# Innate Immunity and Periparturient Well-Being of Late Gestation Holstein Heifers Fed Omnigen-AF® for 60 Days Prepartum

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## INTRODUCTION

Polymorphonuclear neutrophilic leukocytes (PMN) are white blood cells of the innate immune system that serve as the most important defense system of the bovine mammary gland in response to bacterial infection (Burton and Erskine, 2003; Paape et al., 1981). During the periparturient period, PMN function is compromised and deleterious changes in phenotype have been documented (Burton et al., 1995), thus, affecting transition cow resistance to mastitis and overall productivity in the subsequent lactation.

OmniGen-AF® is a nutritional supplement for ruminants shown to influence innate immunity by affecting PMN function and trafficking protein expression. Ryman et al. (2013) recently evaluated dairy heifers on a continuous feeding program with *OmniGen-AF* beginning at 5 months of age through gestation and found that compared with controls, blood PMN from the supplemented heifers exhibited enhanced phagocytic activity, greater reactive oxygen species (ROS) production, and increased L-selectin mRNA expression. These data suggest that supplementing heifer diets with *OmniGen-AF* benefits the innate immune system to protect the mammary gland against bacterial challenges.

## OBJECTIVE

The purpose of this study was to determine if supplementing late gestation heifers with *OmniGen-AF* beginning 60 days prior to calving would be sufficient to restore blood PMN and monocyte function during periparturient immune depression for enhanced resistance to mastitis.

## MATERIAL AND METHODS

Forty Holstein heifers at the University of Georgia Teaching Dairy were serially selected at 5 months of age based on an odd (control) or even (*OmniGen-AF*) ear tag identification number for initial enrollment in the study. Heifers were comingled by age, placed on pasture, and bred by 15 months of age. Only 30 heifers were available for the commencement of the study. At 60 days prior to expected calving, heifers were moved to a far-off pasture and fed the pre-assigned diets: control diet (CT, n = 14) or control diet plus *OmniGen-AF* (OG, n = 16). Heifers remained on assigned diets through calving. Once daily, heifers were locked up in head-lock stanchions along a bunk-line feeding pad and fed a total mixed ration containing either wheat or sorghum silage, and a dry cow grain mix (5 lb/h/d). *OmniGen-AF* was delivered through the dry cow grain mix (10% *OmniGen-AF*, 10% molasses, and 80% grains). Heifers fed the CT diets received the same dry cow grain mix without *OmniGen-AF*. Diets were fed directly on the concrete slab in front of the heifers. Water and bermudagrass hay were available *ad libitum*. At 30 days prepartum, all heifers received non-lactating cow therapy with Spectramast-DC® by intramammary infusion. At approximately 2 to 3 weeks prepartum, heifers were relocated to a close-up pasture and fed a negative dietary cation-anion difference (DCAD) diet until calving. Body weights were taken at 60 and 30 days prepartum to ensure that heifers were within normal growth ranges by age (Hoffman, 1997).

