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Modulation of innate immune function and phenotype in bred dairy heifers during the periparturient period induced by feeding an immunostimulant for 60 days prior to delivery

E.L. Nace[a], S.C. Nickerson[a,*], F.M. Kautz[a], S. Breidling[b], D. Wochele[b], L.O. Ely[a], D.J. Hurley[b]

[a] Department of Animal and Dairy Science, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA
[b] Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

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ABSTRACT

The purpose of this study was to evaluate the effect of a feed additive (OmniGen-AF®, reported to have immune modulating activity) on innate immunity and health events during the periparturient period in dairy heifers when immunity is suppressed. From 60 days prepartum through calving, supplemented heifers ($n = 20$) received OmniGen-AF® daily and were compared with unsupplemented controls ($n = 20$). Blood leukocyte innate immune activity (phenotype markers, phagocytic activity, and reactive oxygen species—ROS production) was measured prior to feeding (60 days prepartum), 30 days later, and on days 1, 7, 14, and 30 postpartum. Adverse health events (udder edema, ketosis, displaced abomasum, and death) and milk production were measured at calving and into early lactation. The fraction of leukocytes with measurable CD62L (L-selectin) on their surface from supplemented heifers tended to be greater during the periparturient period in treated heifers than controls ($p = 0.100$). Likewise, leukocyte phagocytosis of *Escherichia coli* and *Staphylococcus aureus* during this time period tended to be greater in heifers supplemented with OmniGen-AF® ($p = 0.100$). Conversely, ROS production in response to phorbol myristate acetate or when leukocytes were stimulated with killed *S. aureus* lysate tended to be greater among control heifers compared with supplemented animals ($p = 0.100$). Supplemented heifers exhibited fewer incidents of udder edema than controls ($p = 0.030$) and tended to exhibit a lower rate of new cases of mastitis ($p = 0.098$); however, no differences were observed in milk somatic cell counts or level of milk production. Results demonstrate a positive role of OmniGen-AF® in amplifying leukocyte function consistent with antibacterial activity during the periparturient period, and support the continued study of dietary supplementation to enhance mammary gland health in dairy cows.

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1. Introduction

The periparturient period is a critical point in the lactation cycle of the dairy cow because it is a period of depressed immunity, which renders the animal susceptible to diseases such as mastitis (Smith et al., 1985; Burton and Erskine, 2003). Innate immunity is the most important

Abbreviations: FACS, fluorescence activated cell sorter; IMI, intramammary infection; ROS, reactive oxygen species; SCC, somatic cell count.

* Corresponding author. Tel.: +1 706 542 0658/+1 706 340 3367; fax: +1 706 542 2465.
E-mail address: scn@uga.edu (S.C. Nickerson).

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defense system of the bovine mammary gland and relies heavily on polymorphonuclear neutrophilic leukocytes (PMN), which migrate from the blood to the mammary gland in response to bacterial infection (Paape et al., 2000). Macrophages in milk, originating from circulating monocytes, are the predominate cells that act as sentinels to invading mastitis-causing pathogens and recruit the PMN (Paape et al., 2000). From about 2–4 weeks prior to parturition through approximately 2 weeks after birth, PMN as well as mononuclear cell function has been shown to be compromised, and changes in phenotype have been documented (Paape et al., 1981; Burton et al., 1995; Nonnecke et al., 2003; Weber et al., 2004). Strategies aimed at enhancing the immune system of the mammary gland during these periods of immunosuppression might greatly impact the ability of the cow to resist infection.

OmniGen-AF®, a general dietary immunostimulant for ruminants, appears to enhance immunity by stimulating PMN function and trafficking protein expression. Although the basic mechanisms of how the supplement exerts its effects have not been fully elucidated, initial studies demonstrated that the main effect of dietary supplementation is to alter the expression of mRNA transcripts. For example, the down-regulation of genes due to OmniGen-AF® was shown to enrich the oxidative phosphorylation pathway, which suggests that PMN with lower oxidative phosphorylation activity may be more efficient in distributing O_2 toward effector functions such as ROS production (Revelo et al., 2013). Another study suggested that restoration of PMN function by feeding OmniGen during periods of immunosuppression may be dependent on MyD88, which is critical for the development of innate and adaptive immunity by the induction of inflammatory cytokines triggered by TLRs (Ortiz-Marty et al., 2013).

