Associations of cytological endometritis with energy metabolism and inflammation during the periparturient period and early lactation in dairy cows

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ABSTRACT

Multiparous Holstein cows (n = 108) were used to determine the associations of cytological endometritis (CE) with plasma nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) as markers of energy metabolism, calculated energy balance (EB), and plasma haptoglobin (Hp) as a marker of inflammation during the periparturient period and early lactation. Evaluation of endometrial cytology by low-volume uterine lavage was conducted on 1 d between 40 and 60 d post-calving. The incidence of CE among cows sampled was 40%. The area under the curve (AUC) was calculated for both NEFA and BHBA using data collected from 3 wk before to 3 wk after parturition. Data for NEFA and BHBA AUC were stratified into prepartum (wk −3 to parturition) and postpartum (parturition to wk +3) for statistical analysis. Prepartum AUC for neither NEFA nor BHBA was associated with subsequent CE; however, cows that subsequently developed CE tended to have higher postpartum AUC for NEFA and had higher postpartum AUC for BHBA. Consistent with the results for plasma NEFA and BHBA, calculated EB during the prepartum period was not different in cows that did or did not develop CE; however, cows with CE had lower EB during the 6-wk postpartum period compared with cows without CE. Analysis of EB by week (wk −3 to −1 before calving and wk +1 to +6 postcalving) indicated that EB in cows with CE was lower at wk +1, +2, and +3 and tended to be lower at wk +6 than cows without CE. Plasma Hp concentrations were analyzed from wk +1 to +8 of lactation; concentrations of Hp were not different during either wk +1 or the entire postpartum period between cows that did or did not develop CE. These results suggest that lower energy status during the first 3 wk postpartum, but not necessarily systemic inflammation, is associated with subsequent development of CE.

Key words: endometritis, energy status, haptoglobin

INTRODUCTION

The demand for energy in dairy cows is highest in early and peak lactation (Butler and Smith, 1989) and even unusual postparturient increases in voluntary intake cannot satisfy this increased nutrient demand (Bell, 1995). Therefore, dairy cattle do not maintain positive energy balance (EB) during early lactation and hence mobilize body reserves (Butler and Smith, 1989). Furthermore, immunocompetence is decreased during the transition period (Mallard et al., 1998) and the incidence of mastitis, ketosis, digestive disorders, and laminitis is highest around parturition (Ingvartsen et al., 2003). A possibility exists that immunosuppression results from changes in hormones around parturition (Goff, 2008), which may contribute to the high incidence of diseases (Mallard et al., 1998). However, it is difficult to clarify the cause and effect of immune suppression resulting from changes in periparturient hormones because each hormone has positive or negative effects on the immune system (Ingvartsen et al., 2003) and shows different patterns around parturition (Bell, 1995; Ingvartsen and Andersen, 2000).

In addition to the potential effects of hormones, metabolites related to energy metabolism, such as NEFA and BHBA, are also associated with immunocompetence during the periparturient period. Indeed, it has been reported that negative EB status is associated with PMNL function (Hammon et al., 2006) and PMNL energy status (Galvão et al., 2010) in dairy cows. Several studies have suggested an association of uterine diseases with negative EB (Carson, 2009; Dubuc et al., 2010b; Giuliodori et al., 2013a). Among uterine diseases, cytological endometritis (CE) is defined by proportion of PMNL in samples collected by flushing the uterine lumen or by endometrial cytobrush (Dubuc et al., 2010a). Dubuc et al. (2010a) further
distinguished CE from endometritis in that the latter condition is distinct from CE and characterized by purulent vaginal discharge; a given cow may or may not have both conditions. A study conducted using 5 commercial farms showed that CE was highly prevalent and exerted a profoundly detrimental effect on subsequent reproductive performance, such as longer days open (Gilbert et al., 2005), which also was confirmed in larger-scale research with 38 herds (Cheong et al., 2011). Development or incidence of CE also was related to EB, as reflected by elevated NEFA and BHBA in blood (Hammon et al., 2006; Galvão et al., 2010) and development of postpartum ketosis (Cheong et al., 2011); however, more comprehensive associations of CE with calculated EB along with assessment of cumulative elevation of circulating NEFA and BHBA have not yet been elucidated.