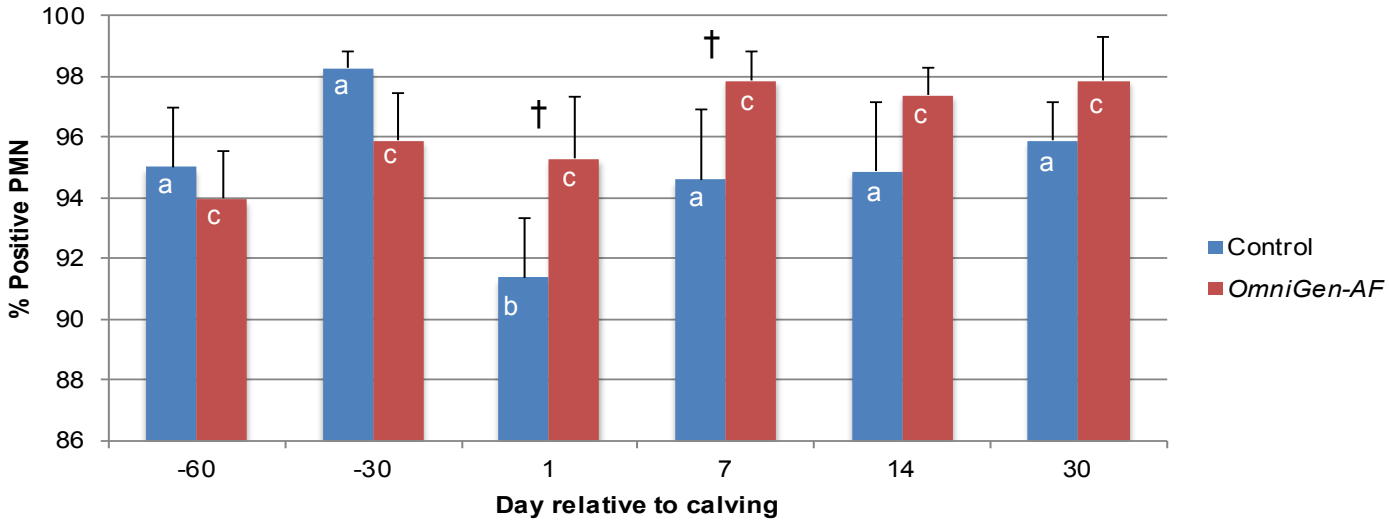
Blood samples were collected from each heifer via jugular vein puncture on day -60 (prior to diet assignment), on day -30 (prior to calving), and on days 1, 7, 14, and 30 postpartum. Blood leukocytes were isolated to determine expression of leukocyte phenotype markers (i.e., L-selectin), phagocytic activity, and ROS production.

At calving, any adverse health events associated with parturition such as a retained placenta, displaced abomasum, ketosis, udder edema, and death were noted. Daily milk production was recorded and milk samples from individual mammary quarters were collected on days 3 and 10 postpartum and analyzed bacteriologically to determine the development of new intramammary infections (IMI). Somatic cell count (SCC) and somatic cell score (SCS) were also determined from each sample.

## RESULTS AND DISCUSSION

**Leukocyte phenotyping:** Leukocytes were assessed for phenotypic expression of several markers including MHC I, MHC II, CD11b, CD11c, CD43, and CD62L (L-selectin). L-selectin expression on PMN from CT heifers was reduced to the lowest percentage of cells on day 1 after calving compared with day -60 ($P = 0.05$) (Figure 1), and expression on monocytes was lowest on day 7 ($P = 0.05$) and 14 ($P = 0.05$) after calving (data not shown). However, L-selectin expression on PMN and monocytes from OG heifers did not decrease during these time points, and expression on PMN tended to remain elevated over control values on day 1 ($P = 0.10$) and day 7 ($P = 0.10$), suggesting a greater ability of PMN from OG heifers to adhere to capillary walls for subsequent extravasation into infected tissue sites.

