008). Although the wooden hutch allows young dairy calves to turn around freely (Love et al., 2016), some CRO were concerned that the ambiguous verbiage of this type of legislation may still affect their housing systems.

Forced modification to such housing may increase the cost of raising calves (California Attorney General, 2008); thus, CRO need to know whether the costs are offset if this alteration improves calf performance and health. Although the effects of increasing space allowance on calf performance are varied in other housing systems (Fisher et al., 1985; Friend et al., 1985; Terosky et al., 1997), conventional space allowance is associated with reduced comfort and locomotor play, as well as increased fearfulness and stereotypic behaviors in calves (Wilson et al., 1999; Jensen and Kyhn, 2000; Tapkı et al., 2006). To our knowledge, very limited data have been reported on calf lying duration, performance, and health responses to increased space allowance in conventional wooden hutches (Macaulay et al., 1995). Therefore, the present research evaluated measures of performance, respiratory immunocompetence, health, and daily activity of calves housed in conventional or modified wooden hutches. First, we hypothesized that increased space allowance would decrease the amount of lying time per day and increase the calf's motivation to consume solid feed, thus influencing growth and feed conversion rates. Because preweaned calves are susceptible to enteric and respiratory disease (Hulbert and Moisá, 2016), we also hypothesized that increased space allowance would facilitate better sanitation and therefore reduce the recovery rate following naturally occurring enteric disease and subclinical signs of respiratory disease. Finally, we hypothesized that increased space allowance would improve respiratory immunocompetence after exposure to subcutaneous and aerosolized ovalbumin.

MATERIALS AND METHODS

General Animal Care, Housing Treatments, and Environment

The present study was conducted from April to July 2011 at the University of California (UC), Department of Animal Science's Feedlot and Environmental Research Facility (Davis). 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), and all procedures were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC Protocol # 16279).

A total of 60 colostrum-fed Holstein bull calves, randomly selected from 120, were used in the present study [total plasma protein at age 4 d = 5.6 ± 1.1 (SD) g/dL; measured via Rhino Clinical hand-held VET 360 Refractometer, Reichert Technologies, Depew, NY]. A commercial calf ranch (in Tulare, CA) selected and purchased calves from 2 different commercial dairies. After 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 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. The CONV hutches had 2 solid inter-barriers that individually separated 3 calves within each structure (1.23 m^2 of space per calf; Figure 1a). The barriers were constructed to the height of each calf's shoulders (approximately 0.9 m high from the floor); hence, calves could make nose-to-nose contact with adjacent calves within each structure, and calves could see and hear other calves surrounding their structure. The MOD hutches were modified to increase space allowance by placing only one 0.9-m-tall barrier in the center of a conventional hutch, which housed 2 calves individually with 1.85 m^2 of space per calf (Figure 1a). For the MAX hutches, both inter-barriers were removed, providing a single calf with 3.71 m^2 of space (Figure 1a); calves in this system could not make nose-to-nose contact with adjacent calves but could still see and hear other calves surrounding their structure. Hutches were made of solid wooden panels (2.44 × 1.52 × 1.37 m) and were elevated 0.2 m above dirt floor with 0.06-m-wide wooden slatted flooring with 0.03-m gaps, positioned across the width of the hutch.

Each hutch type was randomly aligned in rows and all rows were randomly placed into different locations within 4 cattle pen enclosures (**CPE**; Figure 1b) to reduce variation in measures caused by extreme weather conditions (Martin et al., 1975; Hulbert and Ballou, 2012). The CPE were dome-shaped steel structures (Legend Series Cover-All Building, Saskatoon, SK, Canada) covered with a white 100% Marquesa Lana double-stacked weave Dura-Weave cover (Intertape Polymer Group, Montreal, QC, Canada). A mechanical ventilation system supplied fresh air and evaporative cooling through inlet cooling pads (4.88 × 1.22 m) on the east side of the CPE. The mean temperature and relative humidity were monitored using automated loggers (Hobo H8 Pro Series, 2-channels, Onset Computer Corp., Bourne, MA) placed in each CPE. Calves were vaccinated against infectious bovine rhinotracheitis (IBR) virus, bovine virus diarrhea (BVD) virus types 1 and 2, parainfluenza 3 (PI3) virus, and bovine respiratory syncytial virus (BRSV) at the following ages: 18 d (Bovi-Shield GOLD 5; Pfizer Animal Health, New York, NY), 39 d (Bovi-Shield GOLD 4; Pfizer Animal Health), and 86 d (TSV-2; Pfizer Animal Health).

Feeding and Weaning Strategy

The IACUC-approved feeding and weaning strategies used in this experiment followed common feeding protocols in the California commercial calf ranch industry. Calves were bottle-fed milk replacer (**MR**) twice daily at 0600 and 1500 h (Table 1). Milk replacer refusal volumes were documented after every MR feeding. All calves were offered ad libitum access to fresh water in buckets (changed twice daily) placed outside the hutch window (Figure 1a). Calves were also offered commercially available feeds ad libitum (Table 1), and the quantity of feed offered to each calf was recorded daily. A step-down weaning method was applied in 2 blocks, beginning at 52 and 53 d of age. Calves received only the morning MR feeding at the onset of the step-down weaning process, and 11 d later, the morning MR feeding was removed. All calves were individually weighed with a digital scale (PS-500, Salter Brecknell, Fairmont, MN) at 0, 3, 6, 10, and 12 wk of age.

Activity Loggers

Sixteen calves per treatment (n = 16) were randomly selected using the RAND function (Excel, 2007; Microsoft Corp.) to wear activity data loggers at 3, 6, and 10 wk of age. After BW collection, accelerometers (64 k, Onset Pendant G, Computer Corp.) were secured on the medial side of the right or left hind leg above the metatarsophalangeal joint with VetWrap cohesive bandage (3M Products, St. Paul, MN) and nonadhesive grip-surface liner (Con-Tact Brand Grip ultra shelf liner, Kittrich Corporation, Pomona, CA). One day after placement, the accelerometers began recording every 30 s if the calf was in the lying or standing position for 3 consecutive days. Data were downloaded and processed using methods previously described (Ledgerwood et al., 2010; Hill et al., 2013). For each of the 3-d sampling periods, the percent of time per day in the lying position was calculated before analysis. The 3-d sampling periods were treated as subsampling in the model.

Health Monitoring, Scoring, and Treatment

Calves have the greatest risk for enteric disease until 3 wk of age (Hulbert and Moisá, 2016); therefore, calves were scored twice daily for dehydration (enophthalmos), fecal consistency, and hide cleanliness (Table 2) for the first 21 d of the trial. In addition, 3 mL of peripheral whole blood (EDTA) was collected via venipuncture at 3 wk of age and hematocrit, total leukocyte counts, and differential analyses of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were measured using a Procyte analyzer (Idexx Laboratories, Sacramento, CA). Two trained observers scored all calves and if there were any differences between scores at the end of the observation, both evaluators re-observed the calf in question together to agree on the appropriate score. When calves arrived at the UC Davis research facility, the collaborating veterinarian determined whether calves had diarrhea (fecal score 2 to 4; Table 2), were dehydrated (enophthalmos >2; Table 2), or both. Therefore, all calves were prescribed to have MR feeding reduced to the 0600 h feeding and were provided with 4 L of electrolytes (BlueLite C, TechMix LLC, Stewart, MN) in the a.m. and p.m. until d 3 of the trial. If calves had milk refusals or individual fecal scores >3, they were further examined by the veterinarian and treated accordingly. The frequency of milk refusals and antibiotics administered was documented (Table 3). For the 21-d period, the percentage observations

