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Innate immune responses and health of individually reared Holstein calves after placement into transition-pens 23 d after weaning

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Sixty-four Holstein dairy bull calves were all reared in individual calf-hutches and pens until they were randomly assigned to treatments of Grouped (pens of 3, $n=36$ calves) or Control (left in home hutch, $n=8$ calves) at age 68 ± 2·3 d (body weight 74·9 ± 1·5 kg). Blood was drawn at age 66, 70, 74 and 88 d for *ex-vivo* immunological and biochemical analyses. Calf starter intake was measured daily and individual body weights were measured at age 68, 78, and 89 (± 2·3 SD) d. Grouped-calves consumed less starter ($P<0·05$), and weighed 6·4 ± 1·99 kg less ($P<0·05$) than Control-calves by age 89 d. Group housing was a mild stressor, as evident by a transient suppression in neutrophil oxidative burst at age 70 d, but there was a lack of difference in the neutrophil:lymphocyte ratio of peripheral leucocytes and neutrophil expression of L-selectin at age 70 and 74 d. However, grouped-calves had elevated total peripheral leukocyte counts at age 70 d ($P<0·05$) and tended ($P<0·10$) to be greater at age 74 and 88 d. In addition, neutrophil phagocytosis of *Escherichia coli* increased ($P<0·05$) at age 74 d in Grouped-calves. These data indicate that moving calves into transition-pens with 3 calves per group decreases performance, but this may not be due primarily to extreme stress or disease. These data do indicate that it is important that calves have a competent immune system and any potential stressors are limited when they are moved into transition-pens because they are exposed to a wider diversity and (or) load of pathogens.

Keywords: Bovine, commingling, innate immunity.

Dairy calves are typically housed individually after removal from the dam at birth. Individual housing allows for controlled management and prevents the horizontal transmission of disease through calf-to-calf contact (Hepola, 2003; Lundborg et al. 2005). However, after the first few weeks of age, cattle are gregarious animals, and calves express high motivation to interact socially (Bøe & Færevik, 2003; Færevik et al. 2007). This may be one reason why the European Union has mandated that dairy calves greater than 8 weeks of age be housed in groups to provide the calves with the opportunity to perform social behaviours (Council Directive 97/2/EEC), which some consider an improvement of animal welfare (Færevik et al. 2007). In the United States, many states such as California, Arizona and Oregon have passed legislation that prohibits producers from raising veal calves in individual systems (AVMA, 2008). Although this legislation did not include dairy calves, it is conceivable to assume that future propositions and legislations are likely to include dairy calves. Before weaning, calves in groups or pairs may exhibit non-nutritive sucking on each other and competition for milk in systems where milk is limit-fed; therefore, producers prefer to house calves in groups only after they are weaned from milk (de Passille, 2001; Hepola, 2003).

There may be some benefits to group housing; for example, Warnick et al. (1977) observed a greater consumption of calf starter in calves that were group housed after weaning. Furthermore, group-housed calves were less fearful of novel environments and handling stressors at 3 months of age (Bøe & Færevik, 2003). Nonetheless, grouping calves after they have been housed individually may be stressful during a period of potential exposure to novel microbes. When calves are regrouped or an unfamiliar animal is added to the group, it may take up to 15 d for social interactions and locomotor activities to return to basal levels (Bøe & Færevik, 2003). Other researchers have recommended that after dairy calves are weaned at age 5–8 weeks, they should be moved from their individual housing into small 'transition' groups of 3–6 calves per pen (Quigley 2001; Bach et al. 2010). Then at approximately 6 months of age, the calves can be moved into larger groups.

