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Prepartum feeding level and body condition score affect immunological performance in grazing dairy cows during the transition period

Joshua Lange,* Allison McCarthy,* Jane Kay,† Susanne Meier,† Caroline Walker,‡ Mallory A. Crookenden,‡ Murray D. Mitchell,§ Juan J. Loor,# John R. Roche,† and Axel Heiser*[1]
*AgResearch, Hopkirk Research Institute, Grasslands Research Centre, Palmerston North, New Zealand 4442
†DairyNZ Limited, Private Bag 3221, Hamilton, New Zealand 3240
‡DairyNZ Limited, School of Biological Sciences, University of Auckland, New Zealand 1010
§University of Queensland, Centre for Clinical Research, Royal Brisbane and Women's Hospital Campus, Herston, Queensland 4029, Australia
#Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT

Precalving feeding level affects dry matter intake, postcalving energy balance, the risk of hepatic lipidosis and metabolic disease, and gene expression in liver and adipose tissue. These coincide with a higher risk of disease postpartum and, very likely, a failure to reach optimum production as well as reproductive targets. Current interpretation of the available evidence suggest that metabolic stressors affect the immune system of transition dairy cows and lead to reduced immunocompetence. The objective of the current study was to investigate the effect of precalving body condition score (BCS) and level of feeding on immunocompetence during the peripartum period. Twenty-three weeks before calving, 78 cows were allocated randomly to 1 of 6 treatment groups (n = 13) in a 2 × 3 factorial arrangement: 2 precalving BCS categories (4.0 and 5.0, based on a 10-point scale) and 3 levels of energy intake during the 3 wk preceding calving (75, 100, and 125% of estimated requirements). Blood was sampled precalving and at 1, 2 and 4 wk after calving. Cells were analyzed by flow cytometry and quantitative real-time PCR. The numbers of T helper lymphocytes (CD4+), cytotoxic T lymphocytes (CD8+), natural killer cells (CD335+), and γδ T lymphocytes (WC1+) as well as their activation status [IL-2 receptor (CD25)+ cells] were highly variable between animals, but there was no evident effect of BCS, feeding level, or time. All groups presented with an increase in expression of cytokines in unstimulated blood cells in the week after calving, although this was significant only for *IFNG* in the BCS 4.0 group. Analysis of in vitro-stimulated cells allowed 2 general observations: (1) cows with high energy intake precalving (125%) had increased cytokine expression precalving, and (2) all cows had increased cytokine expression in the week after calving. The present study provides evidence that prepartum feed management can affect immunocompetence during the transition period. Considering the current results, optimally conditioned animals might benefit from a restricted precalving diet, whereas underconditioned cows can be fed to requirements.

Key words: immunity, immunocompetence, peripartum period, transition cow

INTRODUCTION

For dairy cows, transitioning from late pregnancy to early lactation poses a significant metabolic challenge (Bell, 1995; Drackley et al., 2005). These metabolic adaptations are likely the reason for immunologic changes (Goff and Horst, 1997; Ingvartsen and Moyes, 2013; Heiser et al., 2015) that contribute to an estimated 30 to 50% of dairy cows experiencing metabolic or infectious disease in the period immediately postcalving (LeBlanc, 2010). Neutrophils and their role in innate immune defense against mastitis have been a focus of previous research (Gilbert et al., 1993; Madsen et al., 2002; Hammon et al., 2006; Graugnard et al., 2012), whereas others have analyzed lymphocyte function, primarily measuring mitogen-induced lymphocyte proliferation (Kehrli et al., 1989; Lessard et al., 2004; Lacetera et al., 2005; Karcher et al., 2008; Schwarm et al., 2013; Heiser et al., 2015; Nightingale et al., 2015). From these studies, it can be concluded that immune function is modulated during the peripartum period, which likely affects susceptibility to infectious diseases after calving. However, there is a need for further analysis of the mechanisms involved and predictive biomarkers to allow identification of susceptible animals in time for preventive intervention.