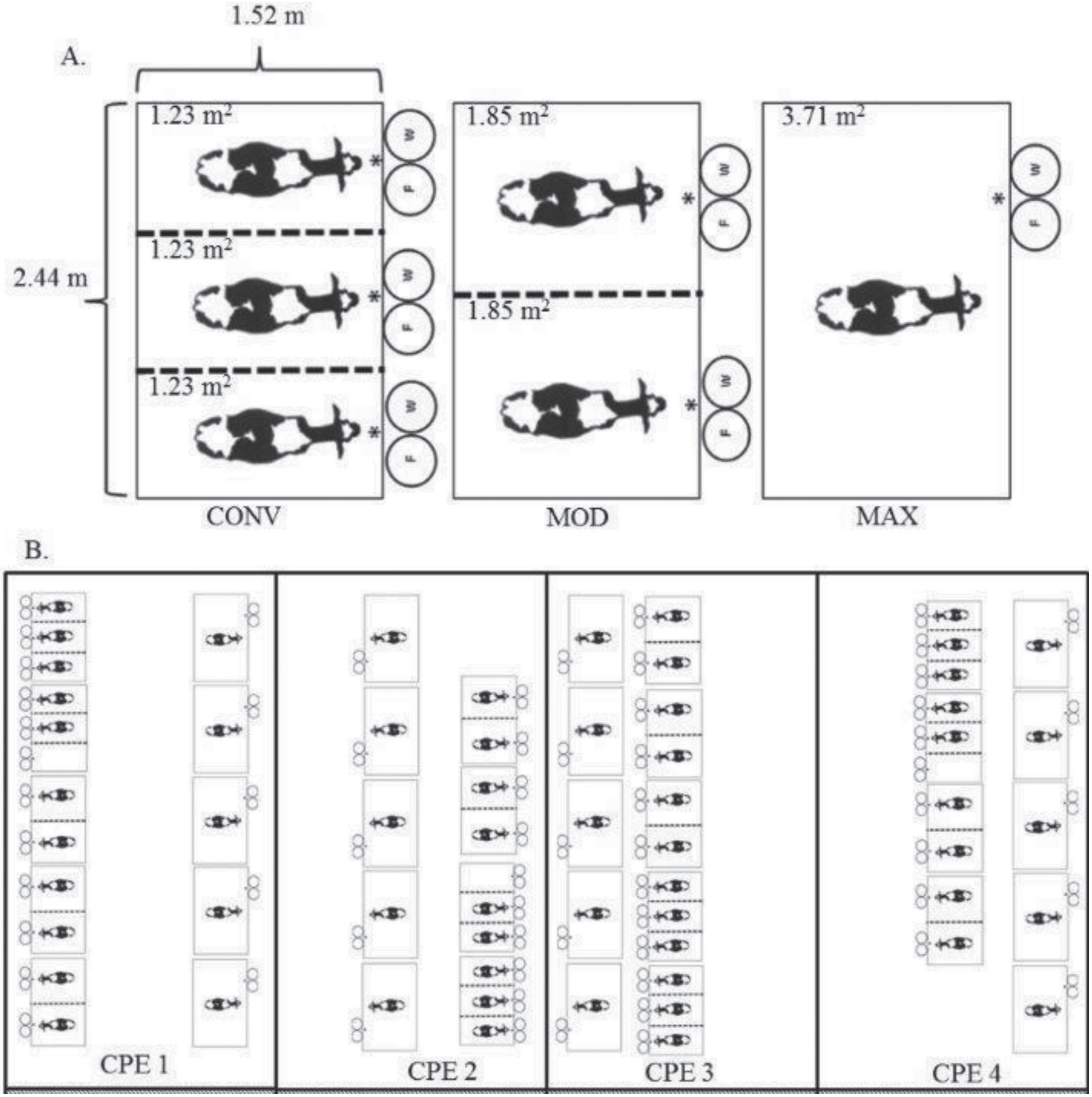

Figure 1. (A) Diagram of wooden calf hutches illustrating modification of conventional (CONV; 1.23 m^2/calf; n = 18) wooden hutches with slatted flooring to increase space allowance by adjustment of the inter-barriers (dashed lines) moderately (MOD; 1.85 m^2/calf; n = 17) or to the maximum (MAX; 3.71 m^2/calf; n = 19). Feeder (F), water (W), and milk bottle (*) holders were also adjusted for each modification. (B) Hutches of each treatment were randomly aligned by row into different locations within each cattle pen enclosure (CPE) and calves were randomly assigned to treatments and CPE at 4 d of age.

for each enteric-disease related score was calculated as single time-point variables. In addition, because all calves reached a fecal score of 3, the number of days for fecal observations to return to <2 was calculated for each calf. Respiratory disease is the second most common factor related to calf mortality (Love et al., 2016); thus each calf's eyes, nose, and ears were examined and scored daily (Table 2) by 2 trained observers. Legs were also examined daily throughout the entire experiment (Table 2) because floor type and the amount of time an animal spends standing may influence joint health (Terosky et al., 1997; Rushen et al., 2007). The collaborating veterinarian diagnosed and treated moderate to severe clinical cases of respiratory illness and joint swelling, and cases were documented (Table 3). However, all calves experienced mild to moderate nasal or eye discharge until 44 d of age. Therefore, percentage observations for this period were calculated as a single time-point for each of these variables.

Respiratory Antigen Challenge Sub-Experiment

All calves were first sensitized in 2 blocks with a subcutaneous injection of 0.5 mg of ovalbumin (**OVA**; Sigma-Aldrich, St. Louis, MO) and 0.5 mg of adjuvant

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]	
		Starter	Grower
Age fed, wk	0 to 9	0 to 9	9 to 12
DM, %	97	92	92
Ether extract (min[3]), %	20	4.8	3.9
CP (min), %	20	18	17.5
Crude fiber (max[3]), %	0.15	5.8	12.8
Calcium (max), %	0.75	0.8	0.9
Calcium (min), %	0.6	0.6	0.7
Phosphorus, %	0.5	0.3	0.25
Vitamin A (min), IU/kg	46,297	26,455	20,944
Vitamin D_3 (min), IU/kg	9,259	—	—
Vitamin E (min), IU/kg	661	—	—
Monensin Na, %	—	0.441	0.441

[1]Calves 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 until step-down weaning that was initiated at 52 and 53 d of age (p.m. milk replacer removed) and completed 11 d later (a.m. milk replacer removed). 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 D_3), D-α-tocopherol acetate (vitamin E), menadione sodium bisulfate complex (vitamin K), vitamin B_{12} 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.

[2]Calves were fed 2 commercial calf feeds ad libitum (Best Start, 5506 ALG calf starter mix [2010-18]-M; 5518 ALG heifer grower mix [2010-15]-M; A. L. Gilbert Company, Oakdale, CA) that provided similar macroingredients and included 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.

[3]min = minimum; max = maximum.

