Immune Function and Energy Status in Holstein Cows with Uterine Infections

D. S. Hammon[1] and J. P. Goff[2]
Pfizer Animal Health[1] and USDA, National Animal Disease Center[2]

Introduction

The postpartum uterus in dairy cows is susceptible to multiple bacterial pathogens and susceptibility to infection appears to be associated with periparturient immunosuppression and energy status. Neutrophils **(PMN)** play an important role as they provide the first-line of cellular defense against bacterial colonization within the uterus. Peripheral blood PMN functions of periparturient dairy cows are impaired relative to non-parturient cattle (Kehrli et al., 1989; Cai et al., 1994). Blood PMN functions begin to decline prior to parturition, reach a nadir shortly after parturition, and slowly return to prepartum levels by about 4 wk postpartum (Kehrli et al., 1989). Although impairment of PMN function in periparturient dairy cows has been established, factors associated with PMN impairment around the time of calving are largely unknown.

Studies have shown a relationship between periparturient PMN function suppression during the periparturient period and retained placenta (**RP**; Kimura et al., 2002) and metritis (Cai et al., 1994) in dairy cows. PMN from cows with RP have decreased migration ability (Gunnink, 1984) and decreased myeloperoxidase activity (Kimura et al., 2002).

Reproductive Consequences of Uterine Infections

Infection and subsequent inflammation of the bovine uterus compromise uterine health and contribute to decreased reproductive efficiency in dairy cows (Coleman et al., 1985; Fourichon et al., 2000). Metritis and endometritis (LeBlanc et al., 2002) are prevalent in dairy herds and contribute to increased days to first breeding, decreased conception rate and pregnancy rate, and increased culling. Subclinical **(SC)** endometritis, based on uterine cytological examination, is also prevalent in dairy cows and has a profound negative impact on reproductive performance. Gilbert et al. (2005) and Hammon et al. (2001) reported that cows with subclinical endometritis had lower conception rates and higher reproductive failure rates, compared to cows without SC endometritis. More recently, Kasimanickam et al. (2004) showed that cows with SC endometritis, based on endometrial cytology examination at 34 to 47 days postpartum, had significantly lower first service and all service conception rates compared to cows without endometritis. Kasimanickam et al. (2004) estimated a loss of $285 per lactation due to uterine diseases. Gilbert et al. (1998) estimated that SC endometritis is likely to cost the dairy industry over $1 billion annually in days open alone.

Energy Status and Immune Function in Periparturient Cows

Decreased dry matter intake **(DMI)** prior to parturition is well documented and is associated with mobilization of lipids, which are released as non-esterified fatty acids **(NEFA)** from adipose tissue (Grummer et al., 2004). Decreased DMI and increased NEFA levels are temporally associated with periparturient immune function suppression and may contribute to impairment of the immune system in dairy cows (Rukkwamsuk et al., 1999). Furthermore, elevated levels of β-hydroxybutyric acid **(BHBA)** and other ketones have been shown to impair important functions of immune cells with possible implications for systemic infections postpartum (Klucinski et al., 1988). Kremer et al. (1993) reported that cows in negative energy balance (with elevated BHBA) prior to experimental infection of the mammary gland experienced more severe mastitis compared to cows with low blood BHBA concentrations, indicating that negative energy balance may predispose cows to severe infections. Hoeben et al. (1997) reported that exposure of PMN to elevated levels of BHBA reduced PMN respiratory burst and they concluded that BHBA may, in part, be responsible for the higher susceptibility to local and systemic infections during the postpartum period. There is also evidence of an association between elevated levels of milk ketones (acetone) and endometritis in dairy cows (Reist et al., 2003).

Immune Response to Uterine Infection

The uterus of the cow is protected from infection by anatomical barriers that limit entry of pathogens into the body and by immune cells, mainly PMN, that engulf and kill the uterine pathogens. These anatomical barriers are breached as parturition approaches and for several weeks after parturition. Therefore, PMN are largely responsible for elimination of bacteria that enter the uterus following parturition. Furthermore, PMN's play an important role in maintaining the health of the endometrium.

