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Research paper

Effects of prepartum stocking density on innate and adaptive leukocyte responses and serum and hair cortisol concentrations

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ABSTRACT

Objectives were to evaluate the effects of prepartum stocking density on innate and adaptive leukocyte responses, serum cortisol and haptoglobin concentrations and hair cortisol concentration of Jersey cows. The cows (254 ± 3 d of gestation) were balanced for parity (nulliparous vs. parous) and previous lactation projected 305-d mature equivalent milk yield and assigned to one of two treatments: 80SD = 80% stocking density (38 animals/48 headlocks) and 100SD = 100% stocking density (48 animals/48 headlocks). Pens ($n = 4$) were identical in size and design and each pen received each treatment a total of 2 times (4 replicates; 80SD: $n = 338$; 100SD: $n = 418$). A sub-group of cows ($n = 48$/treatment per parity) was randomly selected on week 1 of each replicate from which blood was sampled weekly from d −14 to 14 (d 0 = calving) to determine polymorphonuclear leukocyte (PMNL) phagocytosis, oxidative burst, and expression of CD18 and L-selectin, and hemogram. The same sub-group of cows was treated with chicken egg ovalbumin on d −21, −7, and 7 and had blood sampled weekly from d −21 to 21 for determination of serum IgG anti-ovalbumin concentration. Blood was sampled weekly from d −21 to 21 to determine glucose, cortisol, and haptoglobin concentrations in serum. Hair samples collected at enrollment and within 24 h of calving were analyzed for cortisol concentration. The percentage of leukocytes classified as granulocyte and the granulocyte to the lymphocyte ratio were not affected by treatment. Treatment did not affect the percentage of PMNL positive for phagocytosis and oxidative burst or the intensity of phagocytosis and oxidative burst. Similarly, treatment did not affect the percentage of PMNL expressing CD18 and L-selectin or the intensity of expression of CD18 and L-selectin. Concentration of IgG anti-ovalbumin was not affected by treatment. Serum concentrations of haptoglobin and cortisol were not affected by treatment. Similarly, hair cortisol concentration at calving was not affected by treatment. According to the current experiment, a target stocking density of 80% did not improve leukocyte responses compared with 100% target stocking density.

1. Introduction

Overstocking prepartum dairy animals is believed to be a threat to the animals' health and performance because of its effects on feeding and standing behaviors (Hosseinkhani et al., 2008; Huzzey et al., 2006). Recently, our group demonstrated a negative effect of elevated stocking density (100% vs. 80% of headlocks) in the prepartum period on competitive behavior at the feed bunk and lying time (Lobeck-Luchterhand et al., 2015). Furthermore, recent experiments suggest that overstocking pens of commingled nulliparous and parous non-lactating animals affects energy metabolism (Huzzey et al., 2012). Oetzel et al. (2007) suggested that, when prepartum nulliparous and parous animals are commingled, an increase in 10 percentage points in stocking density (headlocks) above 80% results in reduced milk yield among nulliparous animals (0.7 kg/d less milk from 3 to 85 DIM). Despite the mounting evidence, a large percentage of dairy herds still have insufficient feeding and lying space for prepartum dairy cows.

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Dairy ewes housed in high (1.5 m^2/ewe) stocking density from late gestation to mid-lactation had reduced anti-ovalbumin IgG concentration compared with ewes housed in low (3 m^2/ewe) stocking density (Caroprese et al., 2009). Furthermore, ewes that were housed in high stocking density conditions tended to have greater number of aggressive interactions, had reduced milk yield, and had increased milk somatic cell count (Caroprese et al., 2009). The consequences of elevated stocking density during the prepartum period on leukocyte responses, serum and hair cortisol concentrations, and markers of systemic inflammation of Jersey nulliparous and parous animals are unknown.

The hypotheses of the current experiment were that a reduced stocking density (80 vs. 100% of headlocks) would result in reduced serum and hair cortisol concentrations, improved PMNL activity (phagocytosis and oxidative burst), increased expression of adhesion molecules by PMNL (L-selectin and CD18), increased IgG concentration in response to an ovalbumin challenge, and reduced serum concentrations of haptoglobin. Therefore, the objectives of the current experiment were to evaluate whether reduced stocking density (80 vs. 100%) during the prepartum period would improve innate and adaptive leukocyte responses and reduce concentrations of markers of stress and inflammation of periparturient Jersey animals.