Table 2. Scoring system for hydration, feces, hide cleanliness, respiratory illness symptoms, and joint swelling of calves

Measurement	Definition of points allocated					Scores modified from:
	0	1	2	3	4	
Hydration	Normal (<5% dehydration; no eyeball recession; skin tent duration <1 s)	Mild enophthalmos (6 to 8% dehydration; 2 to 4 mm eyeball recession; skin tent duration 1 to 2 s)	Moderate enophthalmos (8 to 10% dehydration); 4 to 6 mm eyeball recession; skin tent duration 2 to 5 s)	Marked enophthalmos (10 to 12% dehydration; 6 to 8 mm eyeball recession; skin tent duration 5 to 10 s)	—	Smith, 2009; Wenz et al., 2010
Fecal consistency	—	Firm	Soft	Runny	Watery	Oliveira et al., 2010; Magalhães et al., 2008; Larson et al., 1977
Hide cleanliness	No feces on calf coat	Some dried feces on calf coat (~25% coverage)	Little dried and wet feces on calf coat (~50% coverage)	Substantial wet and caked on feces (~75% coverage)	Extensive wet and caked on feces (~ >80% coverage)	Terosky et al., 1997
Respiratory illness scores						Lago et al., 2006; Kim et al., 2009
Nasal discharge	No mucous	Mild, cloudy mucous in 1 nare	Moderate, cloudy mucous in both nares, or excessive in 1 nare	Severe and excessive mucous in both nares	—	
Eyes and ears	No discharge	Mild eye discharge	Moderate discharge in both eyes, 1 ear drop	Severe head tilt, both ears dropped	—	
Joint swelling	No swelling	Mild swelling	Moderate swelling	Severe swelling	—	Terosky et al., 1997

Table 3. Morbidity, mortality, and numbers of calves enrolled and reared in conventional (CONV; 1.23 m^2/calf) wooden hutches or hutches modified to increase space moderately (MOD; 1.85 m^2/calf) or to maximum (MAX; 3.71 m^2/calf) allowance

No. of calves:	CONV	MOD	MAX
Enrolled in main experiment[1]	20	20	20
Never treated with antibiotics	10	9	8
Treated with electrolytes during diarrhea outbreak[2]	19	19	20
Refused MR (a.m., p.m.) 1 to 3 times[3]	6	7	4
Lost weight between ages 0 to 20 d	1	1	1
Treated with antibiotics[4] for:			
Enteric disease only	6	7	9
Respiratory illness only	1	1	0
Enteric and respiratory illness	1	0	2
Chronic[5]	0	1	3
Mortality likely due to:			
Transportation[6]	1	1	0
Enteric disease	1	1	0
Occurrence of joint swelling[7]	1	2	6
Used in main experiment analyses[8]	18	17	19
Enrolled in respiratory immune sub-experiment[9]	8	8	8
Used in respiratory immune sub-experiment[10]	6	8	7

[1]Upon arrival at 4 d of age, calves were randomly assigned to housing treatments within a cattle pen enclosure.

[2]Calves with signs of dehydration had milk replacer (MR) reduced to a.m. feedings (age 5 to 7 d) and were provided up to 4 L of electrolytes for up to age 15 d.

[3]Milk replacer refusals were only observed between ages 4 to 16 d; no milk was refused (a.m. or p.m.) after age 16 d, except in a single calf, which was euthanized.

[4]In addition to daily health score assessment by observers, the collaborating veterinarian assessed calf health and treated calves with appropriate antibiotics for diarrhea, signs of respiratory disease, or both. Rectal temperature was taken and if febrile (>39.5°C), antibiotics were administered.

[5]Calves were considered "chronic" if they were treated with antibiotics more than 5 times for enteric disease, joint swelling, or respiratory illness symptoms.

[6]Calves that expired within 1 to 2 d after transportation to the UC Davis Department of Animal Science Feedlot and Environmental Research Facility.

[7]One or 2 swollen joints for 1 to 4 d (scores ranging from 1 to 3). Only a single calf (MAX treatment) was observed with severe joint swelling (in both knees). All incidences occurred after age 6 wk.

[8]Data from calves that died or were considered "chronic" were excluded from all analyses.

[9]Eight calves that were never treated with antibiotics within each treatment were randomly chosen for the respiratory immune sub-experiment.

[10]Data from calves with incomplete lung lavage fluid collection were excluded from final respiratory analyses.

Quil-A (Accurate Chemical, Westbury NY) in 1.0 mL of sterile saline at 39 and 60 d of age. Calves that were treated with antibiotics during the trial were excluded from the sub-experiment. A total of 8 calves were randomly selected from each treatment at age 11 wk to receive an in vivo respiratory challenge. These calves were exposed to 5 mL of 1% aerosolized (nebulizer) OVA in saline for 5 min. Four days after OVA exposure, 9 mL of blood (3 mL in EDTA tube; 6 mL in heparin tube) was collected as described above and bronchoalveolar lung lavages were performed as previously described (Gershwin and Olsen, 1984; Gershwin et al., 2011). Briefly, the bronchoalveolar space was flushed with 60 mL of sterile Hanks' balanced salt solution (**HBSS**), and the bronchoalveolar lavage fluid (**BALF**) was recovered and immediately placed on ice. Samples were centrifuged for 10 min at 1,250 × g at 4°C. The BALF supernatant was collected and stored at −80°C until analyzed. The pellets of BALF samples were measured for cell viability, total leukocyte count, and cell differentials (500 cells/slide), as previously described (Yu et al., 2002). Supernatant was analyzed on 1 plate each for total protein (Bio-Rad Laboratories Inc., Hercules, CA; interassay CV = 6.0%) and lactate dehydrogenase (Roche Applied Science, Indianapolis, IN; interassay CV = 3.6%).

Immunoglobulins specific for OVA (IgG, IgG_1, IgA, and IgE) in plasma and BALF samples were measured by ELISA as previously described (Gershwin and Olsen, 1984; Gershwin et al., 2011). Briefly, microtiter plates were coated with 1 μg of OVA (Sigma-Aldrich) per well. The wells were blocked with 1% rabbit serum albumin for IgG, IgG_1, and IgA assays, and 1% BSA for IgE assays. Plasma samples were diluted 1:20 except in the IgE assay, where a 27.5% saturated ammonium sulfate cut was performed to precipitate out IgG. Lavage samples were not diluted. All samples were analyzed in triplicate.

Conjugated antibodies used for IgG, IgG_1, and IgA assays were rabbit anti-bovine IgG-h+l horseradish peroxidase (HRP), sheep anti-bovine IgG1 HRP (Bethyl Laboratories, Montgomery, TX), or sheep anti-bovine IgA (AbD Serotec, Raleigh, NC), respectively, at 1:10,000 dilutions. For IgE assays, the primary antibody was a 1:100 dilution of mouse anti-bovine IgE, and the secondary conjugated antibody was a 1:7,000 dilution of goat anti-mouse IgG-h+l HRP. Results were expressed as mean absorbance values for all isotypes. The interassay CV for BALF IgG, IgG_1, IgA, and IgE were 1.5, 10.1, 3.1, and 18.3%, respectively; the interassay CV for plasma IgG, IgG_1, IgA, and IgE were 24.6, 13, 9, 4.9%, respectively.

Statistical Analysis

Total plasma protein concentrations at age 4 d and BW at enrollment were used as covariates in all models. Body weight, feed intake, and activity data logger measures were analyzed by restricted-maximum likelihood ANOVA using the Mixed procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). The subject of the repeated statement was calf nested within treatment. Compound symmetry and ante-regressive (1) covariance structures were tested for the within-calf effect in each model, and the most appropriate model was chosen based on the lowest Bayesian information criterion. Single variable data (percentage health score observations for each score and for summed scores, blood cell counts, immunoglobulins, and BALF) were analyzed using a linear mixed model with the fixed effect of treatment and random effect of CPE (Proc Mixed; SAS v.9.2). Before analyses, normality of the residuals was confirmed by evaluating the Shapiro-Wilk statistic using the Univariate procedure of SAS. Pairwise comparisons were performed for each significant effect in a model using Duncan's adjustment to control the familywise type 1 error. In addition, 2 preplanned polynomial contrasts were performed to test whether there were linear or quadratic relationships with increasing space allowance. Least squares means (±SEM) are reported throughout. A treatment difference of $P < 0.05$ was considered significant and $0.05 < P < 0.10$ was considered a tendency. Descriptive data (e.g., milk refusals, antibiotic treatments, mortality) are summarized (Table 3) to provide the scope of inference for the experiment.

