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Effect of prepartum energy balance on neutrophil function following pegbovigrastim treatment in periparturient cows

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ABSTRACT

Treatment with granulocyte colony-stimulating factor (G-CSF) increases polymorphonuclear cell (neutrophil) count and enhances neutrophil function in the periparturient cow. Prepartum undernutrition was hypothesized to reduce the effect of a commercially available recombinant bovine G-CSF product (pegbovigrastim) on neutrophil count and function. Hence this study was undertaken to test the effect of undernutrition for approximately 1 mo before calving on the innate immune response to pegbovigrastim. Cows (n = 99) on pasture were blocked by expected calving date and body condition score and randomly assigned in a 2 × 2 factorial design. The first factor was that cows were fed to exceed energy requirements prepartum (full feeding) or restricted to approximately 85% of prepartum energy requirements (restricted feeding). The second factor was that at approximately 7 d before expected calving date, half the cows in each feed group were injected with pegbovigrastim and the remaining half were injected with saline. Treatments were repeated on the day of calving. Blood samples were collected pre- and postcalving for complete blood count, biochemistry, and in vitro assessment of neutrophil function including phagocytosis, myeloperoxidase release, and oxidative burst. Prepartum energy restriction resulted in lower body weight, a higher proportion of cows with elevated concentrations (i.e., > 0.4 mmol/L) of fatty acids, and higher average β-hydroxybutyrate concentrations before calving relative to fully fed cows. Treatment with pegbovigrastim increased the total white cell, neutrophil, lymphocyte, and monocyte counts. Pegbovigrastim treatment resulted in increased release of myeloperoxidase by neutrophils. Prepartum feeding group did not have an effect, and no feeding group × treatment interaction was observed for any of the white cell counts or functional tests. We concluded that pegbovigrastim treatment results in significant increases in neutrophil count and enhances neutrophil function as indicated by increased myeloperoxidase release. The response to pegbovigrastim was not affected by restricted prepartum energy intake.
Key words: dairy cow, immune function, neutrophil, peripartum energy

INTRODUCTION

The risk of disease in dairy cows is high in the peripartum period (Ingvartsen et al., 2003). Polymorphonuclear cell (neutrophil) functionality decreases at this time, leading to increased susceptibility to mammary and uterine infections (Kehrli et al., 1989; Gilbert et al., 1993; Mallard et al., 1998). Neutrophils in the periparturient cow have reduced chemotaxis, phagocytosis, and oxidative burst (Ingvartsen and Moyes, 2013). The mechanisms for this reduced neutrophil function may include lack of nutrients and negative effects of increased concentrations of some blood metabolites as a result of tissue mobilization associated with negative energy balance (Kehrli et al., 1989; Ingvartsen and Moyes, 2013). Negative energy balance is associated with reduced neutrophil phagocytic function (Moyes et al., 2009). Elevated fatty acid concentrations are associated with changes in oxidative burst capacity of neutrophils (Scalia et al., 2006; Ster et al., 2012). Additionally, elevated BHB concentrations are associated with reduced chemotaxis, reduced milk leukocyte phagocytic activity (Kluciński et al., 1988; Suriyasathaporn et al., 1999), and reduced oxidative burst (Hoeben et al., 1997).

The production of neutrophils is regulated by granulocyte colony-stimulating factor (**G-CSF**; Semerad et al., 2002; Martin et al., 2003; Bendall and Bradstock, 2014). Granulocyte colony-stimulating factor is produced by a variety of cells including monocytes, macrophages, and cells of mesodermal origin including vascular endothelial cells, fibroblasts, and keratinocytes (Demetri and Griffin, 1991; Kehrli et al., 1991). It induces differentiation of progenitor cells into mature neutrophils, shortens maturation time within the bone

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marrow, and alters functionality by increasing phagocytosis and antibody-dependent cell-mediated cytotoxicity and by priming oxidation (Demetri and Griffin, 1991; Dale et al., 1995; Avalos, 1996).

Daily subcutaneous treatment with recombinant human G-CSF resulted in partial protection against intramammary challenge with *Staphylococcus aureus* (Nickerson et al., 1989). A long-acting analog of bovine G-CSF (pegbovigrastim injection, Imrestor, Elanco Animal Health, Greenfield, IN) is commercially available. Pegbovigrastim injection has been shown to increase neutrophil count in peripheral circulation and to increase neutrophil functionality in dairy cattle (Kimura et al., 2014; Hassfurther et al., 2015).

Studies conducted for regulatory approval in New Zealand, the United States, and Europe have demonstrated a lower clinical mastitis incidence in early lactation following pegbovigrastim treatment. However, the magnitude of reduction in mastitis incidence varied substantially between farms. The reasons for this variation is unclear, but one hypothesis is that the response to pegbovigrastim, similar to neutrophil function itself, may be influenced by the metabolic status of the cow, specifically the degree of negative energy balance experienced during the periparturient period.