Upon stimulation by the release of proinflammatory cytokines, such as IL-1, IL-6, and tumor necrosis factor α, from macrophages and monocytes at the site of inflammatory lesions or infection, hepatic acute-phase proteins (APP) are produced and released (Eckersall, 2000). An increase in the circulation of the cytokines stimulates further hepatic APP production (Eckersall, 2000). Haptoglobin (Hp) is a major APP in ruminants and is present in negligible circulating levels in normal animals, but increases over 100-fold on stimulation (Eckersall, 2000). Studies have suggested that increased blood Hp concentration is related to uterine diseases such as metritis (Huzzey et al., 2009; Chan et al., 2010; Dubuc et al., 2010b) and CE (Dubuc et al., 2010b). Fatty liver was reported to induce production of Hp (Murata et al., 2004); however, it has also been suggested that fatty liver may suppress the secretion of important immune proteins, especially APP (Ingvartsen et al., 2003). Collectively, production of Hp appears to be affected by inflammation and EB status during the periparturient period.

The purpose of the current study was to clarify associations of energy metabolism and inflammation with CE during the transition period and early lactation. We hypothesized that cows that developed CE experienced greater negative EB, as reflected by differences in calculated EB and cumulative increase in NEFA and BHBA concentrations along with higher inflammation compared with cows that did not develop CE.

**MATERIALS AND METHODS**

*Experimental Animals and Procedures*

A prospective cohort study was conducted to evaluate associations of CE with energy metabolism and inflammation. All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee (Ithaca, NY) before the onset of the experiment. Data from 108 Holstein cows entering second lactation or greater from the Cornell University Teaching and Research Center Dairy were used for this study. Cows were planned to be recruited to address the present hypotheses from 2 separate experiments (Yasui et al., 2012a,b) evaluating responses to trace mineral nutrition during the periparturient period. To achieve a type 1 error risk of 5% and power of 80%, we aimed to recruit 120 cows from these 2 studies based upon expected differences in circulating NEFA concentrations from previous studies. We anticipated that this sample size also would allow us to detect meaningful differences in circulating BHBA concentrations. Briefly, cows were housed in individual tie-stalls and fed the same prepartum and postpartum basal rations within each experiment. Cows received either various sources of Zn, Cu, or Mn (Yasui et al., 2012b) or Cr-propionate (Yasui et al., 2012a) by daily top-dress once daily beginning at 21 d before expected calving and continuing through either 84 d (Yasui et al., 2012b) or 63 d (Yasui et al., 2012a) postcalving. Cows were milked twice per day (0900 and 2100 h) and milk yields were recorded at all milkings during the postpartum period. Milk samples were collected from all milkings on 1 d per week. Samples were composited and stored at 4°C with a preservative (Bronopol tablet; D & F Control Systems Inc., San Ramon, CA) until analyzed (Dairy One Cooperative Inc., Ithaca, NY) within 24 h for fat, protein, lactose, TS, and MUN using mid-infrared analysis (AOAC International, 2000; method 972.160), and SCC by an optical fluorescent method (AOAC International, 2000; method 978.26). Amounts of feed offered and refused were recorded on a daily basis, and weekly samples of the TMR were analyzed for DM content by drying at 55°C until static weight; the subsequent weekly TMR DM values were used with the daily feed amounts to calculate DMI. Cows were weighed weekly throughout the experiment.