RESULTS

The mean temperature (±SD) across CPE was 17.0 ± 4.80°C, with minimum and maximum temperatures of 5.5 and 29.7°C, respectively. The mean relative humidity (±SD) across CPE was 72.0 ± 21.3%. All calves in the current experiment required veterinary intervention for diarrhea, dehydration, or both, which included electrolyte supplementation for all calves and antibiotic treatment for 29 out of 56 calves (Table 3). Seventeen of 56 calves refused MR at least once, but no milk was refused after 16 d of age (Table 3). Three days after enrollment, 1 calf died from dehydration and 2 calves had severe dehydration and diarrhea. Calves with moderate to severe enteric disease recovered within the first week of the trial after veterinary intervention. After 42 d of age, 9 calves had joint swelling (Table 3). Seven calves experienced joint swelling in the knees, 1 calf experienced swelling in the hocks, and 1 calf experienced swelling in both the knees and hocks.

Performance Measures

Despite their arrival with enteric disease, all but 3 calves (1 per treatment) retained or exceeded their birth weight at 3 wk of age (Table 3), and ADG from 0 to 3 wk of age was 0.206 ± 0.088 kg/d. In addition, BW at 6 wk of age indicated that all calves had tripled their ADG compared with that in the 0 to 3 wk period (Table 4). Overall BW differed across treatments ($P = 0.05$; Figure 2). Calves housed in MAX hutches had the greatest BW by age 12 wk, weighing 5.9 kg more than CONV calves, but MOD calves were not different from CONV calves (Figure 2). Differences across treatments for ADG were observed ($P = 0.039$; Table 4), and increases in space allowance were associated with increased ADG, especially during the 3 to 6 wk period (linear $P = 0.025$; Table 4). We detected no treatment or treatment × period effects for DMI or DMI:gain ($P > 0.05$), although increased space allowance tended to positively influence DMI during the 3 to 6 wk period (Table 4; linear $P = 0.068$) and feed conversion during the 10 to 12 wk period (linear $P = 0.096$).

Lying Position Time

All calves spent over 70% of each day (16.6 ± 0.31 h) in the lying position during the 21 to 23 d behavior sample period, and we detected no differences across treatments (linear and quadratic $P > 0.10$; Figure 3). Space allowance influenced lying time (linear $P < 0.05$; Figure 3) during the 47 to 49 d (preweaning) and 74 to 76 d (postweaning) sampling periods; as space allowance increased, calves spent less time per day in the lying position. The MAX calves spent 4 ± 0.01% more time per day in the standing position than did CONV and MOD calves ($P < 0.05$; 1.0 ± 0.2 h less lying per day; Figure 3) during the 6 and 10 wk sample periods.

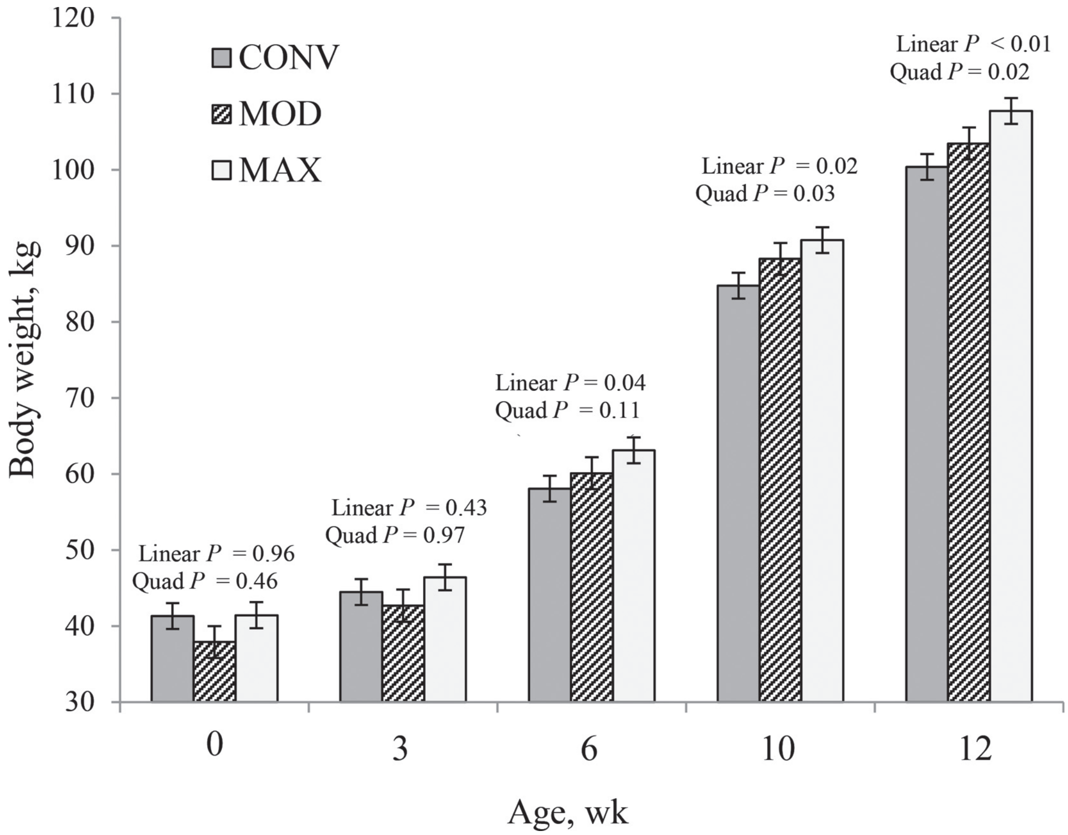

Figure 2. Average calf BW at 0, 3, 6, 10, and 12 wk of age. Calves were reared in conventional (CONV; 1.23 m^2/calf; n = 18) wooden hutches or wooden hutches modified to increase space moderately (MOD; 1.85 m^2/calf; n = 17) or to maximum (MAX; 3.71 m^2/calf; n = 19) allowance. The P-values represent linear contrast significance and quadratic (Quad) contrast significance (CONV vs. MOD and MAX), and the error bars represent the SE of the means. Overall P-values were treatment (TRT) = 0.05, period < 0.001, TRT × period = 0.28.

