Indicators of Immunological Stress and Systemic Inflammation Measured in Healthy Multiparous Peripartum Holstein Cows Fed OmniGen-AF®

C. Nightingale1, M. Sellers1, A. Pepper-Yowell1, M. Ballou1 and J. Chapman2

1Texas Tech University, Lubbock, Texas and 2Phibro Animal Health Corporation, Quincy, IL

INTRODUCTION

Pregnancy and calving are essential events in the production cycle of dairy cows. Although normal and necessary, the periods just before and after calving represent the greatest risk to cow health, affecting subsequent milk yields, reproductive efficiency and herd longevity (Drackley, 1999, Drackley et al., 2005). Hormones involved with the process of parturition, specifically cortisol, are associated with what is described as periparturient immunosuppression. This temporary but unavoidable and necessary ‘programed’ impairment of immune function required for the calving process, is characterized by neutrophilia and a loss of neutrophil function resulting from cortisol's effect on the ability of neutrophils to adequately express L-selectin. If this effect is prolonged and pathogen exposure is high, the opportunity of disease is increased (Burton et al., 1995, Burton and Erskine, 2003). An inflammatory response is also a normal physiological occurrence that is necessary for the onset of labor and the expulsion of the placenta. Recent research has pointed to a link between inflammation and the inflammatory mediators released around the time of parturition and increased incidence of metabolic diseases and impaired subsequent reproductive performance (Hoeben et al., 2000, Huzzey et al., 2009, Nightingale et al., 2015).

Attenuating immune dysfunction and the inflammation process around calving may reduce the risk of disease allowing for greater opportunity to repartition nutrients toward milk and improved reproductive efficiency. In research studies with ruminants and rodents fed OmniGen-AF® and subjected to either a natural or induced immunological stress or a pathogen challenge showed improved innate immune cell function (Wang et al., 2007), responsiveness (Ryman, et al., 2013) and recovery (Ortiz-Marty et al., 2012). These improvements in immune cell function resulted in differences in disease, milk quality and milk yields (Ryman et al., 2013, Holland et al., 2013). To evaluate these same responses in cows that transitioned into milk without a metabolic or disease event, clinically healthy multiparous Holstein cows were used in a study to observe changes in immune and inflammatory markers when OmniGen-AF was fed during the dry period and into early lactation.

OBJECTIVE

To evaluate changes in indicators of immunological stress both pre- and post-partum in healthy multiparous Holstein cows fed OmniGen-AF from dry-off through 28 days in milk.

MATERIALS AND METHODS

Forty-seven multiparous Holstein cows from a large commercial dairy in the Panhandle of Texas were used in the study. Cows were selected and enrolled at dry-off using DairyComp305 records based on expected calving date (-60 ± 3 d) and randomly assigned to one of two experimental diets: the Control diet (CT, n = 24 hd, parity = 2.46) or the Control diet with OmniGen-AF (OG, n = 23 hd, parity = 2.65) at 56 g/head/day. Diets were silage based, fed as total mixed rations (TMR) and offered ad libitum once daily. To ensure appropriate distribution of OG in the TMR's, OG was blended with wheat middlings by a commercial feed company to a quarter pound per head per day inclusion rate. The daily feeding order was: CT diet TMR was mixed and delivered first and then the OG blend was hand-added to the TMR and delivered to the OG assigned cows. Cows averaged 55 ± 3 d dry and at -21 ± 3
days relative to expected parturition, the base diet was changed from the far-off dry cow ration to an anionic close-up ration for both groups. At calving, the CT and OG cows were switched to a lactation diet and continued on their diet assignments through 28 days into milk (DIM). All cows were milked twice daily. Milk and milk components were recorded at approximately 30 day intervals post-partum to 150 DIM.

Whole blood was collected via coccygeal venipuncture from each cow at dry off (-60 ± 3 days relative to calving), at d -30 ± 3 (mid-dry), at calving (d 0; within 25 hours), and days +14 ± 3, and +28 ± 3 relative to calving. Samples were preserved for measures of hematology, blood metabolites and ex vivo leukocyte responses. Neutrophil function was assessed by quantification of surface expression of the adhesion molecule L-selectin (CD62L) and neutrophil oxidative burst capacity, measured as Geometric Mean Fluorescence Intensity (GMFI), when stimulated with an enteropathogenic E. coli.

All cows included in final analysis of immune, health and metabolic statuses were physically normal in appearance and absent of any clinical symptoms of disease or illness. A linear mixed model with repeated measures was fitted with the fixed effects of diet, time and diet by time interaction, using baseline (-60 d) measurements as a covariate. The repeated measure was time and the subject was cow within diet. Significance was declared at \( P \leq 0.05 \) and a tendency when \( 0.05 \leq P \leq 0.15 \).

**RESULTS**

**Neutrophil Responses:** The surface expression of neutrophil CD62L of cows fed the CT and OG diets from dry off through 28 DIM is shown in Figure 1. Neutrophils harvested from the cows fed OG had CD62L surface protein concentrations that were 1.92 times greater (768 GMFI vs 400 GMFI: \( P < 0.001 \)) than the CT fed cows at calving. The CD62L expression values from neutrophils harvested from OG cows were also numerically greater than neutrophils from CT cows at days -30, +14 and +28 relative to calving.