**Figure 1.** Percentage of PMN binding CD62L (L-selectin) antibody



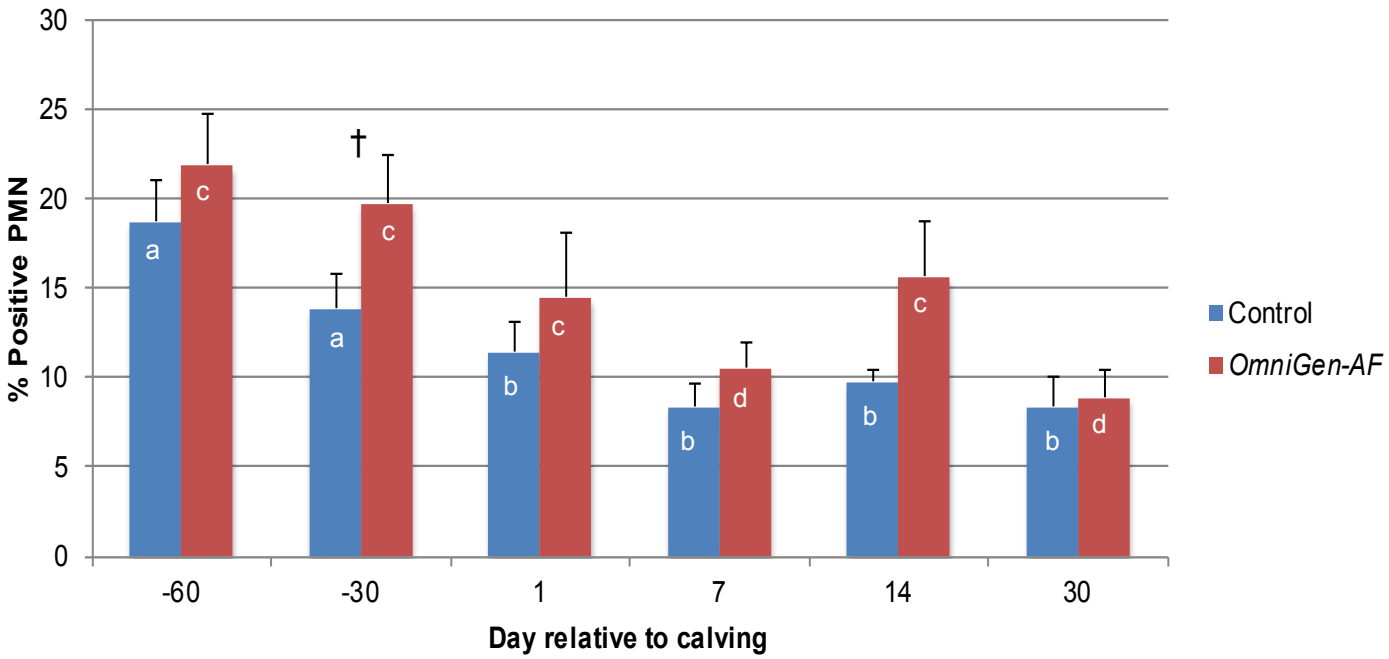

†CT and OG overall means differ ($P < 0.10$). [a,b] CT values for days without a superscript in common differ ($P < 0.05$).
[c] OG values across days do not differ.

These findings suggest that pre-feeding *OmniGen-AF* ameliorated the typical reduction in L-selectin expression observed during the stress of calving. Likewise, Wang et al. (2007) observed that *OmniGen-AF* prevented the decrease in L-selectin expression in sheep that were immunosuppressed with dexamethasone. In two separate studies, Ortiz-Marty et al. (2012) and Nightingale et al. (2013) reported similar findings; mice or cattle pre-fed *OmniGen-AF* prior to corticosteroid exposure maintained L-selectin levels both during and following the event. Results from the present study are also in agreement with Ryman et al. (2013) who observed an increase in L-selectin mRNA expression in blood leukocytes from heifers fed *OmniGen-AF* over a 15-month period compared with control heifers.

**Leukocyte phagocytic activity**: Once leukocytes have migrated into infected tissues, in part, with the assistance of L-selectin, phagocytosis by PMN is the most effective defense against bacterial infection of the mammary gland. However, the phagocytic and bactericidal activities of these cells are especially diminished during the periparturient period (Paape et al., 1981). The results from this trial showed that phagocytic activity of PMN against *E. coli* (Figure 2) and *S. aureus* was significantly lower during the periparturient period compared to day -60 across treatments ($P = 0.05$). However, activity was numerically greater in PMN isolated from OG vs. CT heifers from day -30 prepartum through 14 of lactation, and tended toward significance on day -30 ($P = 0.10$, *E. coli*) and on day 7 ($P = 0.10$, *S. aureus*).

Similarly, monocyte phagocytic activity against *E. coli* and *S. aureus* decreased significantly during the periparturient period, but only in cells harvested from CT heifers ($P = 0.05$). Monocytes from OG heifers showed no decrease in phagocytic activity during this time, and activity tended to be elevated over controls between days -30 and 7.

**Figure 2.** Percentage of PMN that engulfed *E. coli*




†CT and OG overall means differ ($P < 0.10$). [a,b] CT values for days without a superscript in common differ ($P < 0.05$). [c,d] OG values for days without a superscript in common differ ($P < 0.05$).