Table 4. Least squares means and largest SE for total plasma protein (TPP), BW, ADG, DMI, and DMI:gain of Holstein calves reared in conventional (CONV; 1.23 m^2/calf) wooden hutches or hutches modified to increase space moderately (MOD; 1.85 m^2/calf) or to maximum (MAX; 3.71 m^2/calf) allowance

	Treatment (Trt)				$P <$				
Item	CONV	MOD	MAX	Largest SE	Trt	Period	Trt × Period	Linear	Quadratic
No. of calves	17	18	19						
TPP at d 4, g/dL	5.80	5.49	5.55	0.250	0.655	—	—	0.480	0.368
Initial BW, kg	42.14	40.19	41.46	1.195	0.485	—	—	0.672	0.362
Final BW, kg	100.18	102.34	108.99	2.906	0.072	—	—	0.031	0.122
Total DMI, kg/d					0.346	<0.001	0.770		
wk 0 to 3	0.639	0.570	0.645	0.075		—	—	0.955	0.725
wk 3 to 6	1.088	1.153	1.240	0.061		—	—	0.068	0.143
wk 6 to 10	2.404	2.446	2.518	0.090		—	—	0.353	0.473
wk 10 to 12	3.798	3.899	3.996	0.124		—	—	0.246	0.317
ADG, kg/d					0.039	<0.001	0.369		
wk 0 to 3	0.185	0.198	0.261	0.042		—	—	0.183	0.378
wk 3 to 6	0.563	0.668	0.697	0.048		—	—	0.025	0.023
wk 6 to 10	0.985	1.007	1.025	0.046		—	—	0.551	0.603
wk 10 to 12	1.195	1.107	1.324	0.063		—	—	0.139	0.787
DMI:gain					0.420	<0.001	0.430		
wk 0 to 3	3.95	3.85	3.29	0.888		—	—	0.388	0.784
wk 3 to 6	2.00	1.87	1.82	0.107		—	—	0.480	0.907
wk 6 to 10	2.48	2.47	2.56	0.115		—	—	0.123	0.550
wk 10 to 12	3.25	3.59	3.21	0.192		—	—	0.096	0.734

Health

On average, 15 to 20% of the fecal observations were in the soft to runny categories (Figure 4A) during the first 3 wk. Although space allowance did not influence the percent of observations with soft to runny feces, there was a tendency (linear $P = 0.054$) for increased space allowance to increase the number of days for soft feces to return to firm (Figure 4B). The MAX calves had the greatest number of observations in which they scored the cleanest (over 75% of their body visibly free of fecal matter) during the first 3 wk of the experiment ($P < 0.01$; Figure 4C). Space allowance also influenced the number of circulating eosinophils in the 23 d of age blood samples (linear $P < 0.05$; Figure 4D); as space allowance increased, the number of circulating eosinophils decreased.

Five calves required veterinary intervention for respiratory disease (Table 3). These calves had severe respiratory scores (score sum of 3 for cough, eye discharge, nasal discharge, and ear position), and the veterinarian confirmed the respiratory disease after auscultation examination. All calves had mild to moderate nasal and eye discharge until 7 wk of age. Space allowance treatments did not influence the percent of observations of mild to moderate nasal discharge (Figure 5) but positively influenced the percent of observations of calves with mild to moderate eye discharge (linear $P = 0.05$; Figure 5).

Respiratory Immunocompetence Sub-Experiment

Calves selected for the respiratory antigen challenge sub-experiment at 11 wk of age displayed pulmonary inflammation 4 d post-OVA challenge, with the majority of the BALF leukocytes identified as neutrophils (Table 5). The percent of eosinophils in BALF decreased as space allowance increased (linear P-value = 0.01); CONV calves were observed to have over 3 times the concentration of BALF eosinophils compared with MOD and MAX calves ($P < 0.01$; Table 5). Although there were no differences among treatments for plasma and BALF IgG, IgG1 and IgA ($P > 0.10$; Table 5 and Figures 6A and 6B), the CONV calves had 3 times the amount of BALF IgE (quadratic $P < 0.01$; Figure 6B) and tended to have 2 times the amount of plasma IgE (quadratic $P = 0.07$; Figure 6A) compared with MOD calves.

DISCUSSION

Many factors must be considered for calves raised on calf ranches, and housing system plays a vital role in controlling disease transmission, morbidity, and mor-

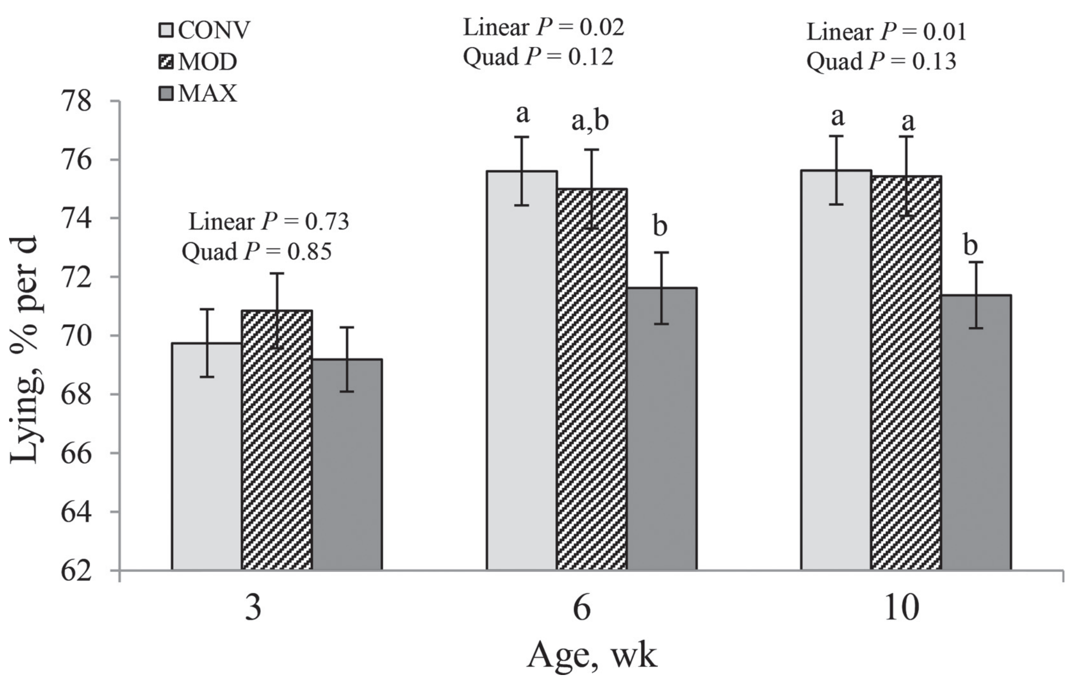

Figure 3. The percentage of time per day that calves were in the lying position at 3, 6, and 10 wk of age. Calves were reared in conventional (CONV; 1.23 m^2/calf; n = 18) wooden hutches or wooden hutches modified to increase space moderately (MOD; 1.85 m^2/calf; n = 17) or to maximum (MAX; 3.71 m^2/calf; n = 19) allowance. The P-values represent linear contrast significance and quadratic (Quad) contrast significance (CONV vs. MOD and MAX), and the error bars represent the SE of the means. Least squares means with different letters (a, b) differ ($P < 0.05$).