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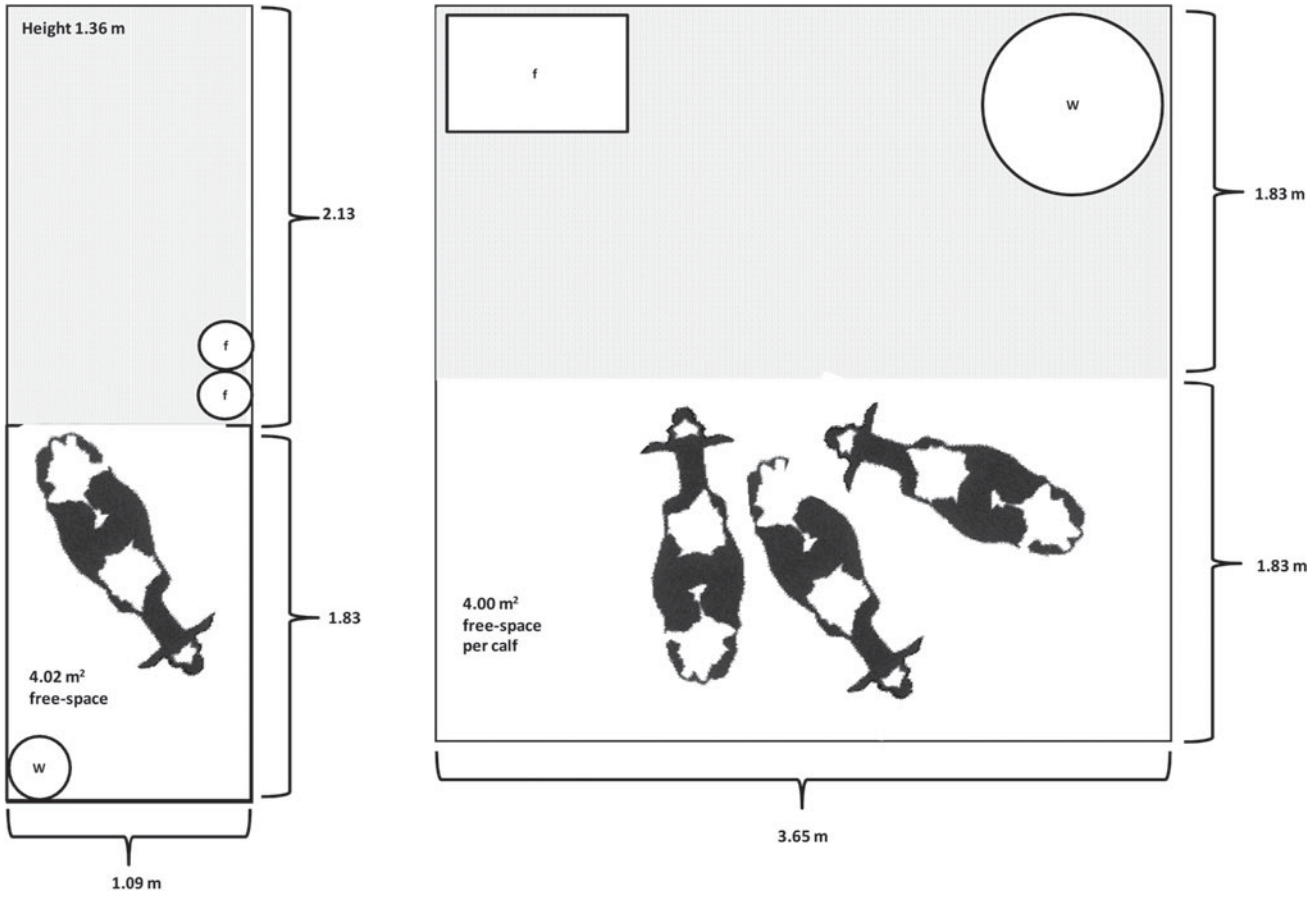

Fig. 1. All calves were reared in enclosed, commercial 2·13 × 1·09-m polyethylene calf hutches (left panel) (Agri-Plastics, Tonawanda NY, USA) attached to 1·83 × 1·09-m pens, which provided each calf with 4·02 m^2 of free space. At age 68 ± 2·3 d, grouped-calves were moved to pens of 3 (n = 36) or they remained in hutches (Control; n = 28). Grouped-calf pens (right panel) were 3·65 × 3·65 m with at least half of the pen shaded. In the shaded portion of the pen, calves had a 91·44-cm diameter water-bucket (w) and a 91·44 × 60·96-cm feeder (f) placed in each corner, providing 0·45 m of free bunker space and 4·00 m^2 of free space per calf.

Recommendations and previous research into 'commingling' or placing calves into small groups have primarily focused on behaviour and performance. Very little is known about how grouping calves into transition-pens will influence innate immune responses. It is well-documented that stress influences the immune system of dairy calves and may increase the relative risk of disease (Amadori et al. 1997; Hulbert et al. 2011a, b). We hypothesized that calves in transition-pens will be a mild stressor and have acute effects on innate immune function. Therefore, the objectives of the current study were to determine the influences of grouping dairy calves in small-grouped, transition-pens after they had been reared individually on innate immune responses and production performance.

Materials and Methods

Animals, housing and treatments

The experiment was conducted in May 2010. All animal procedures were reviewed and approved by the Texas Tech University Animal Care and Use Committee. Sixty-four Holstein, bull calves (24–48 h after birth) were purchased from two local commercial dairies over a 7-d period. All calves were fed 3·8 l of pooled colostrum from their respective dairy within 12 h of birth and all calves were transported approximately 60 km to the Hilmar Cheese/Agri-Plastics Calf Research Facility at Texas Tech University in New Deal, TX. Calves were housed with straw-bedding in commercial 2·13 × 1·09-m polyethylene calf hutches (Agri-Plastics, Tonawanda, NY, USA) attached to 1·83 × 1·09-m pens, which provided each calf with approximately 4·02 m^2 of free space (Fig. 1). Calves were fed 3·8 l of a 20% all milk protein/20%-fat milk replacer (Land O Lakes, Minneapolis, MN, USA) and weaned by age 45 d as previously described (Hulbert et al. 2011a, b). Calves had *ad-libitum* access to an 18·9% CP steam-flake corn/soybean meal based calf starter (Hulbert et al. 2011a) and water. The quantity of calf starter was recorded and adjusted daily for an approximate 10% refusal. During this experiment, weather was variable; therefore, weather information in relation to the trial timeline was collected from a local weather station (Weather