In order to kill bacterial pathogens in the uterus, PMN's must carry out a highly orchestrated and complex chain of events. This process involves the binding of immunoglobulins or complement components to receptors on the surface of PMN to initiate phagocytosis, after which the PMN becomes activated. Activation of the PMN typically involves a large increase in oxygen consumption and hexose monophosphate activity with subsequent generation and release of large amounts of superoxide anion to the cell surface and to the phagosome, which then spontaneously converts to hydrogen peroxide. These compounds alone are toxic to many bacteria, but as the phagosome fuses with primary granules of the PMN, myeloperoxidase is released. Myeloperoxidase catalyzes the reaction between hydrogen peroxide and chloride (and other halides) anions to form hypochlorite. Hypochlorite reacts with tyrosine and other residues of bacterial proteins to kill bacteria.

Research has provided evidence that PMN function is impaired in cows that develop uterine infections. Sheldon (2004) reported that *E. coli*, as well as their products, in addition to *A. pyogenes* inhibit the phagocytic activity of PMNs. Cai et al. (1994) reported that cows with metritis had decreased blood PMN function. In their study, they showed a reduction in PMN cytochrome C activity prior to parturition and a reduction in PMN myeloperoxidase activity after parturition, compared to clinically normal cows. They also reported a decline in circulating PMN after parturition in cows with metritis. Zerbe et al. (2002) studied the influence of *E. coli* and *A. pyogenes* on PMNs and their functional abilities. They determined that high-grade uterine contaminations were always associated with the presence of both bacteria. *E. coli* and its products were found to cause functional depression of PMN. Products of both bacteria were also found to accelerate the death of PMN in vitro with the products of *E. coli* being slightly more potent than the products of *A. pyogenes*.

PMN Function, Energy Status, and Uterine Health

In a recent study, we used 83 periparturient cows to compare PMN functions and energy status between cows with uterine health disorders and normal uterine health. Blood samples were collected at 1 wk prepartum, the week of calving, and at 1, 2, 3, 4 and 8 wk after calving for PMN function determination. Blood samples for NEFA and BHBA were collected via the coccygeal vein twice weekly, 4 to 5 h after morning feed was offered, from 2 wk prepartum until 5 wk postpartum. Dry matter intake for each cow was determined daily from 2 wk prepartum until 5 wk postpartum.

PMN Function

We used two measures of PMN killing, blood PMN myeloperoxidase activity and cytochrome C reduction, to determine immune functions around the time of calving in cows with puerperal metritis, SC endometritis, or normal uterine health. Cows with puerperal metritis (occurring between 0 and 14 d after parturition) and SC endometritis (diagnosed at 28 ± 3 d postpartum) had significantly lower PMN myeloperoxidase than cows with normal uterine health activity beginning prior to parturition and extending through the early postpartum period. Cows with puerperal metritis had significantly lower PMN cytochrome C reduction around the time of calving compared to cows with SC endometritis and cows with normal uterine health (Figure1-A and B).

These results are in general agreement with Cai et al. (1994) who showed that cows with metritis had decreased cytochrome C reduction activity prior to parturition, compared to clinically normal cows. However, Cai et al. (1994) reported that PMN myeloperoxidase activity declined only after parturition; whereas the decline occurred prior to parturition in our study. Cai et al. (1994) also reported a decline in circulating PMN after parturition, but not before, in cows with metritis compared to normal cows. Numbers of circulating PMN were not recorded in our study. Zerbe et al. (2002) reported that PMN from uterine lochia (but