To provide a broader analysis of immune function in transition cows in this study, we used flow cytometry

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[1]Corresponding author: axel.heiser@agresearch.co.nz

and a combination of in vitro stimulation and fluorescence-based quantitative real-time PCR (**qPCR**) to analyze the cellular composition of peripheral blood mononuclear cell (PBMC) subsets from pasture-based dairy cows as well as the immunological performance of these cells. Immunological performance was investigated by analyzing expression of mRNA encoding for selected cytokines after in vitro stimulation. Interferon-γ (*IFNG*) is critical for innate and adaptive immune responses against viral and intracellular bacterial infections and is also a key activator of macrophages (Schroder et al., 2004). Tumor necrosis factor (*TNF*) regulates immune cells, with variable effects. Dysregulation of *IFNG* and *TNF* is associated with several diseases (Locksley et al., 2001). Interleukin-17 (*IL17*) acts similarly to *IFNG* and synergistically with *TNF*. It is produced by T lymphocytes and supports migration of monocytes and neutrophils to the site of inflammation (Jin and Dong, 2013). In contrast to these proinflammatory cytokines, IL-10 (*IL10*) is considered an antiinflammatory cytokine (Couper et al., 2008).

Despite a significant number of studies investigating the metabolic and molecular changes associated with dietary energy intake before calving (Schmitt et al., 2011; Graugnard et al., 2012; Ji et al., 2014; Akbar et al., 2015), the underlying mechanisms are still unclear. Roche et al. (2013) reported significant effects of calving BCS on cow metabolic profiles, supporting the hypothesis that the effect of precalving level of feeding is dependent on precalving BCS (Roche et al., 2015). The objective of the current study was to investigate the effect of precalving BCS and level of feeding on immunocompetence during the peripartum period with the long-term goal of developing feeding recommendations for improved immune function.

MATERIALS AND METHODS

Animals were sourced from an experiment undertaken at Scott Farm (Hamilton, New Zealand; 37°46′S 175°18′E) between January and October 2013 that was reported by Roche et al. (2015). After passing a clinical examination, 150 cows were enrolled in the experiment on January 21, 2013. Cows were allocated randomly to 1 of 6 treatment groups (13 cows per group) in a 2 × 3 factorial arrangement: 2 precalving BCS categories (4.0 and 5.0; based on a 10-point scale, where 1 is emaciated and 10 obese, **B4** and **B5**, respectively; Roche et al., 2004) and 3 levels of energy intake during the 3 wk preceding calving (75, 100, and 125% of estimated requirements, **F75**, **F100**, and **F125**, respectively; Roche et al., 2005). Although cow allocation to treatment was random, groups were assessed to ensure they represented typical New Zealand dairy cows from a management and genetic perspective and were balanced for age, breed, BCS at the time of enrollment, and expected calving date. Age at enrollment was 4.0 ± 1.4 yr (mean ± SD). To generate the 2 BCS treatment groups before the end of lactation, feeding levels were manipulated starting on February 1 (205 DIM). Therefore, 3 groups of cows were assigned to gain, maintain, or lose BCS. All animals grazed an allowance of fresh pasture and were supplemented with pasture silage, corn silage, and palm kernel expeller. From 23 ± 5.6 d precalving, cows were offered fresh pasture at 75, 100, or 125% of their estimated ME requirements (Roche et al., 2005). Further details of the approach to establish treatment groups are described elsewhere (Roche et al., 2015). The mean calving date was July 11 ± 10.2 d. All animal manipulations were in accordance with the New Zealand Animal Welfare Act (1999) and had approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Blood Samples

Blood was sampled on multiple days via coccygeal venipuncture at approximately 0800 h. To allow analysis of immunocompetence for a period starting before calving and covering an extended time after calving (4 wk) while maintaining feasibility and considering animal welfare, samples were taken at 4 time points. One sample each per time point (and 13 animals per treatment group) was taken at 1 to 17 d before calving (**Pre**), 3 to 11 d (wk +1), 12 to 18 d (wk +2), and 25 to 35 d (wk +4) after calving. Blood was collected into evacuated blood tubes containing lithium heparin as an anticoagulant (Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ). Following a procedure that showed good results in a previous study (Heiser et al., 2015), samples were shipped over night at ambient temperature to the Hopkirk Research Institute (Palmerston North, New Zealand) for further processing.

Phenotypic Analysis by Flow Cytometry

Peripheral blood leukocyte populations were analyzed using flow cytometry. The following antibodies were used: natural killer (**NK**) cells were identified using bovine CD335 (A488 conjugated, clone AKS1; Storset et al., 2004); γδ-T lymphocytes were identified using anti-WC1 [fluorescein isothiocyanate (**FITC**)-conjugated, clone CC15; Clevers et al., 1990]; T helper cells were identified using bovine CD4 (AlexaFluor 647-conjugated, clone CC8; Bensaid and Hadam, 1991); cytotoxic T lymphocytes (**CTL**) were identified using bovine CD8 (FITC-conjugated, clone CC63; MacHugh and Sopp, 1991); cells expressing the IL-2 receptor (CD25) were