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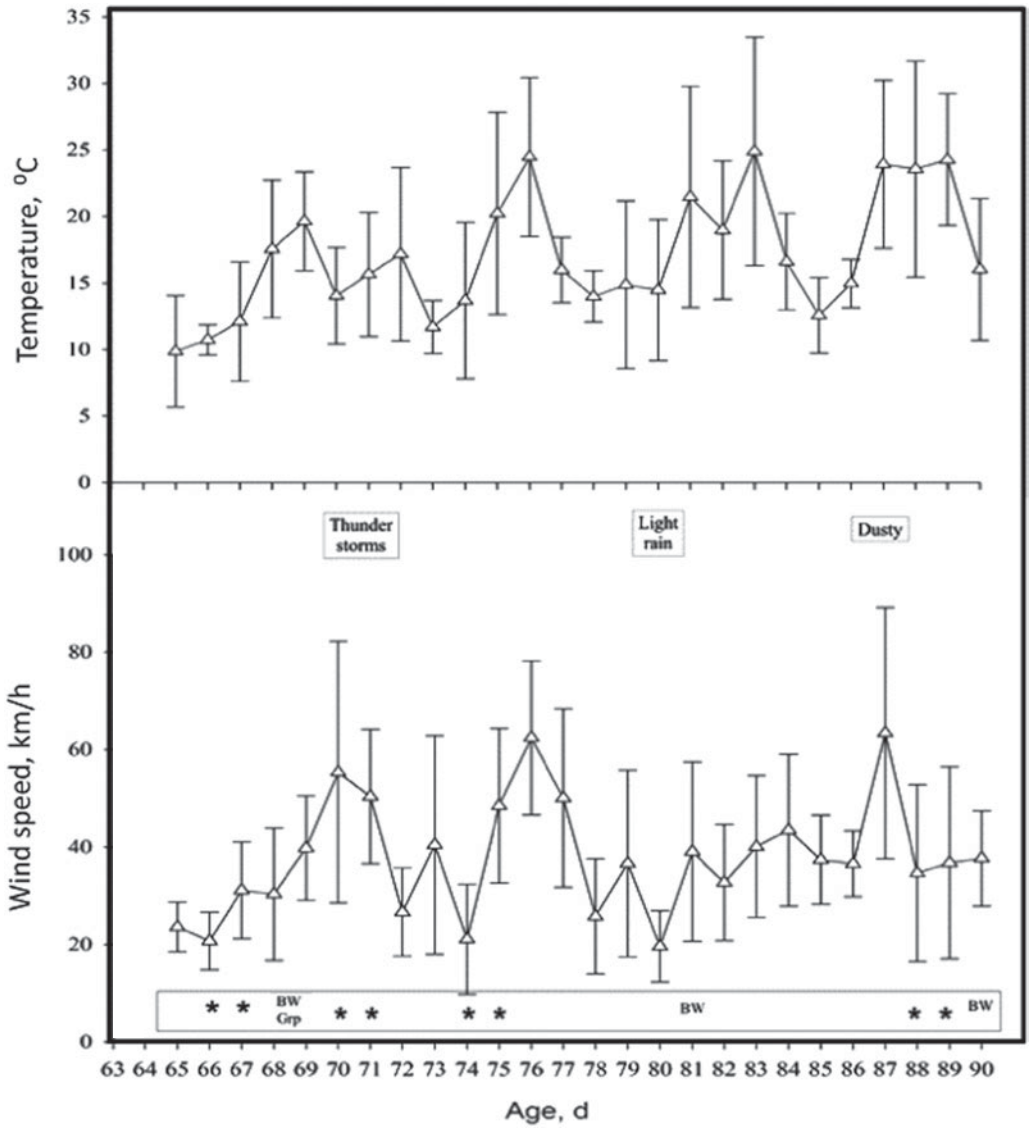

Fig. 2. Daily (mean ± SD) environmental temperatures (top panel) and wind speed (mean ± SD; bottom panel) and timeline (above *x*-axis) of handling calves during the experiment. Asterisk indicates when blood was sampled in two blocks. Body weights (BW) were measured and grouped calves (Grp) were place in pens of 3 ($n = 36$) or were left in home calf hutches (Control; $n = 28$).