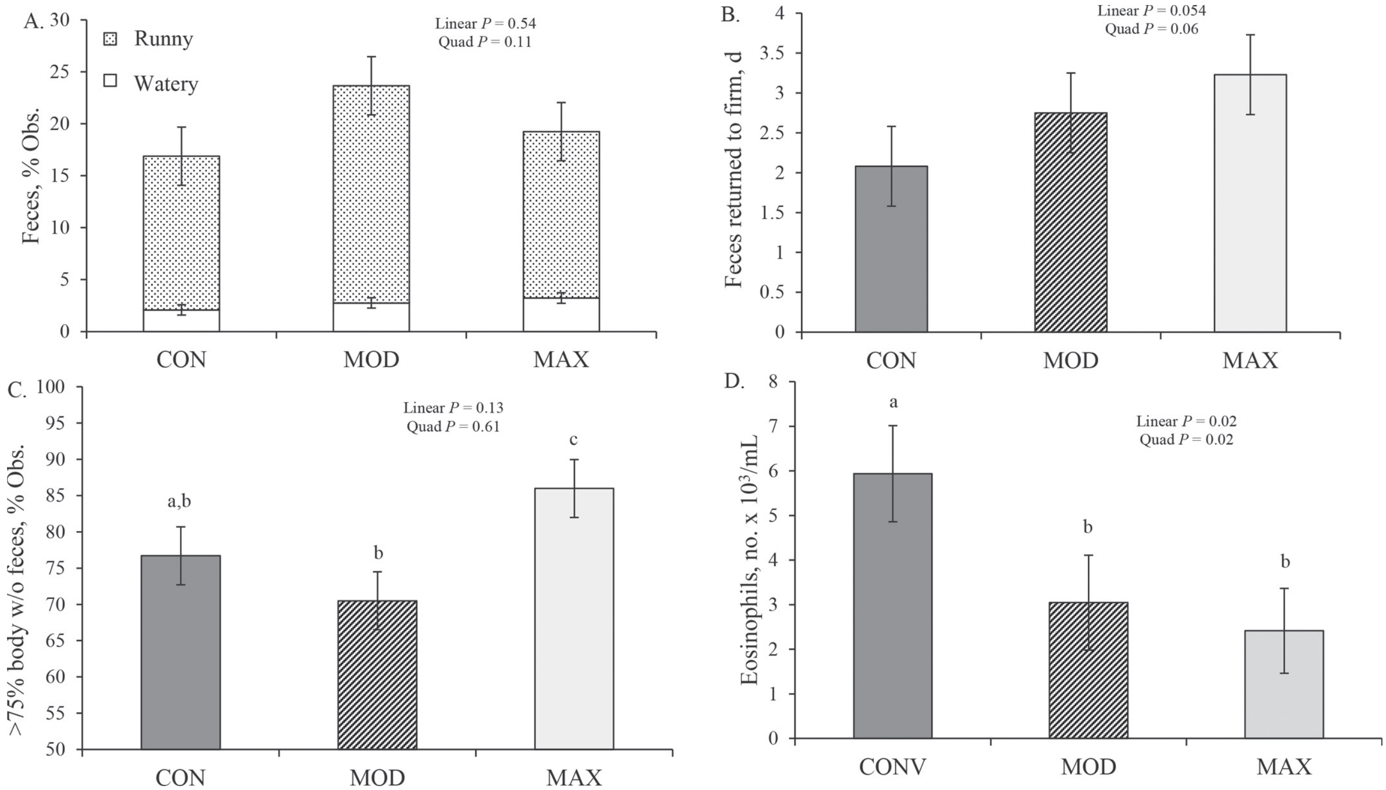

Figure 4. Means of daily recorded diarrhea-related measures for calves for the first 21 d of the experiment. The data represent (A) percent of observations (Obs., collected daily) that calves had fecal scores ≥3; (B) number of days for a calf's feces to return to a normal consistency (score = 1) after the first runny or watery score was observed (score ≥3); (C) percentage of observations when the calf's hide, face, and legs were >75% free from fecal matter; and (D) number of peripheral eosinophils at age 23 d. Calves were reared in conventional (CONV; 1.23 m^2/calf; n = 18) wooden hutches or wooden hutches modified to increase space moderately (MOD; 1.85 m^2/calf; n = 17) or to maximum (MAX; 3.71 m^2/calf; n = 19) allowance. The *P*-values represent linear contrast significance and quadratic (Quad) contrast significance (CONV vs. MOD and MAX), and the error bars represent the SE of the means. Least squares means with different letters (a, b, c) differ ($P < 0.01$).

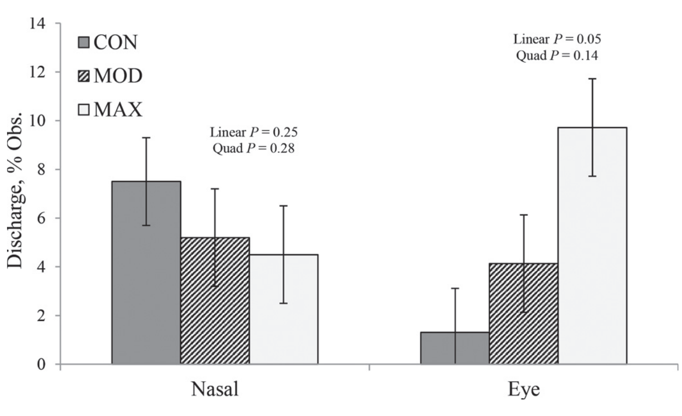

Figure 5. Mild to moderate nasal or eye discharge (score ≥1) was observed for the first 40 d of the experiment (% observations, Obs.) in calves reared in conventional (CONV; 1.23 m^2/calf; n = 108) wooden hutches or wooden hutches modified to increase space moderately (MOD; 1.85 m^2/calf; n = 17) or to maximum (MAX; 3.71 m^2/calf; n = 19) allowance. The *P*-values represent linear contrast significance and quadratic (Quad) contrast significance (CONV versus MOD and MAX), and the error bars represent the SE of the means.

tality (Anderson, 1998; Walker et al., 2012). However, calf producers need to know whether space allowance will affect calf health, productivity, and behavior if legislative requirements are enforced. Numerous studies have investigated the behavior and performance of calves housed in pens of increased size in barns (Fisher et al., 1985; Terosky et al., 1997; Wilson et al., 1999), but remains a gap in our knowledge regarding calf lying behavior, performance, and health responses to increased space allowance in commonly used conventional wooden hutches.

The neonatal period for calves is by far the most vulnerable stage for enteric disease, as calves are most susceptible to diarrhea during this period (Constable, 2004; USDA, 2010; Hulbert and Moisá, 2016). Over 50% of mortality in dairy calves was related to diarrhea from 1996 to 2007 (USDA, 2008, 2010). A recent survey indicated that 18% of calves at CRO experience morbidity due to enteric disease, and 73% of these calves receive antibiotics due to severe diarrhea (Walker et al., 2012). All calves in the present study were diagnosed

Table 5. Proportions of cells in whole blood and bronchoalveolar lavage fluid (BALF) from weaned calves 4 d after challenge with ovalbumin[1] at age 11 wk

	Treatment[1,2]				Contrast P-value[3]	
Item	CONV	MOD	MAX	SEM	Linear	Quadratic
No. of calves	6	8	8	—	—	—
Whole blood[4]						
Hematocrit, %	32.2	31.4	31.5	0.85	0.50	0.47
Leukocytes, no. $\times 10^6$/mL	10.3	9.9	10.6	1.31	0.80	0.96
Neutrophils, %	34.4	37.8	37.5	5.44	0.64	0.62
Lymphocytes, %	50.1	47.5	48.1	5.03	0.71	0.71
Monocytes, %	14.3	12.9	13.4	1.29	0.42	0.46
Eosinophils, %	1.1	1.6	0.8	0.63	0.80	0.86
Basophils, %	0.2	0.1	0.01	0.22	0.62	0.61
BALF[5]						
Lavage volume recovered, mL	41.3	43.8	39.8	1.69	26	0.79
Leukocyte viability, %	86.9	88.5	87.5	1.92	0.76	0.53
Leukocytes, no. $\times 10^6$/mL	2.6	3.0	2.1	0.93	0.83	0.39
Total protein,[6] µg	6.9	7.6	6.9	2.31	0.81	0.88
Lactate dehydrogenase,[5] µU	35.7	51.2	51.5	17.23	0.28	0.33
Macrophages, %	23.3	26.4	18.9	6.28	0.96	0.67
Monocytes, %	7.9	7.7	6.5	1.53	0.71	0.92
Neutrophils, %	64.9	63.6	72.0	8.32	0.82	0.82
Lymphocytes, %	2.7	1.9	2.4	0.74	0.69	0.36
Eosinophils, %	1.2[a]	0.4[b]	0.3[b]	0.32	0.01	0.01

[a,b]Within a row, means with different superscripts are different ($P = 0.05$).