Plasma samples were obtained weekly (Yasui et al., 2012b) or 3 times per week (Yasui et al., 2012a) via coccygeal blood vessel puncture from 3 wk prepartum through 3 wk postpartum (wk −3 through wk +3) and analyzed for NEFA and BHBA. Plasma concentrations of NEFA and BHBA were analyzed by enzymatic analyses (NEFA-C; Wako Pure Chemical Industries, Osaka, Japan; and BHBA dehydrogenase; kit no. 310, Sigma Chemical, St. Louis, MO). Energy balance was calculated using NRC (2001) equations from wk −3 through wk +6 as follows:

\[
\text{EB} = (\text{DMI}) - (\text{DE})
\]

\[
\text{DE} = \text{Intake} \times \text{TS} \times \text{NDF}
\]

\[
\text{DMI} = \text{Intake} \times \text{TS} \times \text{NDF}
\]
Prepartum EB = (DMI × NE\textsubscript{L} diet) − [(BW\textsuperscript{0.73} × 0.08) + {[(2 × 0.00159 × days pregnant − 0.0353) × (calf BW/45)/0.14] × 0.64}];

Postpartum EB = (DMI × NE\textsubscript{L} diet) − {(BW\textsuperscript{0.75} × 0.08) + [(0.0929 × milk fat % + 0.0563 × milk true protein % + 0.0395 × milk lactose %) × milk yield]}.

Plasma samples collected once weekly from wk +1 through wk +8 also were analyzed for concentrations of Hp. Plasma concentrations of Hp were measured by enzymatic analysis (Phase Range Haptoglobin Assay; Tridelta Diagnostics Ltd., Morris Plains, NJ). All spectrophotometric measurements were conducted using a VersaMax tunable microplate reader (Molecular Devices LLC, Sunnyvale, CA).

Evaluation of endometrial cytology by low-volume lavage (Gilbert et al., 2005) was determined on all cows on 1 d between 40 and 60 d postcalving as previously described (Cheong et al., 2011). The perineum of the cow was cleansed and a 64-cm Flex Tip sterile plastic infusion pipette (Exodus Breeders Corp., York, PA) was manipulated through the cervix into the uterus. Sterile saline solution (20 mL) was injected into the uterus and agitated gently via the rectum; then, a sample of the fluid was aspirated. The recovered fluid was centrifuged using a cytocentrifuge directly onto a glass slide. After drying, the slides were fixed and stained using a rapid Romanowsky-type staining procedure and examined at 400× magnification. Two hundred cells were counted from each slide, and results were expressed as the percentage of PMNL in total cells (excluding erythrocytes). All the slides were read by the same investigator (T. Yasui). Incidence of CE was diagnosed with cut-off point of 10% PMNL (Cheong et al., 2011).

Statistical Analyses

We were interested in evaluating the relationships of cumulative increases in NEFA and BHBA during the periparturient period with subsequent development of CE. Therefore, the area under the curve (AUC) was calculated for both NEFA and BHBA concentrations using PROC EXPAND of SAS (version 9.1; SAS Institute, Cary, NC), using cubic spline interpolation and the trapezoidal rule. The AUC of NEFA and BHBA for each cow was subjected to ANOVA using PROC MIXED of SAS. Data for EB and plasma Hp were subjected to repeated-measures ANOVA using PROC MIXED of SAS and the REPEATED statement. Four covariance structures were tested: compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive and the covariance structure that resulted in the smallest Akaike information criterion was used. The degrees of freedom for PROC MIXED were estimated using the Kenward-Roger option in the model statement. Fixed effects included CE (yes or no), time (week or day only for REPEATED analysis), and the 2-way interaction between them (only for REPEATED analysis). The random effect was cow or cow nested within CE only for REPEATED analysis and the PDIF option was used to identify differences within individual weeks for the analysis of EB. Least squares means and standard error of the mean were reported. Statistical significance was declared at \( P < 0.05 \) and trends were discussed at 0.05 < \( P < 0.15 \).

RESULTS AND DISCUSSION

The incidence of CE among cows analyzed was 40%; 43 cows developed CE and 65 cows did not. This is slightly higher than the level in a review by Sheldon et al. (2009), who reported that about 30% of cattle have subclinical endometritis diagnosed at about 5 wk postpartum; however, the prevalence of CE among herds was reported to range from 37 to 74% in 5 herds, with an average of 53% (Gilbert et al., 2005) and 4.8 to 52.6% in 38 herds, with 25.9% as the average (Cheong et al., 2011). The incidence of CE in the current study appears to have been typical of that existing in commercial dairy farms. Furthermore, authors who specifically exclude cases with purulent vaginal exudate may underestimate the incidence of CE.