Underground, 2010) and the daily mean temperatures (mean ± SD), wind speed (mean ± SD) and notes were reported (Fig. 2). At age 66 ± 2·3 d, all calves were stratified by age and body weight and randomly assigned to 1 of 2 penning-strategy treatments: Groups of 3 (Grouped; $n = 36$ calves in 12 pens) and Control ($n = 28$, remained in hutches). Because calves were randomized, there were no differences in age 4 d total serum protein between treatments (5·04 ± 0·21 SEM g/dl). At age 68 d, all calves were handled to collect body weights (BW), and subsequently, Grouped-calves were moved using a trailer and tractor, 405 m from their home-hutches to their respective pens. All calves were also weighed at age 78 and 89 d. Grouped-calf pens were allowed 4·00 m^2 of free space per calf (Fig. 1).

Blood collection and analyses

Nine millilitres of peripheral whole blood (6-ml and 3-ml heparin vacutainers) were collected via jugular venepuncture immediately before assignment of treatments (age 66 ± 2·3 d), as well as age 70, 74, and 88 ± 2·3 d. Samples had to be collected into 2 blocks on 2 consecutive days to accommodate logistics of running the immune function assays. The block (A or B) was assigned randomly to calves within their treatment ($n = 18$ Grouped, and $n = 14$ Control per block). The 3-ml vacutainers from each calf were analysed for haematocrit, total leucocyte counts, and differentiated analyses of neutrophils, lymphocytes, and monocytes using a Cell Dyn 3700 with automated 50-sample

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loader and vet-package software (Abbot Laboratories, Abbot, IL, USA). In addition, the neutrophil:lymphocyte cell ratio (N:L) was calculated.

Plasma analyses

Plasma was collected after centrifugation of 3-ml vacutainers at 1200 ***g*** and stored at −80 °C until analysed for cortisol, glucose and haptoglobin concentrations. Circulating concentrations of cortisol were determined using a commercially available competitive-binding Chemiluminescence-ELISA kit (Searchlight- Aushon BioSystems Inc., Billerica, MA, USA). The intra-assay variation was 5·8% and inter-assay variation was 7·2% for the cortisol assay. Plasma glucose was analysed by commercially available enzymic, colorimetric kits (Stanbio Laboratory, Boerne, TX, USA). Control serum (Randox Laboratories) was used to calculate the inter- and intra-assay coefficients of variation of 4·0 and 3·8, respectively. Plasma haptoglobin concentrations were determined by measuring haptoglobin/haemoglobin complex by the estimation of differences in peroxidase activity (Hulbert et al. 2011a). Results were expressed in arbitrary units resulting from the absorption reading at 450 nm × 100. The intra- and inter-assay variations were 1·8 and 1·3% for this assay.

Neutrophil function

Neutrophils were analysed by dual-color flow cytometery (QuantaSC MPL, Beckman Coulter, Fullerton, CA, USA) for simultaneous phagocytic and oxidative burst capacities of peripheral blood neutrophils in response to an *Escherichia coli* (*Esch. coli* 0111:H8) using methods as previously described (Hulbert et al. 2011a). *Esch. coli* were heat-killed and labelled with propidium-iodide-label and the conversion of dihydrorhodamine (Invitrogen) to rhodamine was used to measure the oxidative burst response. Neutrophils that were positive for both oxidative burst (OB+) and phagocytosis (PG+) were gated using a FL-1 by FL-3 scatter-plot. The percent neutrophils that exhibited both oxidative burst and phagocytosis (OB+PG+) were measured for cell percentage and geometric mean fluorescence intensity for oxidative burst (FL-1) and phagocytosis (FL-3).

Neutrophil L-selectin was assayed as previously described (Hulbert et al. 2011a) using primary anti-bovine CD62L monoclonal antibody (VMRD, Pullman, WA, USA) and a F(ab′)2 antimouse IgG:FITC labelled secondary antibody (AbD Serotec Raleigh, NC, USA). Using the flow-cytometer analysis software neutrophil L-selectin was measured using total geometric mean fluorescence intensity (FL-1).

Whole blood LPS stimulation

Whole blood was stimulated using methods as previously described (Hulbert et al. 2011a). Briefly, whole blood was diluted in cell-culture media and stimulated at a final concentration of 1 μg/ml of LPS (*Esch. coli* O111:B4; Sigma-Aldrich, St Louis, MO, USA) and incubated for 24 h. The supernatant fraction was collected and stored in −40 °C freezer until analysed for tumour necrosis factor-alpha (TNF-α) using a commercially available ELISA (DY2279E; R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay coefficients of variation were 3·2 and 4·1%, respectively. The sensitivity of this assay was 125 pg/ml.

