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## The effect of prepartum feeding and lying space on metabolic health and immune function

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### ABSTRACT

The determinants of metabolic and reproductive health disorders in the peripartum period and the degree to which feeding and lying space and management can influence health are only partially understood. The objective of this randomized controlled study was to determine whether providing noncompetitive feeding and lying access in the close-up dry period improves health and immune function. Forty-eight Holstein cows of all parities were randomly assigned to a treatment group of 6 to 10 cows in 1 pen with either 80% cows to stalls and 90 cm of feeding space per cow (understocked) or 120% stocking density and 45 cm of feeding space per cow (overstocked) for 3 wk before expected calving. All cows wore electronic data loggers to monitor daily standing and lying time. Video recordings representing d 7 to 9 after group formation were reviewed, and a competition index (C\_Ind) was calculated for each cow by dividing the number of times a cow displaced another as an actor by its total number of actor and reactor displacements. Cows were categorized as high success (C\_Ind  $\geq 0.6$ ), moderate success ( $0.4 \leq \text{C\_Ind} < 0.6$ ), or low success (C\_Ind  $< 0.4$ ). Weekly blood samples measured nonesterified fatty acids,  $\beta$ -hydroxybutyrate, calcium, glucose, albumin, aspartate aminotransferase, bilirubin, haptoglobin, insulin, and insulin-like growth factor-1 from 3 wk before to 5 wk after calving. Measures of innate immune function (neutrophil phagocytosis and oxidative burst) were assessed at -2, -1, 1, 2, 3, and 5 wk relative to calving. Liver biopsies were collected at wk 1 and 3. Cows in the understocked group spent significantly more time per day lying; the back-transformed least squares means and 95% confidence interval were 14.8 h (13.9–15.6) versus 12.8 h (12.0–13.7). Controlling for parity, there was no difference between treatments in  $\beta$ -hydroxybutyrate,

nonesterified fatty acids, glucose, insulin, insulin-like growth factor 1, aspartate aminotransferase, bilirubin, or haptoglobin concentrations. Throughout the study, cows in the understocked treatment had higher mean calcium and tended to have higher albumin and at 3 wk after calving tended to have lower mean liver triacylglycerol content. Overall, there was no treatment effect on phagocytosis, but cows with a higher C\_Ind in the understocked treatment group had greater oxidative burst function. There was no effect of treatment on endometritis. Despite increased competition and lower lying time, the expected harmful effects of crowding and competition on metabolic indicators and innate immune function were mostly not observed. Although this does not refute the importance of access to feeding and lying space, these results indicate that metabolic and reproductive health is more complex than can be explained solely by exposure to what are understood to be best practices for space allowances.

**Key words:** transition cow, grouping strategy, immune parameter

### INTRODUCTION

The peripartum period in dairy cows is characterized by a period of negative energy balance, diminished DMI, insulin resistance, lipolysis, and weight loss of varying severity (Goff and Horst, 1997; De Koster and Opsomer, 2013). Furthermore, diminished immune function typifies the peripartum period, a time when the majority of cows have bacterial contamination of the uterus that must be promptly eliminated (Sheldon et al., 2002). The innate immune system, specifically neutrophils as the first responders, is the primary means of cellular defense against bacterial colonization in the uterus. Impairment of neutrophil function has been documented beginning 2 wk before calving, reaching a nadir 1 wk postpartum with slow recovery in the early weeks of lactation (Kehrli et al., 1989). Mitigating this immune suppression would be auspicious as it contributes to common production-limiting diseases important to animal welfare and profitability.

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Differences in innate immune function in the weeks ahead of calving have been recognized in several reproductive diseases. Metritis and endometritis have been associated with reduced neutrophil migration, phagocytosis, and oxidative burst function (Hammon et al., 2006) and reduced tumor necrosis factor  $\alpha$ , a proinflammatory mediator prepartum (Kim et al., 2005). These changes are present weeks before disease becomes manifest, coincident with the onset of insulin resistance and lipolysis. Cows in greater negative energy balance as measured by diminished DMI, higher nonesterified fatty acids (NEFA), and higher BHB and in particular those that go on to have metritis or endometritis have more pronounced impairment of at least some immune functions (Hammon et al., 2006; Galvão et al., 2010). High NEFA concentrations are a risk factor for ketosis and fatty liver and may have direct effects on neutrophil function as demonstrated in vitro by Scalia et al. (2006). Despite the emerging understanding of these relationships, among cows with similar nutrition and management it is unclear what determines the incidence of metabolic and reproductive disease.