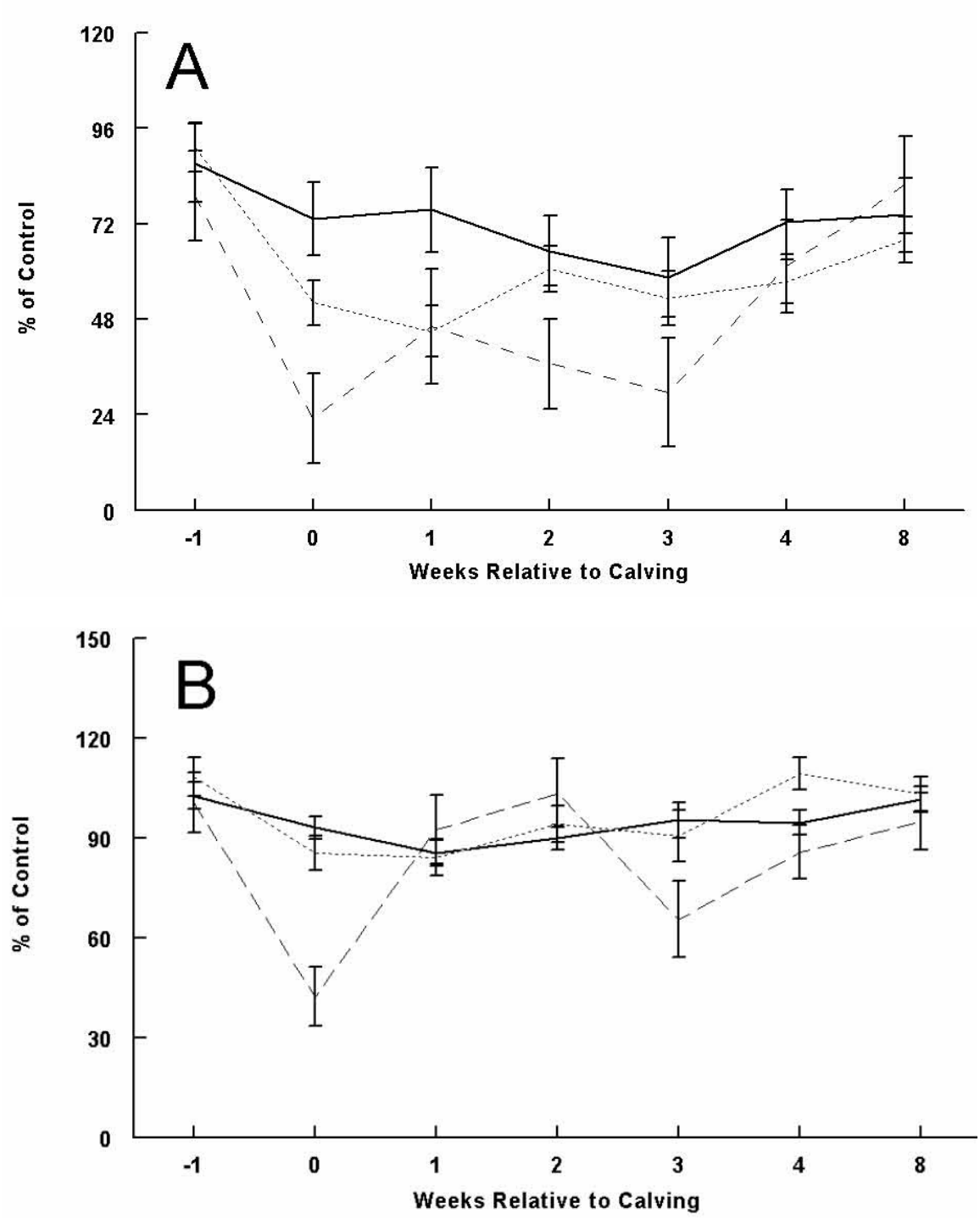

Figure 1. Myeloperoxidase activity of blood PMN (A) and Cytochrome C reduction of blood PMN (B) from dairy cows classified as having puerperal metritis (--, n = 18), subclinical endometritis (…, n = 43) or normal uterine health (__, n = 22). Cytochrome C reduction and myeloperoxidase activity shown is the percentage of response of PMN obtained from subject animals, compared to the response of PMN obtained from the internal laboratory standard steers samples obtained at the same time. Values shown are means ± SEM.

not PMN from blood) of cows with endometritis caused by *E.coli* and *Arcanobacterium pyogenes* had altered phenotype and decreased antibody-independent cellular cytotoxicity, compared to healthy cows. Ours is the first report of declining PMN myeloperoxidase activity occurring in cows prior to parturition in cows that would develop metritis and the first report linking PMN impairment in periparturient cows prior to or at calving with development of SC endometritis 3-4 wk later.