Statistical analysis

All data were analysed by restricted maximum likelihood ANOVA using the MIXED procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA). Prior to ANOVA, all data were tested for normality of the residuals by evaluating the Shapiro–Wilk statistic using the UNIVARIATE procedure of SAS. Data that were not normally distributed were either log- or root-transformed before mixed model analysis. Baseline measurements taken before assigning penning strategy treatments (age 66 d) were tested as a covariate in all statistical models. The appropriate covariance structure was chosen for the within-calf nested within treatment for all models using the smallest Schwarz Bayesian Criterion. For all blood, plasma, and immune response data, a linear, mixed model with the fixed effects of treatment, time, and the interactions of treatment × time and block × treatment × time were fitted. The random effect was calf nested within treatment × block. Prior to statistical analysis, performance data were calculated for each pen. The statistical model for the performance data included the fixed effects of treatment, time, and the interaction of treatment × time. The random effect was pen nested within treatment. A Kenward–Roger correction was applied to the denominator degrees of freedom to obtain appropriate standard errors and *F* statistics for each model. Pair-wise comparisons were performed at each time using a sliced-effect multiple comparison approach with a Tukey–Kramer adjustment. A treatment difference of $P \leqslant 0{\cdot}05$ was considered significant, and $P \leqslant 0{\cdot}10$ was considered a tendency.

Results

There was a tendency for Grouped-calves to have less feed intake from age 66 to 78 d ($P = 0{\cdot}06$) and less feed intake from age 79 to 89 d ($P < 0{\cdot}01$; Table 1) than Control-calves. Consequently, Grouped-calves had lighter BW than Control-calves by age 89 d ($P < 0{\cdot}05$; Table 1), and had less average daily gain (ADG) by age 78 d ($P < 0{\cdot}04$) and for the overall period ($P < 0{\cdot}01$; Table 1) than Control-calves. In addition, Grouped-calves F:G ratios were not different from **Control-calves** for age 66–78 d ($P = 0{\cdot}81$), but for the age 79–89 d period, Grouped-calves had greater F:G than Controls ($P < 0{\cdot}01$; Table 1). Control-calves had greater plasma glucose concentrations than Grouped-calves ($P < 0{\cdot}05$; Table 2).

Although plasma cortisol concentrations did not increase above baseline concentrations, Control-calves had greater

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Table 1. Performance data for grouped (pens of 3) and control calves

	Treatment			
	Group	Control	Largest SEM	*P* value
N	36	28		
Age at start, d	65·9	66·2	2·3	0·54
68 d of age BW, kg	75·4	74·4	1·49	0·61
68–78 d of age				
BW 78 d of age, kg†	84·3	84·6	1·68	0·89
ADG, kg/d†	0·89	1·02	0·047	0·04
FI/d, kg /d‡	2·79	3·09	0·080	0·06
F:G kg/kg‡	3·18	3·13	0·173	0·81
79–89 d of age				
BW 89 d of age, kg	95·3	101·7	1·99	0·02
ADG, kg /d	0·91	1·42	0·059	<0·01
FI/d, kg /d	2·99	3·75	0·173	<0·01
F:G kg/kg	3·50	2·66	0·163	<0·01
Overall, 68–89 d of age				
ADG, kg /d	0·900	1·24	0·041	<0·01
FI/d, kg /d	2·90	3·45	0·140	0·02
F:G kg/kg	3·27	2·80	0·098	<0·01

†For body weight (BW) and average daily gain (ADG), the individual animal was the experimental unit ($n=36$ Grouped and $n=28$ Control)
‡For feed intake (FI) and feed:gain (F:G), the average feed intake and gain/d was calculated and the pen was the experimental unit ($n=12$ Grouped and $n=28$ Control)

plasma cortisol concentrations (15·3 ± 1·89 SE ng/ml) than Grouped-calves (10·4 ± 1·64 SE ng/ml) at age 74 d ($P<0·01$). With the exception of neutrophil oxidative burst response ($P<0·05$; Fig. 3b), Grouped-calves did not have acutely (age 70 or 74 d) suppressed measures of innate immune responses ($P>0·10$; Table 2; Figs. 3a, 4a, b). In contrast, Grouped-calves had greater total circulating concentration of leucocytes at age 70 d ($P<0·05$; Fig. 5), which persisted from age 74 to 88 d inclusive ($P<0·10$). Neutrophil phagocytosis was increased at age 70 d among Grouped-calves ($P<0·01$; Fig. 3a) compared with Control-calves.

At age 88 d, Grouped-calves had elevated neutrophil:lymphocyte ratio ($P<0·05$; Fig. 4a) and lower neutrophil L-selectin expression ($P<0·05$; Fig. 4b). No other measures of innate immune competence were different between Grouped- and Control-calves at age 88 d. There was no treatment or treatment time effects on plasma haptoglobin concentrations during the study ($P>0·10$; Table 2). In all calves, haptoglobin concentrations increased at age 70 d, decreased at age 74 d, and were greatest at age 88 d ($P<0·05$; Table 2).