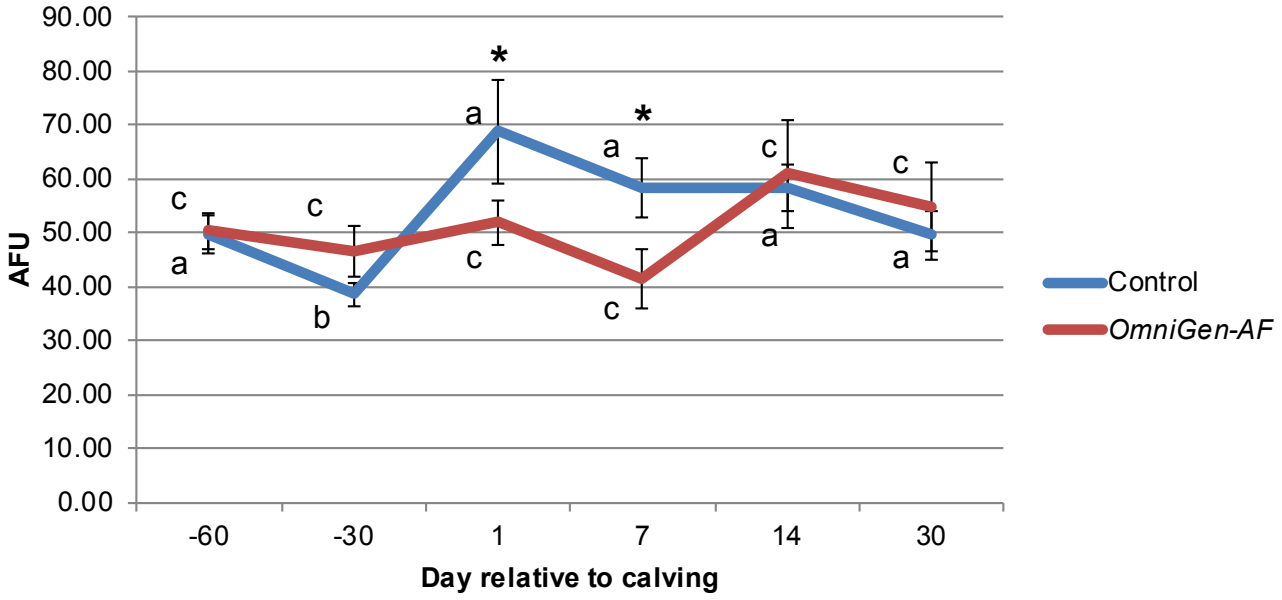
The decreased phagocytic activity through day 7 reflects the inhibitory effect of the stress associated with calving. Thus, the feeding of *OmniGen-AF* for 60 days prepartum appeared to ameliorate any immunosuppressive effects. In support of the increased leukocyte phagocytic activity observed, Corbett et al. (2008) found that PMN harvested from lactating cows receiving *OmniGen-AF* for 61 days showed significantly increased phagocytosis of *S. uberis* compared to PMN collected from controls. Likewise, Eubanks et al. (2012) found that heifers fed *OmniGen-AF* for 30 or 60 days during gestation exhibited greater leukocyte phagocytic activity compared to control heifers.

**Reactive oxygen species (ROS) production:** Once bacteria are attached to the PMN surface and the phagocytic process has begun, ROS (hydroxyradicals, singlet oxygen, oxygen halides, hydrogen peroxide, nitrogen oxide) are released that kill ingested bacteria (Burvenich et al., 1994). Production of ROS, however, is typically decreased during the periparturient period, which may increase susceptibility to mastitis (Mehrzad et al., 2002).

Prior to diet assignment, both groups of heifers exhibited similar levels of ROS production in response to stimulants such as killed *S. aureus* lysate (Figure 3). However, during the periparturient period, CT heifers produced more ROS than OG heifers, then both groups decreased to day -60 values by days 14 and 30 postpartum. For example, in response to *S. aureus* lysate, PMN from CT heifers exhibited a significantly higher ROS capacity on day 1 ($P = 0.05$) and on day 7 ($P = 0.05$) than OG heifers.