Results for the AUC for NEFA and BHBA during the prepartum and postpartum periods for cows that did or did not subsequently develop CE are reported in Table 1. Prepartum AUC for neither NEFA (1,518 vs. 1,374 \( \mu \text{Eq/L} \times d; P = 0.21 \)) nor BHBA (39 vs. 38 mg/dL \( \times d; P = 0.81 \)) was different for cows that subsequently developed CE. However, postpartum AUC for NEFA (5,391 vs. 4,427 \( \mu \text{Eq/L} \times d; P = 0.11 \)) tended to be increased and postpartum AUC for BHBA was increased (72.0 vs. 58.9 \( \mu \text{Eq/L} \times d; P = 0.049 \)) for cows that subsequently developed CE (Table 1). Consistent with these results, prepartum EB was not associated with subsequent development of CE (\( P = 0.61 \)); however, cows that developed CE had lower (\( P = 0.02 \)) postpartum EB than those that did not develop CE (−3.8 vs −1.9 Mcal/d; Table 2). Further analysis of EB within individual weeks showed that the EB in cows with CE was lower at wk +1 (−8.1 vs. −4.9 Mcal/d; \( P = 0.01 \)), wk +2 (−7.9 vs. −5.5 Mcal/d; \( P = 0.04 \)), and wk +3 (−4.9 vs. −2.7 Mcal/d; \( P = 0.048 \)) and tended to be
lower at wk +6 (0.9 vs. 2.5 Mcal/d; \( P = 0.10 \)) than cows without CE (Figure 1).

Given the lack of association of prepartum variables, particularly plasma NEFA that has been associated previously (Hammon et al., 2006) with endometritis, a discussion of sample size is warranted. Data for circulating concentrations of NEFA and BHBA from studies in our laboratory (Piepenbrink and Overton, 2003a,c; Piepenbrink et al., 2004) were used previously to calculate AUC values for both NEFA and BHBA during the periparturient period and correlated with liver composition and in vitro liver metabolism (Piepenbrink and Overton, 2003b). Based upon the means and standard deviations of these values, sample size estimations suggested that approximately 40 cows per group would be required to detect a difference of 25% between groups for postpartum BHBA and approximately 60 cows per group would be required to detect a difference between groups of 25% for prepartum NEFA. Originally, 121 cows were enrolled in the 2 studies from which these data were obtained; however, several cows were removed from the data set because of severe health programs at parturition or difficulty with obtaining uterine lavage. Therefore, data from 108 cows were available for this analysis. In the Hammon et al. (2006) study, cows that developed subclinical endometritis had NEFA concentrations ranging from 40 to 80% greater than healthy cows. In our study, the magnitude of the difference between groups for prepartum NEFA was about 10%, suggesting that the sample size was significantly underpowered during the prepartum period to detect these smaller differences in NEFA than those reported previously.

Our findings during the postpartum period were consistent with other previous studies, which showed that cows with CE had elevated plasma NEFA or BHBA concentrations during early lactation (Hammon et al., 2006; Dubuc et al., 2010b; Galvão et al., 2010). However, this was the first study to show that CE is most closely associated only with postpartum EB. Because Galvão et al. (2010) showed that plasma BHBA was higher at calving and lower at 14 DIM in cows with metritis than those with CE and that glycogen content in blood PMNL was lower at calving in cows with metritis than those with CE, it can be speculated that uterine diseases except for CE are associated with

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**Table 1.** Calculated area under the curve (AUC) for plasma NEFA and BHBA concentrations during the periparturient period for cows categorized as either negative or positive for cytological endometritis (CE)

<table>
<thead>
<tr>
<th>Item</th>
<th>CE</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>65</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>NEFA AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk −3 to −1</td>
<td>1,374</td>
<td>1,518</td>
<td>0.21</td>
</tr>
<tr>
<td>wk +1 to +3</td>
<td>4,427</td>
<td>5,391</td>
<td>0.11</td>
</tr>
<tr>
<td>BHBA AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk −3 to −1</td>
<td>38</td>
<td>39</td>
<td>0.81</td>
</tr>
<tr>
<td>wk +1 to +3</td>
<td>59</td>
<td>72</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1− = cows without CE; + = cows with CE.  
2AUC for 3 wk before parturition.  
3AUC for 3 wk after parturition.