Energy Status

In our study, cows with puerperal metritis or SC endometritis had significantly lower DMI beginning 1 wk prior to parturition, compared to cows with normal uterine health (Figure 5-A). Cows with puerperal metritis or SC endometritis had significantly higher NEFA levels (Figure 5-B) beginning 2 wk prior to parturition and significantly higher BHBA levels wk 1 to 4 after parturition (Figure 5-C), compared to cows with normal uterine health.

Energy Status and Immune Function

The mechanisms responsible for PMN function impairment in periparturient dairy cows are poorly understood. The metabolic challenges associate with late gestation and the onset of lactation could be responsible in part for PMN function impairment during this time (Kimura et al., 1999). Data from our study suggests an association between energy status prior to calving and PMN function impairment in periparturient dairy cows. Elevation of NEFA (Figure 3) and suppression of DMI (Figure 4) prior to parturition were associated with suppressed blood PMN myeloperoxidase activity during the periparturient period. Blood PMN myeloperoxidase activity and plasma NEFA concentration in the days around calving were negatively correlated (Figure 2), suggesting that cows experiencing negative energy balance prior to or around calving are predisposed to periparturient immune suppression. Furthermore, cows with low (< 20.5 lb/d) prepartum DMI had significantly ($P < 0.01$) lower PMN myeloperoxidase activity compared to cows with high (> 29.5 lb/d) prepartum DMI (Figure 4).

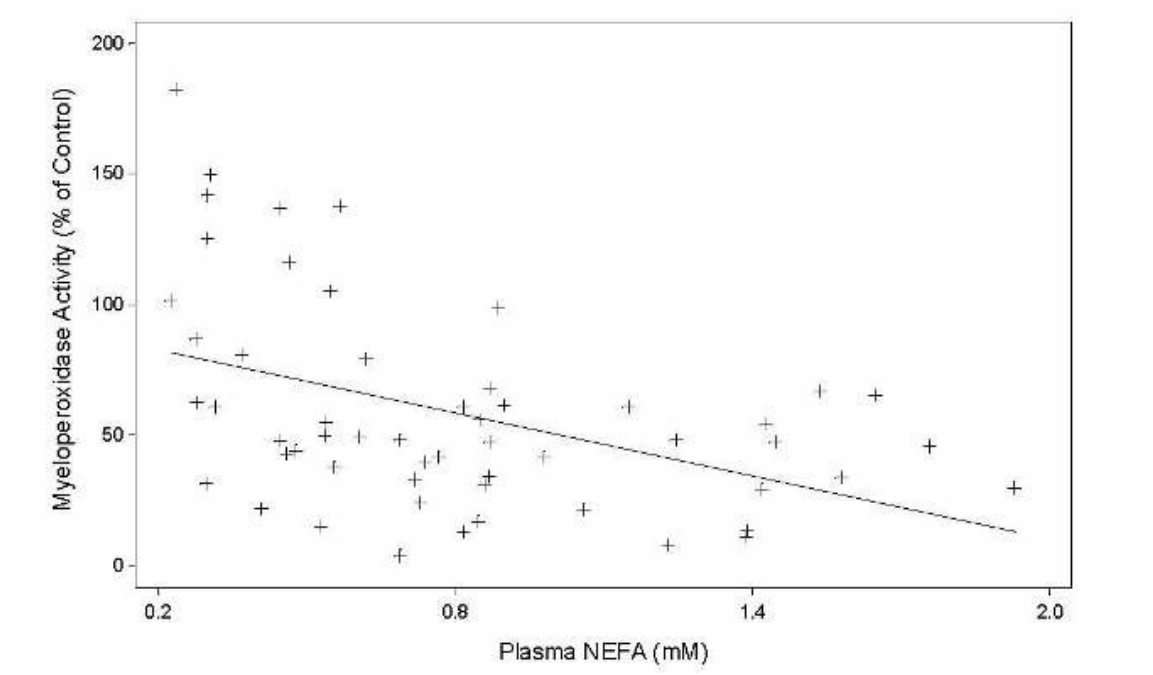

Figure 2. Relationship between PMN myeloperoxidase activity and plasma nonesterified fatty acid (NEFA) concentration (B, $r = 0.44$, $P < 0.001$).