The objective of this study was to test the hypothesis that response to pegbovigrastim, as assessed by neutrophil count and in vitro neutrophil functional assays, is modulated by negative energy balance in the peripartum period.

MATERIALS AND METHODS

The study was undertaken following approval of the animal ethics committee of AgResearch, Ruakura, New Zealand. Multiparous cows (n = 99; 48 Holstein Friesians, 13 Jerseys, and 38 Holstein Friesian × Jersey) on pasture all the time at a research dairy facility (Massey University, Palmerston North, New Zealand) were enrolled. Cows were blocked by week of expected calving, ranked on BCS, and randomly assigned within block in a 2 × 2 factorial design.

On 3 occasions, at weekly intervals, cows due to calve in 4 to 5 wk were assessed by a veterinarian, and cows with grossly evident signs of disease were excluded (n = 2; Figure 1). Remaining cows were assigned to either a full ration (**FR**) or restricted ration (**RR**) at 31.4 (SD = 5.5) and 30.9 (SD = 4.9) d before calving, respectively. The FR cows were offered (on a DM basis) 2 kg of pasture [ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*)], 3.2 kg of pasture hay, 6.1 kg of maize silage, and 0.2 kg of a trace element vitamin mix (NutriMin Springer cow balancer Hi Mg, Nutritech International Ltd., New Zealand, batch number 506072) per cow per day. This ration was calculated (Rumen8, Department of Agriculture, Western Australia; http://www.rumen8.com.au/) to provide approximately 120 MJ of ME per cow per day, or 122% of prepartum energy requirements. The RR group was offered 1.33 kg of pasture (ryegrass/white clover), 2.3 kg of pasture hay, 4.2 kg of maize silage, and 0.2 kg of trace element vitamin mix per cow per day. This diet provided approximately 82 MJ of ME per cow per day, or 85% of prepartum energy requirements.

Cows were managed by placing temporary electric fencing longitudinally down a paddock and feeding the 2 groups side by side. The area of pasture offered was adjusted to ensure that the required intakes were achieved. The maize silage, hay, and trace element mix was fed once daily in concrete troughs near the dairy parlor. The 2 feed groups were fed separately, but using the same feed mix. The feed was mixed in a wagon that had incorporated scales, and the mass mixed was recorded each day.

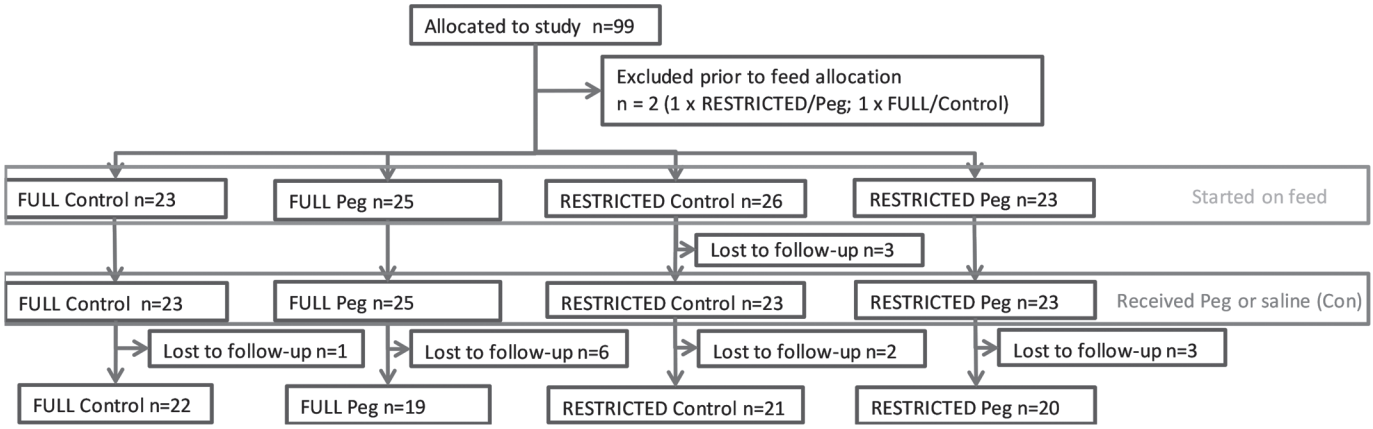

Figure 1. Number and fate of cows allocated to restricted ration or full ration feeding precalving and either pegbovigrastim (Peg) or saline (Control) at approximately 7 d before due calving date and the day of calving. Color version available online.