Discussion

Rearing dairy calves individually for the first few weeks of life is common practice to provide calves with individual care as they adapt to the *ex-utero* environment as well as to prevent the horizontal transmission of disease (Warnick et al. 1977; Lundborg et al. 2005). Producers often observe decreased performance during the grouping phase, which may be due to stressors related to the change from being individually raised to being placed in large groups. In the current study, feed intake, ADG and feed efficiency were reduced among Grouped-calves when compared with Control-calves. Data also indicated that the Grouped-calves had not fully acclimatized to the group-housing within the 21-d observation period. Differences in the housing environments in the current experiment may explain some of the performance variation (Hepola et al. 2006). Although space allowance for each calf was equal between treatments, the Control-calves had more protection from the windy environment, while all calves were exposed to the same temperatures. The individual hutch was enclosed; whereas although the group pens were equipped with shade, the pen design did not include a wind-barrier for calves. Because weather conditions were variable, especially wind speed, Grouped-calves probably reduced feed intake and therefore reduced feed efficiency.

The reduced performance among Grouped-calves could also be due to an increased locomotor activity and decreased inactivity. In fact, other researchers found that grouped-housed or paired calves spent less time feeding and more time performing other locomotor behaviours, while individually and isolated calves spent more time in recumbency (Warnick et al. 1977; Chua et al. 2002). Therefore, Grouped-calves in the current study may have had increased locomotor activity. Coinciding with variable weather conditions, increased activity probably contributed to decreased efficiency and utilization of metabolizable energy for growth. Similarly to our findings, dry matter intake decreased during post-weaning grouping, especially in calves that were fed extra milk replacer during preweaning (Terré et al. 2007). However, others (Babu et al. 2004; Hepola et al. 2006 observed increased starter consumption and dry-matter feeding-related behaviours in calves grouped at an earlier age, 0–2 d, after birth. Therefore, it remains to be determined the earliest age when calves raised in individual, outdoor hutches should be introduced to group housing, and the present data and previous literature suggest that group-housing earlier than 66 d of age may improve performance measures. This is an area that warrants further research.

Performance measures are not the only determining factors for whether or not a housing condition is deemed 'stressful' (Amadori et al. 1997). Moving calves into small-grouped transition-pens 23 d after weaning was probably a mild stressor and transient, as evidenced by only a suppressed neutrophil oxidative burst at age 70 d. Suppressed neutrophil oxidative burst has been associated with other stressors, such as early weaning of dairy calves (Hulbert et al. 2011a), castration of dairy calves (Pang et al. 2009), and transportation of beef calves (Hulbert et al. 2011c). In the current study, the stressor from moving calves into pens was probably mild because there was no treatment effect on either neutrophil L-selectin expression or the N:L at age 70 and 74 d. Previous research indicated that both reduced neutrophil L-selectin expression and elevated plasma N:L were sensitive measures of stress among cattle (Hulbert et al.

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Table 2. Daily means of blood measurements of all calves ($n = 64$) before (age 66 d) and after Grouped-calves were placed in pens

	Treatment		Largest	Age, d				Largest	*P* values		
	Group†	Control	SE	66‡	70	74	88	SE	TRT	Day	TRT*Day
Whole blood measures											
WBC, $\times 10^{-6}$/ml§	9·3	8·5	0·33	7·9	8·6[a]	8·5[a]	9·6[b]	0·33	0·06	<0·01	0·92
N:L¶	0·59	0·54	0·05	0·53	0·62[a]	0·49[b]	0·60[a]	0·05	0·61	<0·01	0·08
Haematocrit,%	27·6	28·2	0·28	28·1	27·8[a]	27·3[a]	28·6[b]	0·28	0·12	<0·01	0·27
Cortisol, ng/ml	12·5	14·6	1·40	17·4	16·1[a]	13·0[a,b]	11·6[b]	1·60	0·30	0·02	0·01
TNF-α, pg/ml††	1530·5	1488·4	177·0	1820·7	1541·0[a]	1240·8[b]	1746·5[a]	208·50	0·79	<0·01	0·29
Haptoglobin, OD ×100‡‡	1·86	1·79	0·14	0·94	1·65[a]	1·44[b]	2·39[c]	0·15	0·9	0·02	0·49
Glucose mg/dl	91·2	96	1·47	98	88·4[a]	93·0[b]	99·4[c]	2·00	0·02	<0·01	0·44
Neutrophil function											
L-selectin, GMFI§§	72·6	81	9·12	68·2	55·2[a]	60·8[a]	122·9[b]	9·65	0·71	<0·01	0·05
OB+PG+,%¶¶	83·7	83·0	0·5	83·8	84·7[a]	82·2[b]	82·8[b]	0·56	0·24	<0·01	0·14
OB, GMFI†††	2170·5	2231·9	52·6	2077·6	2101·0[a]	2116·0[a]	2510·3[b]	60·80	0·16	<0·01	0·16
PG, GMFI‡‡‡	138·9	136·7	2·54	96·03	127·9[a]	170·3[b]	157·1[c]	3·24	0·37	<0·01	<0·01