PMN myeloperoxidase activity in cows with low prepartum DMI was suppressed prior to parturition, declined through wk 1, and remained suppressed until wk 3. In contrast, PMN myeloperoxidase activity in cows with high prepartum DMI was high prior to parturition, relative to cows with poor prepartum DMI, and declined only slightly around the time of parturition.

The results of our study differ from the study of Stabel et al. (2003) who reported no difference in PMN myeloperoxidase activity between prepartum cows with decreased DMI and cows that were stuffed through ruminal canulas to maintain DMI, despite the observation that cows allowed to express the typical decline in prepartum DMI had elevated NEFA levels; which was prevented by stuffing the cows with feed. However, Hoeben et al. (1997) reported that subketotic concentrations of BHBA significantly reduced PMN respiratory burst activity *in vitro*, as measured by a chemiluminescence assay; but elevated BHBA had no effect on either myeloperoxidase activity or cytochrome C reduction. To our knowledge, our's is the first report linking impaired PMN function with suppression of DMI and elevation in NEFA prior to parturition. Previous studies have reported a relationship between negative energy balance accompanied by elevated ketone levels during early lactation, and periparturient diseases, including metritis (Erb and Gröhn, 1988; Gröhn et al., 1989; Correa et al., 1993). Hill et al. (1985) showed an association between accumulation of lipids in liver and increased length of bacterial shedding in cows with mastitis. Kaneene et al.