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**Table 2.** Energy balance (EB) during the 3-wk prepartum period and 3-wk postpartum period for cows categorized as either negative or positive for cytological endometritis (CE)

<table>
<thead>
<tr>
<th>Item</th>
<th>CE</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>n</td>
<td>65</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk −3 to −1</td>
<td>8.6</td>
<td>9.0</td>
<td>0.6</td>
</tr>
<tr>
<td>wk +1 to +3</td>
<td>−1.9</td>
<td>−3.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

1− = cows without CE; + = cows with CE.  
2Wk = week effect.  
3Energy balance during 3 wk before parturition, calculated according to NRC (2001): \( EB = (DMI \times NE_{\text{diet}}) - [(BW^{0.75} \times 0.08) + \{(2 \times 0.00159 \times \text{days pregnant} - 0.0353) \times (\text{calf BW/45}) \times 0.14) \times 0.64]}\).  
4Energy balance during 3 wk after parturition, calculated according to NRC (2001): \( EB = (DMI \times NE_{\text{diet}}) - \{(BW^{0.75} \times 0.08) + [(0.0929 \times \text{milk fat %}) + 0.0563 \times \text{milk true protein %}) + 0.0395 \times \text{milk lactose %}) \times \text{milk yield}]}\).
prepartum energy deficit and that a later energy deficiency is linked to CE. Indeed, Carson (2009) and Dubuc et al. (2010b) determined that elevated prepartum NEFA concentrations were positively associated with development of metritis, whereas the concentrations of postpartum NEFA were not after accounting for BHBA values. Cheong et al. (2011) found that cows that developed postpartum ketosis had 3.8 times higher odds of having CE than cows that did not.

However, other studies showed mixed results for associations of uterine diseases with energy status. A recent study reported that increased prepartum NEFA and postpartum BHBA concentrations, but not NEFA concentration during the immediate postpartum period, were associated with development of clinical endometritis (Giuliodori et al., 2013a). Furthermore, Giuliodori et al. (2013b) reported that cows with metritis had higher blood BHBA concentrations than healthy cows immediately after parturition; however, neither prepartum BHBA and NEFA nor NEFA concentrations immediately postpartum were associated with the development of metritis. Hammon et al. (2006) reported that cows with metritis had increased NEFA concentrations for both prepartum and postpartum periods and had increased BHBA concentrations after parturition compared with healthy cows.

Because increased liver triglyceride in the first and second week after calving is associated with decreased functional capacities of PMNL derived from the blood and uterus (Zerbe et al., 2000), the extent of body fat deposition before calving may be another factor to affect uterine immunity. This may explain why cows that are fat at calving have a higher incidence of infectious diseases such as endometritis (Zerbe et al., 2000). However, Reist et al. (2000) showed that cows with the same moderate BCS before and at calving had different metabolic responses such that serum and milk BHBA concentrations during the first 6 wk of lactation, but not before calving, were higher in cows with late-onset ovulation than in cows with early-onset ovulation, suggesting that other metabolic or endocrine factors can cause higher secretion of energy-related metabolites after calving. β-Hydroxybutyrate is known to suppress the function and proliferation of leukocytes (Hoeben et al., 1997, 1999). Sheldon et al. (2009) suggested that postpartum reductions in neutrophil function are most marked in high-producing dairy cows that have compromised energy metabolism after parturition, which may predispose cows to the establishment of uterine disease. Nevertheless, it is reported that cows with metritis and mastitis had lower PMNL function during the prepartum period (Cai et al., 1994). Although our
findings support the idea that development of CE is associated only with postpartum energy status, further studies are needed to elucidate how the stage, duration, and severity of negative EB affects subsequent uterine immunity.