Although ROS production by PMN from CT heifers was significantly lower on day -30 than at day -60 ($P = 0.013$), and not different thereafter, ROS generated by PMN isolated from OG heifers was more stable and showed no significant differences across times (Figure 3).

**Figure 3.** Maximum ROS generating capacity of PMN reacting to killed *S. aureus* lysate




*CT and OG overall means differ ($P < 0.05$).
[a,b]CT values for days without a superscript in common differ ($P < 0.05$). [c] OG values across days do not differ.

This sequence of events suggests that although the two heifer groups started with no significant differences in ROS production, the continuous feeding of *OmniGen-AF* over time modulated the inflammation process more effectively, thus allowing the OG heifers to manage the stress associated with parturition, hence the lower and more stable ROS production. Following *OmniGen-AF* removal at the time of calving, both heifer groups exhibited similar ROS production from days 14 through 30 of lactation.

It has been suggested that when ROS is produced adequately but not excessively, collateral cell and tissue damage is minimized, as uncontrolled generation of ROS upon the initiation of phagocytic activity is harmful for many cell systems (Mehrzad et al., 2002). The lower, and more controlled or stable generation of ROS by PMN from OG heifers in the present study may be a safety mechanism to minimize collateral mammary cell and tissue damage in early lactation.

**Health events, mastitis, SCC, and milk production**: Although 40 heifers were selected for the study, heifer numbers were reduced to 30 for a variety of reasons including recurring resistance to sampling procedures (bleeding), abortion, displaced abomasum, bloat, leg injury and culling.

An analysis of adverse health events revealed a tendency ($P$ = 0.10) for a greater incidence of health issues among CT heifers (88%) compared with OG heifers (64%). Likewise, when the number of events per heifer was analyzed, there tended ($P$ = 0.10) to be more events among CT (1.69) vs. OG heifers (1.07). Among the health events recorded, only mammary edema was significantly different between the groups (CT = 75%, vs. OG = 36%, $P$ = 0.05). Although not significantly different, OG heifers exhibited fewer incidents of ketosis (0.14 vs. 0.31), displaced abomasum (0.14 vs. 0.19), death (0.14 vs. 0.31), and a lower overall adverse event score (2.21 vs. 3.44) compared with CT heifers.

New IMI rates among heifers postpartum were slightly more prevalent among CT heifers than OG heifers (50% vs. 36%), likewise, on a mammary quarter basis, there was a tendency ($P$ = 0.10) for more infected quarters per heifer in CT than OG heifers (0.93 vs. 0.36).

SCC from quarter milk samples taken 3 days postpartum were slightly lower for CT (685 x $10^3$/ml) vs. OG (963 x $10^3$/ml) heifers; however, by day 10 postpartum, no differences were detected (CT= 470 x $10^3$/ml; OG = 471 x $10^3$/ml). Likewise, SCS from quarter milk samples taken 3 days postpartum were slightly lower for CT (5.04) vs. OG (5.51) heifers; however, by day 10 postpartum, no differences between CT (4.66) and OG (4.32) heifers were observed.

The average milk production from day 7 through day 35 postpartum was not different between the CT and OG fed heifers (28.8 kg/day vs. 28.8 kg/day, respectively). Similarly, CT and OG fed heifers' milk yields on day 7 (21.5 kg, 21.0 kg) and day 35 (34.2 kg, 33.3 kg) were not different.

**SUMMARY**

The time around calving has been characterized as a period when the innate immune system is immunosuppressed, leading to increased incidences of IMI and other infectious diseases in dairy cows (Smith et al., 1985). In this study, the feeding of *OmniGen-AF* for 60 days prepartum to replacement dairy heifers resulted in bovine blood leukocytes that were more resistant to the effects of stress while maintaining the ability to effectively migrate, phagocytize bacteria, and regulate ROS production during the periparturient period. Collectively, these actions may have resulted in a reduction in the recorded adverse health events and new IMI at calving. These data suggest that the use of *OmniGen-AF* can provide a low cost intervention to modulate the mammary gland immune response by helping to control mastitis and other postpartum infectious diseases, creating greater opportunities for improved animal welfare and profitability.

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