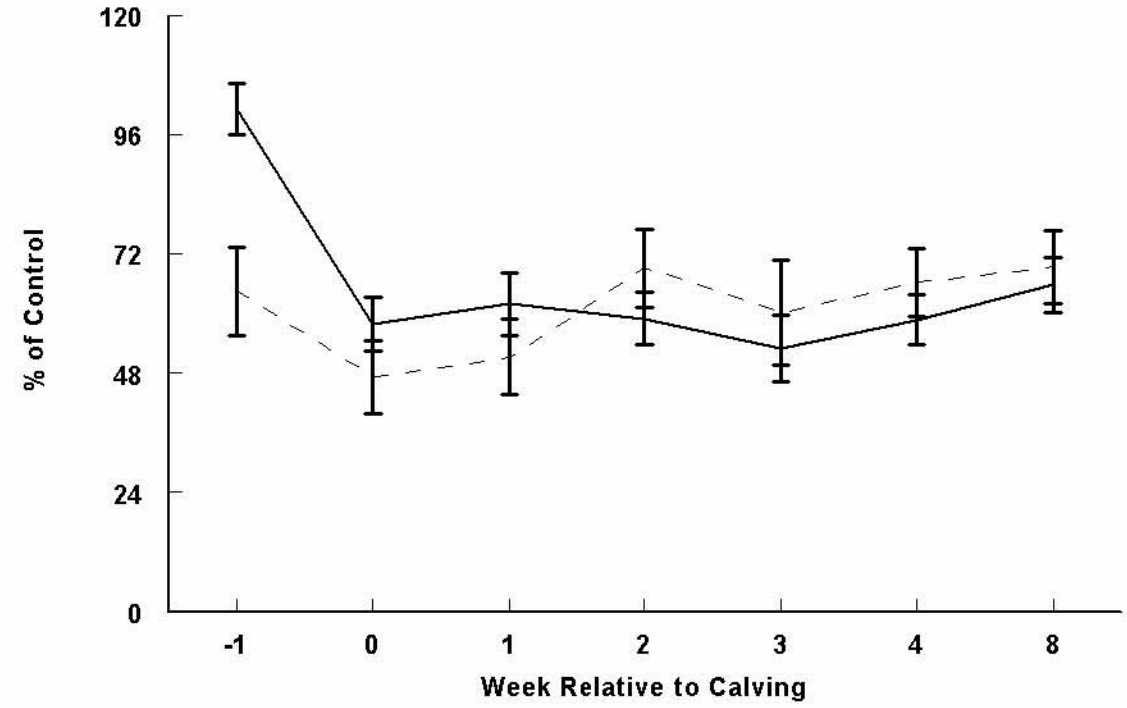

Figure 3. Myeloperoxidase activity of PMN from dairy cows with elevated non-esterified fatty acids (NEFA) (> 0.4 mEq/L) prior to parturition (- -, n = 20) compared to cows without elevated NEFA prior to parturition (__, n = 63). Myeloperoxidase activity shown is the percentage of response of PMN obtained from subject animals, compared to the response of PMN obtained from the internal laboratory standard steer samples obtained at the same time. Values shown are means ± SEM.

(1997) reported that prepartum fat mobilization and serum lipoprotein metabolism were related to increased risk of metritis and RP. In contrast, Jorritsma et al. (2000) reported no difference in the incidence of endometritis in cows with high (> 50 mg/g) and normal liver triacylglycerol contents. Reist et al. (2003) reported that elevated milk acetone concentrations, but not serum BHBA, were associated with an increased risk for endometritis diagnosed using vaginoscopy. Although there appears to be an association between negative energy balance in early lactation and infectious diseases during early lactation, the underlying mechanisms for this association remain unclear. Our study supports and advances these observations by demonstrating that metabolic disturbances occurring before calving predispose cows to later uterine health disorders. Furthermore, our study provides evidence of an association between decreased prepartal DMI and elevated prepartal plasma NEFA concentration with subsequent development of SC endometritis.

Conclusions

In summary, studies suggest that some uterine health disorders are associated with impairment of PMN function and negative energy status that begins prior to calving and extends into early lactation. Our study provides evidence that impaired PMN function around the time of parturition is associated with nutrient deficiencies that occur prior to parturition.

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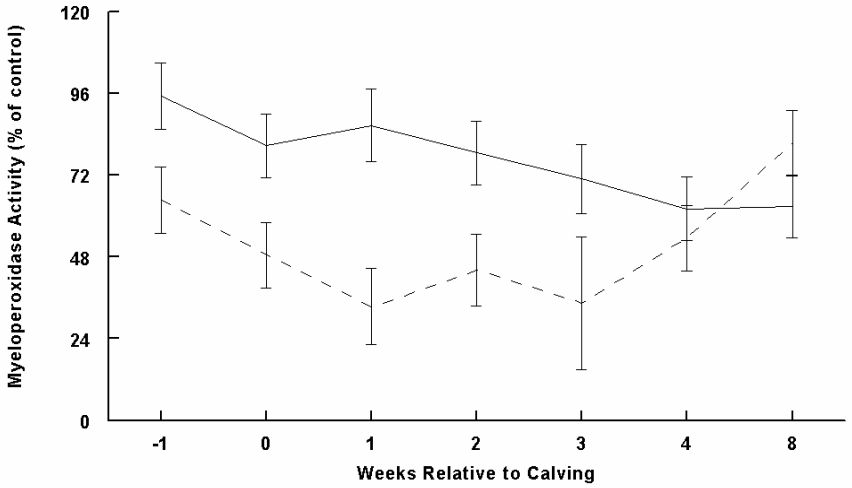

Figure 4. Myeloperoxidase activity of blood PMN from cows within the highest quartile (-) of prepartal dry matter intake (DMI) compared to cows within the lowest quartile (- -) of dry matter intake during the 3 wk period prior to parturition. Myeloperoxidase activity shown is the percentage of response of PMN obtained from subject animals, compared to the response of PMN obtained from the internal laboratory standard steers samples obtained at the same time. Indicates values for cows in lowest quartile DMI are significantly ($P < 0.05$) lower than for cows in highest quartile DMI. Values shown are means ± SEM.