In ruminants, Forsberg (Omnigen Research, LLC., Corvallis, Oregon, personal communication) found that PMN harvested from lactating cows receiving OmniGen-AF® for 60 days showed significantly increased phagocytosis of *Streptococcus uberis* compared to PMN of unsupplemented controls. Likewise, Wang et al. (2004) showed that OmniGen-AF® increased L-selectin (a PMN cell surface protein that promotes diapedesis and migration to infection sites) mRNA expression in pathogen-challenged sheep, which suggested that this immunostimulant enhanced the potential capacity of PMN to enter infection sites and kill bacteria. In a subsequent study, Wang et al. (2007) determined that OmniGen-AF® increased the expression of L-selectin and IL-1β mRNA in sheep that had been immune suppressed with dexamethasone. Thus, OmniGen-AF® appeared to ameliorate the immunosuppressive effects similar to those encountered around parturition by enhancing PMN activity and function.

More recently, Ryman et al. (2012, 2013) evaluated dairy heifers on a continuous feeding program with OmniGen-AF® (compared to unsupplemented controls) that were vaccinated against *Staphylococcus aureus* mastitis at 6 months of age followed by boosting every six months. The trial was conducted to determine if daily feeding of OmniGen-AF® would enhance the antibody titer response to this vaccine in these young heifers. They also examined the function and phenotype of leukocytes 30 and 60 days after initiation of continuous feeding of these heifers. OmniGen-AF® induced no improvement in antibody titers during calfhood or throughout the first pregnancy; however, heifers receiving extended continuous feeding of OmniGen-AF® exhibited enhanced PMN phagocytic activity against *Escherichia coli* and *S. aureus*, and greater reactive oxygen species (ROS) production compared to unsupplemented controls 30 and 60 days after supplementation. In addition, PMN L-selectin mRNA expression was significantly increased in heifers receiving continuous feeding with the supplement, and IL-8R mRNA expression was enhanced, but not significantly. These data suggest that supplementing heifer diets continuously with OmniGen-AF® stimulated the innate immune system in an effort to protect against bacterial challenge.

In the present study, we wished to determine if short-term daily supplementation with OmniGen-AF® beginning approximately 60 days prior to calving, would be sufficient to induce the previously observed enhancements in leukocyte function. It was our hypothesis that OmniGen-AF® would enhance blood PMN and monocyte function and provide a phenotype consistent with reduced periparturient immune depression, which may enhance resistance to mastitis during this time.

2. Materials and methods

2.1. Animals used in the study

Control ($n = 20$) and OmniGen-AF® treated ($n = 20$) Holstein heifers were serially selected at 6 months of age based on an even (treated) or odd (control) ear tag identification number for inclusion in the study, commingled by age, placed on pasture, and bred by 15 months of age. As the pregnant heifers reached 60 days prior to expected calving date, they began receiving a supplementation (OmniGen-AF® at the rate of 4 g/45 kg of body weight/day) or a control diet as described by Ryman et al. (2013).

Briefly, heifers were locked-up along a bunk-line feeding pad with head-locks and fed once daily a total mixed ration (TMR) based on wheat or sorghum silage and 2.3 kg of dry cow grain mix. The grain mix contained the following in kg/t: rolled corn (790), soybean meal (100), dicalcium phosphate (7.8), Clarifly® (4.3), salt (3.8), a trace mineral pack (4.4), vitamins A, D, and E (4.4), Zinpro performance minerals® (3.4), and limestone (2.0). At feeding time, treated heifers received the recommended dose of OmniGen-AF® delivered through the dry cow grain mix as a topdress at the rate of 4 g OmniGen-AF®/45 kg of body weight/day. Based on this rate, the supplement contained 10% Omnigen-AF, 10% molasses (to allow for binding), and 80% grain mix. Control heifers received a supplement of only the grain mix. The treatment and control diets were fed directly on the concrete slab in front of the heifers.

The average body weight, which was recorded each month starting 60 days prepartum, determined the amount of OmniGen-AF®/grain mixture that treatment groups received. This average corresponded to a designated amount of OmniGen-AF®/grain mix that was fed once a day. In addition to the grain supplement, heifers were given 22.7 kg/head/day of wheat or sorghum silage depending

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