An environment that limits DMI and lying time may have enduring effects on transition cow health. Proudfoot et al. (2009) demonstrated that with increased competition in transition pens, displacements at the feed bunk increased and cows ate less in the week before calving and spent more time standing in the week after calving. Many prepartum pens do not have a stable group structure, and additions may be weekly or more frequently. After regrouping, lactating dairy cows moved to a new pen had reduced resting time and feed intake and more displacements from the feed bunk (von Keyserlingk et al., 2008). Cook and Nordlund (2004) have suggested that weekly additions to close-up pens result in similar disruption of social interactions among prepartum cows. The relationships among cow grouping, group size, bunk space, and competition for feed have been reviewed with the recommendation that bunk space not be limited to avoid DMI reductions (Grant and Albright, 2001). Huzzey et al. (2007) found that cows with severe metritis ate 2 to 6 kg of DM less than healthy cows in the 2 to 3 wk before clinical signs of disease. Lower feed intake prepartum is associated with increased NEFA and hepatic lipid accumulation (Grummer, 1993). Whether impairment of immune function or reproductive disease risk can be influenced through management has not been well tested despite the recognized associations between a competitive environment and DMI. Two studies have looked at prepartum stocking density in Jersey cows previously. A stable prepartum group with no further additions during the close-up period compared with a prepartum

pen with weekly additions to maintain stocking density (100% of stalls and 91.6% of headlocks) did not affect NEFA or BHB concentrations, retained placenta, metritis, or the percentage of neutrophils positive for phagocytosis or oxidative burst (Silva et al., 2013a,b). A second study that examined stocking pens at 80% versus 100% headlock stocking density and 86% versus 109% stalls found that treatment did not affect NEFA or BHB concentrations, retained placenta, metritis, or purulent vaginal discharge (Silva et al., 2014).

The objective of this study was to test whether providing noncompetitive access to feeding and lying modifies metabolic health and immune function. A secondary objective was to determine whether differences in metabolic health and immune function could be explained using a measure of social status in the group. The hypothesis was that providing greater feeding and lying space would reduce social stress and thereby improve measures of adaptation to negative energy balance and innate immune function.

## MATERIALS AND METHODS

### *Animals, Housing, and Management*

Twenty nulliparous and 28 parous (total  $n = 48$ , parity  $2.0 \pm 1.2$ ) Holstein dairy cows housed in prepartum groups in a freestall facility at the former University of Guelph Dairy Research Centre (Guelph, ON, Canada) were enrolled in this study. Cows were managed according to the guidelines set by the Dairy Farmers of Canada and the National Farm Animal Care Council (DFC-NFACC, 2009), and the animal utilization protocol was approved by the University of Guelph Animal Care Committee. Cows were enrolled in the study 21 to 28 d before expected calving date and monitored until 5 wk after calving, from October 2012 to October 2013. Weeks were corrected to actual calving date. Groups consisted of 6 to 10 cows depending on the availability of calvings at the farm. Prior to group formation and after calving, cows were housed individually in tiestalls. The precalving diet was a controlled-energy TMR diet ( $NE_L = 1.37$  Mcal/kg of DM) delivered once daily at 0600. After calving, all cows were fed a TMR ration 3 times per day (0900, 1300, and 1500 h) and were milked twice daily (0530 and 1600 h). The diets fed were identical between treatment groups pre- and postpartum (Table 1). A sample size of 20 cows per group was planned based on a difference of 15 units ( $SD = 16$ ) between treatments for the percentage of cells activated by phorbol myristate acetate in an oxidative burst assay or percentage of neutrophils that phagocytosed  $\geq 1$  fluorescent bead, both evaluated by flow cytometry.

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