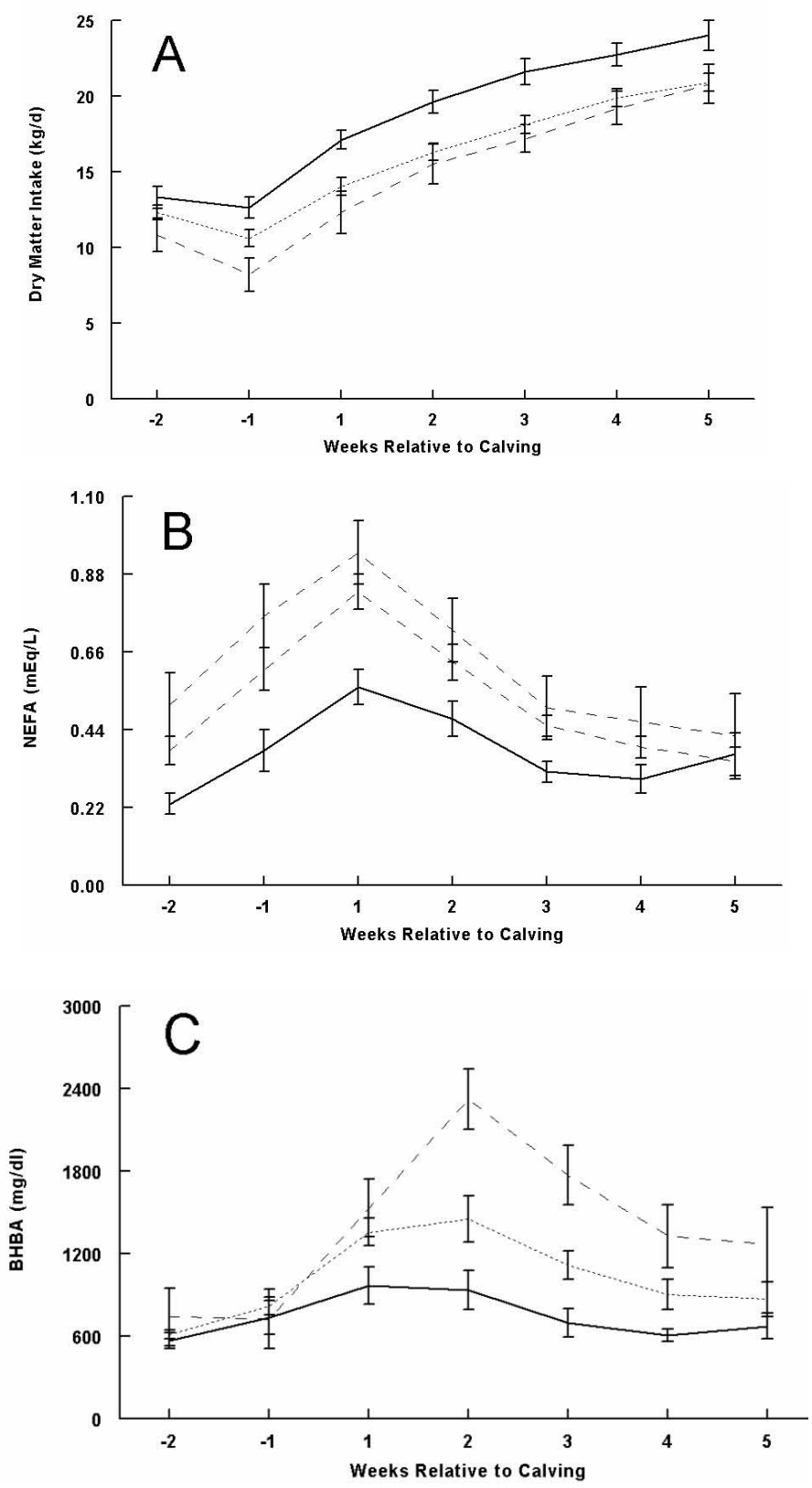

Figure 5. Dry matter intake (A), plasma non esterified fatty acid concentration (B, NEFA), and plasma ß-hydroxybutyric acid concentration (C, BHBA), from dairy cows classified as having puerperal metritis (--, n = 18), subclinical endometritis (…, n =43) or normal uterine health (__, n =22). Values shown are means ± SEM.

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