Neutrophil to lymphocyte ratio (N:L) was used in this study to evaluate the ability of neutrophils to maintain normal function around the time of calving. An increase in the N:L ratio was detected from day -30, at calving (d 0) and at 28 DIM in the CT fed cows, indicating a loss of L-selectin expression capability, thereby reducing the ability of neutrophils to adhere to vessel walls and migrate to sites of infection. This trend was the inverse for cows fed OG, with the N:L ratio observed to be less at calving (\( P < 0.001 \)) and at 14 DIM.

**Inflammatory Markers:** Haptoglobin (Hp), an acute phase protein, produced in response to any inflammation process or immunological challenge increased at calving in both the CT and OG cows. However at 14 DIM the OG cow's Hp levels were significantly lower than the CT cows (104 µg/ml vs. 176 µg/ml; \( P < 0.002 \)) suggesting that OG cows had reduced inflammation. No differences were detected between the CT and OG fed cows at 28 DIM (Figure 3).
Elevated plasma levels of tumor necrosis factor alpha (TNFα) are common prior to calving and into early lactation. Whole blood harvested from CT and OG fed cows was cultured and stimulated with lipopolysaccharide (LPS) from *E. coli* 0111:B4 were observed to follow this trend (Figure 4). However, whole blood cultures from the OG cows had a 2.7 fold greater increase of TNFα secretion immediately after calving (+2.7 fold, *P* < 0.10) and remained greater (4.6 fold) through 14 DIM (*P* < 0.048) than those from CT cows. This data suggests that OG cows may have leukocytes that are better prepared to defend against any non-specific pathogen. No differences were observed in TNFα concentrations by 28 DIM between the CT or OG cows.

**Blood Metabolites, Milk and Milk Components:** Blood metabolites used to assess the nutritional status of transition cows were measured. These included non-esterified fatty acids (NEFA), B-hydroxybutyrate (BHBA) and blood urea nitrogen (BUN). Cows used in the final analyses were clinically healthy, therefore all blood metabolites evaluated were within the expected normal ranges.

In general, plasma NEFA levels increased from d -30 to calving and declined from 14 to 28 DIM for both the CT and OG cows (*P* < 0.001). However, NEFA concentrations tended to be greater in the OG cows compared to the CT, at d -30 (252 µEq/L vs. 193 µEq/L) and at calving (492 µEq/L vs. 418 µEq/L, *P* < 0.109), but had a tendency to be less at days 14 and 28 postpartum. BHBA concentrations were not different between the CT and OG cows and followed a similar pattern, increasing from d -30 to 14 DIM and then declining through 28 DIM. The BUN concentrations tended to be greater in the OG cows from d -30 to calving (OG = 13.4 mg/dL, CT = 12.3 mg/dL, *P* < 0.10) but were less than the CT cows at days 14 and 28 postpartum.

No differences in milk production (kg/d) were detected at the first test-day (*P* = 0.781) or overall through 150 DIM (*P* = 0.913) between the CT (44.9, 47.4) and OG (44.9, 46.8) dietary treatments. Similarly, no differences were observed between the CT and OG cows for milk fat % (3.37 vs. 3.38), milk fat yield (1.50 kg/d vs. 1.51 kg/d), milk protein % (2.95 vs. 2.92) or milk protein yield (1.31 kg/d vs. 1.31 kg/d). Cows fed OG had a tendency (*P* = 0.065) for a lower overall SCC from calving through 150 DIM (OG = 54,000 cells/ml vs. CT = 131,000 cells/ml) compared to the CT cows. Cows fed OG had all test-day SCC counts of <100,000 cell/ml compared to the CT cows which had 4 or 5 test-day counts above 125,000 cells/ml.

**SUMMARY**

Immunosuppression and inflammation are normal physiological events associated with parturition in dairy cows and have been linked to increased incidences of infectious and metabolic diseases. Reducing or attenuating the associated effects from these two events could potentially allow dairy cows to transition into milk with a reduced risk for disease and a more efficient response to an infection with less tissue damage.

To study the effect of feeding OmniGen-AF from dry off through early lactation on innate cell response and the inflammation process around the time of calving, forty-seven clinically healthy multiparous cows from a commercial dairy unit were used. In this experiment, the cows fed OG from dry off through 28 DIM were observed to have a more functional innate immune response at the day of calving compared to CT cows as measured by neutrophil surface expression of L-selectin (*P* < 0.001), neutrophil to lymphocyte ratio (*P* < 0.001) and tumor necrosis factor alpha (*P* < 0.10). The ability of neutrophils to generate an oxidative burst was similar between the CT and OG fed cows. However, neutrophils from OG cows demonstrated a more rapid response as calving approached which was maintained through 28 DIM. In addition, haptoglobin, a measure of systemic inflammation was found to be lower (*P* < 0.002) at 14 DIM in the OG verses the CT cows. Results from this study suggest that feeding OmniGen-AF beginning at dry off and continuing through early lactation enhanced innate immune cell function plus attenuated the effects of the normal inflammatory response associated with calving in transition dairy cows.
REFERENCES