† Calves were all weighed and grouped-calves were moved to pens ($n = 36$; 3/pen) at age 66 ± 2·3 d or were left in their home hutches (control $n = 8$)
‡ Data for age 66 d was considered baseline and used as a covariate in the model
§ Circulating white blood cell counts
¶ Neutrophil:lymphocyte ratios, log-transformed *P*-values
†† Tumor-necrosis factor-alpha (TNF-α) from the supernatant of whole blood stimulated with 1 μg/ml of lipopolysacharride (LPS) for 24 h
‡‡ Optical density, root-transformed *P*-values
§§ Geometric mean fluorescent intensity
¶¶ The percentage of neutrophils that were oxidative burst (OB) positive and phagocytosis (PG) positive
††† Oxidative burst (OB) geometric mean fluorescent intensity of OB+PG+ neutrophils
‡‡‡ Phagocytosis geometric mean fluorescent intensity of OB+PG+ neutrophils

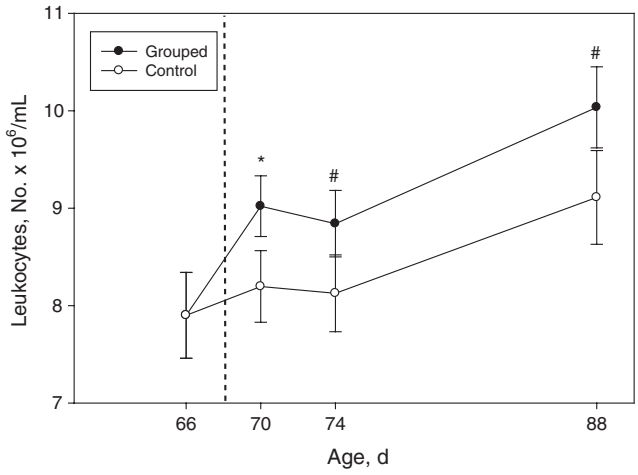

Fig. 3. Peripheral blood leucocyte counts (mean± SE) before (age 66 d) and after calves were penned in groups of 3 at age 68 ± 2·3 SD (group, $n = 36$) or were left in home calf hutches (control, $n = 28$). treatment × time sliced effect $^{*}P < 0{\cdot}05$; $^{\#}P = 0{\cdot}09$.

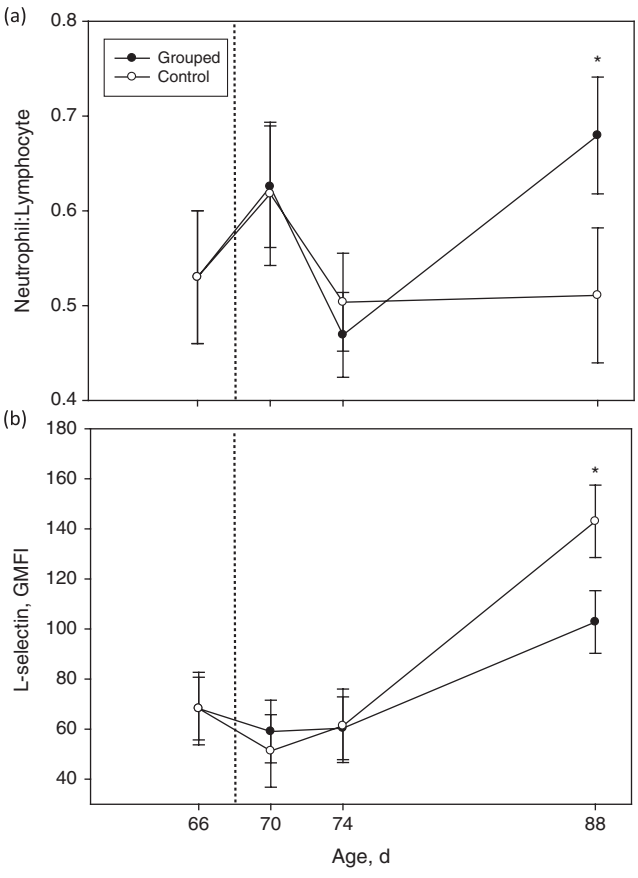

Fig. 4. (a) Peripheral neutrophil to lymphocyte ratios and (b) neutrophil L-selectin geometric mean fluorescence intensity (GMFI; mean ± SE) before (age 66 d) and after calves were penned in groups of 3 at age 68 ± 2·3 SD (Group, $n = 36$) or were left in home calf hutches (control, $n = 28$). Treatment × time sliced effect $^{*}P < 0{\cdot}05$.