2. Materials and methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (#1105B99113).

2.1. Cows, housing, and feeding

Cows used in the current experiment ($n = 96$) are a sub-group of animals used in a larger experiment ($n = 756$; Silva et al., 2014). Detailed description regarding facilities, management, and nutrition may be found in Silva et al. (2014). Briefly, the experiment was conducted from October 2012 to December 2013 in a commercial dairy located in Southern Minnesota. Throughout the experiment, Jersey cows were housed in cross-ventilated free-stall barns. During the prepartum period (d −28 to 0; d 0 = calving) nulliparous and parous animals were housed separately in one of four free-stall pens that were identical in size and design. All experimental pens had 44 deep sand bedded free-stalls (230 cm length × 107 cm width × 114 cm height) with a head-to-head double row configuration. The width of stall space for each cow or stall divider was determined by side-lunging pipe loops with wide opening between the top and bottom pipes. Stalls were located between two main alleys (feed alley and stall alley) in the center of each pen occupying approximately 32% of total pen area. The feed bunk area of the pens had 48 self-locking metal headlocks (61 cm width each headlock) trough where cows had access to the feed bunk and were free to withdraw at any time.

As cows demonstrated signs of calving (discomfort, restlessness, tail twitching, and visualization of the allantoic sac through the vulva) they were moved to a box stall. During the immediate postpartum period (1 to 21 ± 3 DIM) cows were grouped by parity (primiparous vs multiparous) and housed in free-stall pens with 240 stalls and 260 headlocks. After calving, cows from different treatments were commingled in the same pen but primiparous and multiparous cows were kept separate throughout the lactation. From 1 to 21 ± 3 DIM, pens were stocked at 91.6% and 100% of headlocks and stalls, respectively. Stocking density from 21 DIM to the end of the lactation varied between 110 and 120% of headlocks and between 119% and 130% of stalls. Artificial lighting was provided during the prepartum (24 h of light) and postpartum (16 h of light and 8 h of dark) period. From enrollment to calving a different TMR was fed to nulliparous and parous animals once a day. From 1 DIM until drying-off primiparous and multiparous cows were fed the same TMR. Composition of TMR fed in the prepartum and immediate postpartum (1 to 21 ± 3 DIM) periods are described in Silva et al. (2014).

2.2. Treatments

At enrollment, cows were balanced for parity (nulliparous = 340 or parous = 416) and previous lactation 305-d mature equivalent milk yield (parous) and were assigned to one of the four study pens. Treatment applied to the study pens in the first replicate was determined by a coin toss. Animals were assigned to the 80% stocking density (80SD, $n = 2$ pens and 4 replicates) or 100% stocking density (100SD, $n = 2$ pens and 4 replicates) based on headlocks. Twice weekly, thereafter, groups of 2–15 cows (median = 9 cows) were moved to the 80SD and 100SD pens to re-establish the desired stocking density. At the start of each replicate and on the days of movement of new cows to the study pens, the desired stocking densities were 80% of headlocks, 86.3% of stalls, and 9.2 m^2/cow for the 80SD treatment and 100% of headlocks, 109% of stalls, and 7.2 m^2/cow for the 100SD treatment. At the end of each replicate, a new 80SD and 100SD group started but pens were switched to avoid location bias. There were a total of 8 replicates (4 replicates and 2 pens/treatment per replicate). Thus, each pen had the 80SD and 100SD treatments twice during the experiment. Numbers of cows in each pen were counted twice daily during the prepartum period by study personnel and daily stocking densities were calculated as the number of cows in the pen divided by the number of headlocks or stalls.

On the first day of each replicate a sub-group of cows was chosen for determination of innate leukocyte responses and concentration of antibodies ($n = 5$–7 cows per treatment per replicate). The selection of these cows was based on BCS and gestation length, in order to assure that sampling occurred on the same date for all cows. Ninety six cows ($n = 48$/treatment) were used for evaluation of innate and adaptive leukocyte responses, hemogram, serum concentrations of glucose, haptoglobin, and cortisol, and hair cortisol concentration.

2.3. Body condition and locomotion scores

At enrollment and 1 ± 1, 28 ± 3, and 56 ± 3 d postpartum animals were scored for body condition (1 = emaciated and 5 = obese; 0.25 unit increment as described by Ferguson et al., 1994) and locomotion (1 = normal locomotion and 5 = severely lame; as described by Sprecher et al., 1997; Fig. 1).

2.4. Hemogram and innate immune responses and antibody concentration assays

Blood sampled on d −14 ± 3, −7 ± 3, 0 ± 3, 7 ± 3, and 14 ± 3 were used for hemogram (Fig. 1). Samples collected into evacuated tubes with EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) were analyzed using a Vet Scan HM2 (Abaxis, Union City, CA). Complete blood count was performed but only data referent to concentration of granulocytes relative to total leukocytes and the granulocytes to lymphocytes ratio are reported.

Ex vivo innate leukocyte response was evaluated on d −14 ± 3, −7 ± 3, 0 ± 3, 7 ± 3, and 14 ± 3 (Fig. 1) as described by Hulbert et al. (2011). Samples were collected into heparinized evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Expression of L-selectin and CD18 by peripheral PMNL was determined by indirect immunofluorescence staining. Briefly, the assay consisted of incubating 200 μL of whole blood at 4 °C for 30 min