[1]Calves were challenged at age 39 and 60 d with 0.5 mg of ovalbumin (OVA) subcutaneously. At 76 and 77 d of age, 8 calves from each treatment were challenged with 1% aerosolized OVA in saline for 5 min (5 mL) using a nebulizer.

[2]Calves were reared in conventional (CONV; 1.23 m^2/calf; n = 18) wooden hutches or wooden hutches modified to increase space moderately (MOD; 1.85 m^2/calf; n = 17) or to maximum (MAX; 3.71 m^2/calf; n = 19) allowance.

[3]P-values represent linear contrast significance and quadratic contrast significance (CONV versus MOD and MAX).

[4]Blood samples were collected before BALF collection on 80 and 81 d of age, 4 d after OVA exposure.

[5]The BALF was collected by flushing 60 mL of sterile Hanks' balanced salt solution (HBSS) into the bronchoalveolar space of each calf. Data from calves with <36 mL of fluid recovery were not included in the analyses in this table (2 calves in the CONV treatment).

[6]Lavage volume recovered after flushed with 60 mL of HBSS.

with diarrhea, dehydration, or both, and were treated accordingly by the veterinarian (Table 3). In 2006, it was estimated that nearly 1 in 4 preweaned US dairy calves (23.9 ± 1.7%) were affected with an enteric disorder and one-third of calves were estimated to be treated with antibiotics before weaning (USDA, 2010; 1,077 US dairy operations surveyed in 2006). For this study, we speculate that transportation contributed to the incidence of enteric disease and dehydration. Nonetheless, this experiment's scope of interest applies to a significant proportion of dairy calves in the United States, as it is estimated that nearly 1 out of 10 dairy heifers and most dairy-beef calves are transported to CRO before they reach 1 wk of age (Hulbert and Moisá, 2016). The enteric disease and dehydration experienced by calves at the onset of the present study likely increased variation for lying time, milk refusals, feed consumption, and, consequently, BW gain. Therefore, more research is needed on a larger number of calves to better understand the influence of space allowance on these measures in the first 3 wk of life. At 3 wk of age, all calves spent 70% of the day in the lying position (16.8 h/d), which is similar to reports in the literature (Wilson et al., 1999; von Keyserlingk et al., 2006). There were no differences in lying time among space allowance treatments during the 3 wk of age sampling period. We speculate that this observation may be due to the enteric and dehydration status of most calves, as limited research has reported decreased activity in calves experiencing enteric infections (Panivivat et al., 2004; Borderas Tordesillas, 2009) or respiratory diseases (Hanzlicek et al., 2010; Theurer et al., 2013). However, our findings at 3 wk of age may also indicate that space was not a limiting factor for lying versus active behavior in this experiment. In models of "subclinical" sickness (low doses of endotoxin), between-calf variation for lying duration was sufficiently great among 23-d-old calves that researchers were not able to use behavior measures alone to distinguish subclinical calves from saline-treated calves; thus, they had to use other indicators of sickness, such as rectal temperature (Borderas et al., 2008). In addition, it is possible that recovering neonatal calves are not likely to use extra space for active behaviors, but this cannot be confirmed without further investigation on a larger population of calves. Nonetheless, even among healthy calves, lying time per day is greatest during the first few weeks of life, and the rate at which lying time declines with age is also dependent on the housing and feeding system (Panivivat et al., 2004; von Keyserlingk et al., 2006; Hill et al., 2013).

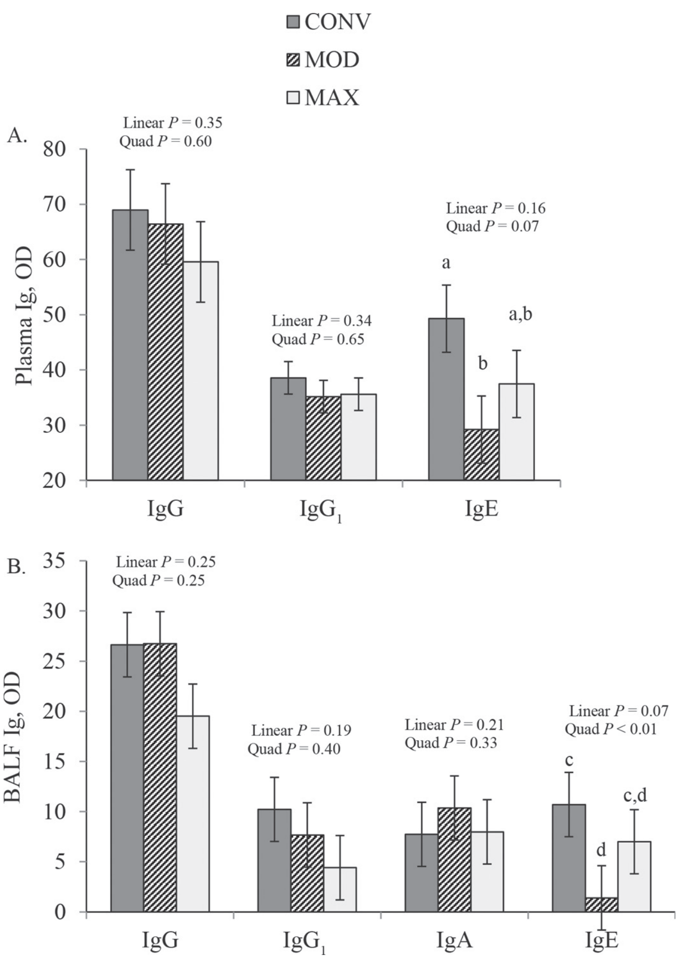

Figure 6. (A) Plasma and (B) bronchoalveolar lavage fluid (BALF) immunoglobulin concentrations [measured in arbitrary units of optical density (OD) × 100 or 1,000] of a subset of calves from each housing treatment of conventional (CONV; 1.23 m^2 space/calf; n = 6), a moderate increase in space allowance (MOD; 1.85 m^2 space/calf; n = 8) or maximum space allowance (MAX; 3.71 m^2 space/calf; n = 7). Samples were taken 4 d after calves were challenged with 1% aerosolized ovalbumin at 11 wk of age. The *P*-values represent linear contrast significance and quadratic (Quad) contrast significance (CONV vs. MOD and MAX), and the error bars represent the SE of the means. Letters a and b indicate a tendency ($P = 0.07$) for LSM to differ; letters c and d indicate that LSM differed ($P < 0.01$).

After the 1 to 3 wk period, all calves recovered from the bout of diarrhea. During the 6 and 10 wk behavior sampling periods, increased space allowance influenced calves' lying time, which has been reported in previous work. For example, Tapkı et al. (2006) reported that healthy calves with more space in an indoor barn displayed decreased lying time with increased space allowance (1.5, 2.25, and 4 m^2 of space). This observation has a logical explanation: calves in conventional space allowance may start to "outgrow" their space and be less motivated to stand in their home environment. In the current experiment, growth rate rapidly increased among all calves between the 3 and 6 wk periods, and space allowance positively influenced BW after 6 wk of age. Jensen and Kyhn (2000) observed that when calves housed in 1.5-m^2 pens were provided a temporary increase in space, such as during an open field test, they became more motivated to display locomotor play behavior than calves that were reared with more space (2.2-, 3-, and 4-m^2 pens). This observation could be tested for calves in the wooden hutch system for future work. Hill et al. (2013) used the same automated logging systems as in the current experiment; they reported that dairy calves housed with 2.8 m^2 of space had decreased lying time with age at a rate of less than 0.04% per day. Therefore, the MAX calves were not expected to have more than a 0.9% decrease in total lying time. Although CONV and MOD calves could turn around at the 3 and 6 wk sample periods, their body size in comparison to the space allowance may have decreased their motivation to stand.