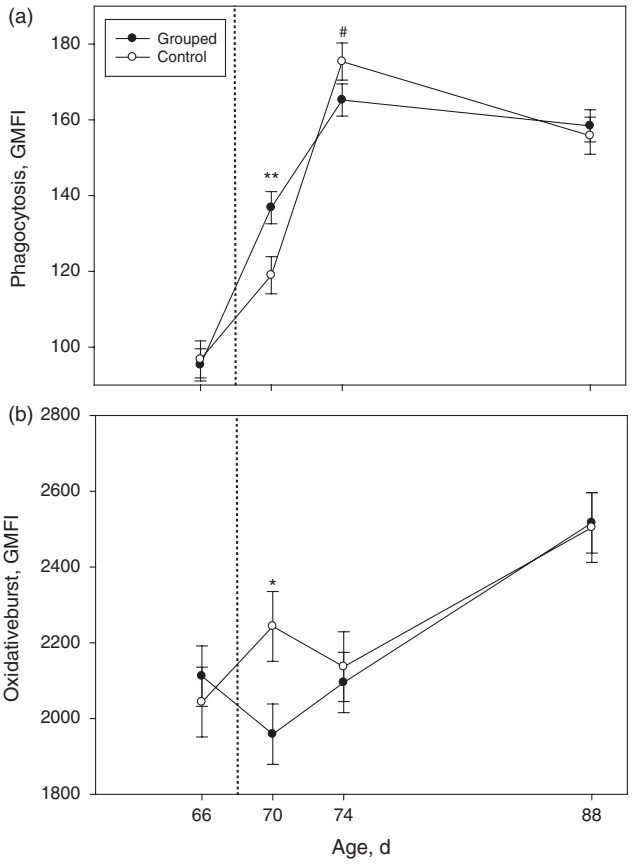

Fig. 5. Peripheral neutrophil geometric mean fluorescence intensity (GMFI; mean± SE) of (a) phagocytosis and (b) oxidative burst before (66 d of age) and after calves were penned in groups of 3 at age 68 ± 2·3 d (Group, $n = 36$) or were left in home calf hutches (Control, $n = 28$). Treatment × time sliced effect $^{**}P < 0{\cdot}01$; $^{*}P < 0{\cdot}05$; $^{\#}P < 0{\cdot}08$.

2011a, b, c). Despite a lack of a treatment effect on either neutrophil L-selectin expression or plasma N:L, relative to baseline measurements at age 66 d, all calves had decreased neutrophil L-selectin and increased N:L at age 70 d. In addition, all calves had decreased TNF-α from LPS-stimulated whole blood cultures and increased plasma haptoglobin concentrations at age 70 d when compared with age 66 d. These immunological and biochemical changes indicated that all calves may have experienced an acute stress, regardless of treatment. All calves were handled and weighed before the Grouped-calves were moved to their pens; therefore, the stress at age 70 d was at least partially due to handling stress.

Leucocyte numbers in Grouped-calves at age 70 d were increased and remained elevated throughout the study. The leucocytosis of Grouped-calves is most likely due to an increased immunogenic stimulation from either the new environment and/or through direct contact with novel calves. Although Grouped-calves had increased circulating

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leucocyte pool, this probably did not contribute substantially to the reduced performance, as the total leucocyte pool remains only a small fraction of the total body mass (Klasing, 1998). Nonetheless, Lochmiller & Deerenberg (2000) argued that even though the total leucocyte pool quantitatively is only a small fraction of total body mass, an activated acute phase response can contribute to decreased performance and feed efficiency. In the present study, no calves showed any clinical signs of disease and there was no difference in the plasma concentration of the acute phase protein, haptoglobin, between Grouped- and Control-calves, which suggests that the decreased performance of Grouped-calves was not associated with a substantially activated acute phase response. The immunogenic stimulation during this transition-phase may benefit cattle once they are moved in larger groups because of a greater diversity of immunological memory (Færevik et al. 2007); therefore this warrants further research.

If moving calves into transition pens was stressful, investigators expected that by age 88 d, Grouped-calves would have acclimatized to their new environment and the innate immune responses normalized. However, all calves were exposed to dusty and windy conditions immediately prior to the last blood sample collection on age 88 d. All calves had increased haptoglobin concentrations, haematocrits, neutrophil L-selectin expression and oxidative burst. At age 88 d, it is probable that weather-conditions played a role in Grouped-calves' innate immunity as indicated by increased N:L and decreased neutrophil L-selectin compared with Control-calves. All calves experienced the same weather conditions; therefore this dissimilarity in neutrophil adhesion between treatments is probably due to the difference of wind barriers in each housing treatment. Therefore, investigators propose that transition pens with an enclosure may help reduce any additional weather-related stressors when calves are likely to be exposed to novel micro-organisms.

Legislation in both the European Union and the United States could possibly outlaw individual-housing systems in dairy calves completely. This would greatly impact dairy calf production systems and potentially calf well-being. It is important that future research aims to determine the earliest age at which dairy calves should be placed into groups, the size of the group, and the type of housing. In addition to using performance measures, physiological end-points such as innate immune measures and health should be used to assess the effectiveness of these management strategies.

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