Plasma Hp concentrations during the first 8 wk postpartum were not associated with subsequent development of CE (Table 3). Analysis of samples at 1 wk postpartum also found no relationship between subsequent CE and the concentration of plasma Hp (Table 3). Increased Hp is known to be a marker of inflammation in the week after calving in dairy cows (Humblet et al., 2006). Association of Hp with uterine diseases also has been reported. Chan et al. (2010) showed that cows that developed metritis had higher concentration of blood Hp for 6 mo postpartum than healthy cows. Huzzey et al. (2009) and Dubuc et al. (2010b) reported that high levels of Hp in blood within the first week postpartum were associated with development of metritis and Dubuc et al. (2010b) further reported associations of Hp during the immediate postpartum period with CE. Therefore, we expected that plasma Hp concentration would be higher during early lactation or right after parturition in cows that developed CE than cows that did not. Other production diseases during the transition period, such as ruminal acidosis, which causes inflammatory response (Mulligan and Doherty, 2008), might have been confounding factors for values of plasma Hp because blood Hp concentration is known to be increased in ruminal acidosis conditions (Jacobsen et al., 2004; Khafipour et al., 2009). Further, each APP has a different extent of response to the same inflammatory stimulation (Jacobsen et al., 2004) and has a different pattern of response according to the stage of inflammation (Humblet et al., 2006). Therefore, consideration of other health problems and various APP may be needed to investigate the association of CE with the inflammatory response. On the other hand, Hp is known to be induced in cows with fatty liver syndrome (Murata et al., 2004; Guzelbektes et al., 2010). Uchida et al. (1993) reported that the detection rate of serum HP was significantly higher at parturition compared with before and after parturition, and the detection was in concurrence with higher cortisol and NEFA in blood. Also, Hiss et al. (2009) showed that blood NEFA sampled at 2 wk postpartum and blood BHBA sampled at 1 wk prepartum were associated with greater concentration of Hp in serum and milk during the prepartum through early lactation period. However, our analysis did not find postpartum concurrent change in plasma Hp concentration with plasma NEFA or BHBA concentration. Huzzey et al. (2011) reported that prepartum NEFA and Hp concentration in plasma was positively correlated, but the correlation coefficient was low. Those authors pointed out that NEFA, cortisol, and Hp are interrelated but these relationships are complex and vary depending on the physiological status of the cow. Therefore, the non-association of Hp with energy metabolism in the current analysis may result from the correlations among energy metabolism, endocrine system, and inflammatory response.

**CONCLUSIONS**

Prepartum AUC for NEFA and BHBA were not different between cows that did or did not subsequently develop CE in this study; however, postpartum AUC for NEFA tended to be increased and for BHBA was increased for cows that subsequently developed CE. Consistent with the results for plasma NEFA and BHBA concentration, calculated EB during the prepartum period was not different in cows that did or did not develop CE; however, cows with CE had lower EB during the 6-wk postpartum period compared with cows without CE. Analysis of EB by week indicated that EB in cows with CE was lower at wk +1, +2, and +3 and tended to be lower at wk +6 than cows without CE. Concentrations of plasma Hp were not different during either wk +1 or the first 8-wk lactation period between cows that did or did not develop CE. These results sug-

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**Table 3.** Plasma haptoglobin (Hp) during the 8-wk postpartum period for cows categorized as either negative or positive for cytological endometritis (CE)

<table>
<thead>
<tr>
<th>Item</th>
<th>−</th>
<th>+</th>
<th>SEM</th>
<th>CE</th>
<th>Wk&lt;sup&gt;2&lt;/sup&gt;</th>
<th>CE × Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>65</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hp, mg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk +1 to +8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.78</td>
<td>0.73</td>
<td>0.05</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>wk +1 only&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.98</td>
<td>0.99</td>
<td>0.09</td>
<td>0.95</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>1</sup>= cows without CE; + = cows with CE.

<sup>2</sup>Wk = week effect.

<sup>3</sup>Represents plasma samples collected weekly from 1 wk postpartum through 8 wk postpartum.

<sup>4</sup>Represents plasma samples collected from 1 wk postpartum only.
gest that decreased energy status during the first 3 wk postpartum, but not elevated systemic inflammation, is associated with subsequent development of CE.

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