Tapkı et al. (2006) suggested that increased lying time is associated with decreased energy expenditure and may improve protein and ME efficiency. Researchers found that calves in small, indoor pens (1.5 m^2 space/head) were less active and 11% heavier than calves with larger pens (2.25 and 4 m^2 space/head) but they observed no differences in DMI. In contrast to this suggestion, MAX calves were the most active at 6, 10, and 12 wk of age and were the heaviest calves at the end of the trial. In addition, MAX calves tended to consume the most starter diet in the 3 to 6 wk period. Similar to our findings, Fisher et al. (1985) found that weaned calves raised at 0.98 m^2 space/head had lower BW and less-efficient feed conversion at age 6 to 10 wk compared with calves raised with more space (2.01 m^2 space/head). Nonetheless, the motivation to stand and eat solid feed during this phase may play an important role in performance and health once calves are removed from the hutch and placed into groups (commingling). Thus, the long-term effects of hutch space allowance on performance measures need to be investigated.

Although we observed no performance differences among treatments during the first 3 wk of the experiment, calves reared in the MAX space allowance were observed to have more days with a cleaner body than CON and MOD calves. This was likely due to the large amount of space in the MAX treatment that provided more fecal-free areas for calves to lie down. Nonetheless, there may be other contributors to these observations. For example, Borderas et al. (2008) reported that low-dose endotoxin caused a decrease in auto-grooming bouts. In the current experiment, MAX calves may have also groomed more than CONV and MOD calves, but this warrants further investigation, as auto-grooming was not directly measured.

Cleanliness may have contributed to the number of peripheral eosinophils at age 3 wk. Marked increases in peripheral blood eosinophils are associated with parasitic activity in the digestive tract (Granstrom et al., 1990), but total leukocyte counts and the number of eosinophils in the blood in the present experiment were within normal ranges (Santos et al., 2002; Mohri et al., 2007). Nonetheless, increased eosinophils might be indicative of subclinical challenges from immune stimulation due to greater exposure to feces. For example, Terosky et al. (1997) reported that calves provided 1.36 m^2 space/calf were dirtier, had greater eosinophils at age 4 wk, and groomed themselves more than calves with even less space (1.00 or 1.18 m^2/calf) than that of the CONV treatment in the current experiment. Wilson et al. (1999) reported that manure accumulation on coats was associated with increased peripheral eosinophils for veal calves housed in stalls with space allowances similar to the MOD and CONV hutches. Fisher et al. (1985) noted that calves housed in the 2.01 m^2/calf treatment had lower blood eosinophils than controls (0.97 m^2/calf) at 5 and 7 wk of age. We speculate that because MAX calves were cleaner, they had less chance of oral exposure to immune stimulants, a decreased risk of pathogen exposure, and therefore, had fewer peripheral eosinophils present.

Respiratory immunocompetence of calves before their first commingling is very important to reduce the risk of postweaning morbidity and mortality. Bovine respiratory disease (**BRD**) is a challenge for economic losses in the cattle industry, and it is well known that housing and management strategies can influence the risk of BRD in calves (Waltner-Toews et al., 1986; Curtis et al., 1988; Love et al., 2014). During the current experiment, 5 calves experienced respiratory illness, but for the rest of the herd, there were no incidences of severe eye, nose, and ear scores. However, all calves had mild to moderate nasal and eye discharge from d 6 to 44. The MAX calves were observed to have the greatest occurrence of mild to moderate eye discharge, but these incidences were not combined with other clinical symptoms (elevated rectal temperature, cough, and eye and ear condition) and were not identified as having BRD (Apley, 2006; Lago et al., 2006; Love et al., 2014). As mentioned previously, space allowance positively influenced hide cleanliness, and therefore likely reduced oral exposure to immune stimulants; however, the greater incidences of mild to moderate eye discharge may indicate that increased activity may have caused more aerosolized irritants in MAX hutches than the CONV hutches.

Aerosolized irritants may not have contributed to incidences of respiratory illness but could have influenced overall pulmonary health and immunity. Hence, it was important to capture the sensitivity of the pulmonary immune system among calves housed in the 3 space allowances. The CONV calves evaluated in the respiratory challenge sub-experiment had the greatest percentage of BALF eosinophils compared with the other treatments. Eosinophil infiltration into lung tissue is associated with acute inflammation and remodeling of pulmonary tissues, which may cause chronic restriction in pulmonary airways, especially if there are subsequent challenges with antigens or chemical and climate stressors (Hooper et al., 2013). The MAX calves had the lowest percentage of eosinophils in BALF, which suggests that these calves had less pulmonary inflammation from the OVA challenge than CONV calves. It seems counterintuitive that although MAX calves had the most observations with eye discharge, the sub-experiment MAX calves had the least amount of pulmonary eosinophil infiltration. We speculate that MAX calves experienced a more mucosal and humoral-based immune response than a cellular immune response to aerosolized antigens. Eosinophil infiltration into the lungs is also highly correlated with IgE concentrations (Gershwin and Olsen, 1984; Bowersock, 1998; Gershwin, 2009); therefore, it was not surprising that in addition to the greatest percentage of BALF eosinophils, CONV calves had the highest concentrations of plasma and BALF OVA-specific IgE. Although the other OVA-specific immunoglobulin isotypes (IgA, IgG, IgG_1) are important for priming the immune system to boost subsequent vaccinations against targeted pathogens (Bowersock, 1998), the OVA-IgE isotype in BALF fluid is associated with the passive cutaneous anaphylaxis reaction in cattle (Gershwin and Olsen, 1984; Gershwin, 2009). It is not apparent to us which component of the increased space allowance environment influenced this finding; therefore, more research is needed to identify the chemical or climate stressors (or lack thereof) associated with these housing types. Nonetheless, increased space allowance of wooden hutches may improve calf pulmonary immunity and health.

An increase in space allowance of calves housed in hutches may improve calf health and performance, especially for those calves that experience enteric disease in the preweaning phase. The MAX hutches provided the most sanitary environment during the diarrhea bout, and calves may benefit from less fecal coverage. The performance benefits from increased space allowance were not observed until after the calves recovered from enteric disease. All calves increased in BW, but an increased BW relative to decreased space allowance appeared to impede the motivation of CONV and MOD calves to stand, and lying time increased. The MAX calves, however, did not change the amount of time they spent lying, even though they were the largest calves by the end of the experiment. This may have been attributed to more space allowance, thus MAX calves were more likely to stay motivated to stand and perform normal maintenance behaviors, such as solid feed consumption. The pulmonary health of calves at age 11 wk, just before commingling, appeared to be influenced by space allowance. Increasing space for calves housed in wooden hutches may decrease the risk for hyperresponsive pulmonary inflammation. A change in space allowance may increase housing costs for calves, so the outcome of improved health, sanitation, and performance associated with increased space allowance will need to be considered in economic models. Further research on a larger scale operation is needed to determine if pre- and postweaning treatment costs would decrease with increased space allowance.

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