Prepartum plane of energy intake affects serum biomarkers for inflammation and liver function during the periparturient period

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ABSTRACT

Serum collected from multiparous Holsteins (n = 73) in a previous experiment was used to determine the effect of prepartum plane of energy intake on metabolites related to inflammation and liver function in periparturient cows. Prepartum diets were in a 3 × 2 factorial arrangement over the far-off (d −65 to −26 before expected parturition) and close-up (d −25 relative to parturition until parturition) dry periods. During the far-off period, 2 diets were fed for ad libitum intake (ADLIB) to supply either 100% (100E) or 150% (150E) of National Research Council recommendations for net energy for lactation for mature cows in late gestation. For the third dietary far-off treatment, the 150E diet was fed at restricted intake (REST) to supply 80% (80E) of recommendations. During the close-up period, cows were fed a diet either at ADLIB or REST to supply 150% of net energy for lactation or 80% of net energy for lactation requirements, respectively. Beginning at parturition, all cows were fed a lactation diet through 56 d in milk. Cows fed 150E tended to accumulate more liver lipid postpartum; peak lipid accumulation occurred in all groups on d 14 postpartum. Cows fed 150E tended to have lower serum Ca on d 1 after calving, and cows fed REST had higher Ca than those fed ADLIB. Cows fed 150E tended to have higher serum bilirubin prepartum compared with other groups. Feeding REST in the close-up period resulted in higher bilirubin prepartum compared with ADLIB; bilirubin was positively associated (r = 0.34) with lipid accumulation postpartum. Feeding REST resulted in lower serum vitamin A (an indirect measure of retinol binding protein) prepartum compared with ADLIB, especially when coupled with 80E. Postpartum paraoxonase was negatively associated (r = −0.27) and ceruloplasmin was positively associated (r = 0.21) with liver lipid accumulation postpartum. A larger spike in haptoglobin was observed on d 1 and 7 postpartum for cows fed 100E and 150E during the far-off period followed by REST during the close-up period. The ratio of albumin to globulin in serum was higher for cows fed 100E than for those fed 150E both prepartum and postpartum. Liver activity index indicated poorer liver function for cows fed 150E in the far-off period regardless of close-up diet. Negative liver function was also noted for cows fed 80E and REST. Cows in the 100E group fed ADLIB or REST had positive liver activity index postpartum. Avoidance of gross overconsumption of energy prepartum, particularly during the far-off period, appears best to reduce systemic inflammatory signals and improve liver function. 

Key words: inflammation, liver, acute phase proteins, transition period

INTRODUCTION

Optimal liver function is crucial to the success of dairy cows in transitioning from the dry period to early lactation. During the periparturient period, cows enter a state of negative energy balance and mobilize body stores from adipose tissue to support lactation. Non-esterified fatty acids (NEFA) released from adipose tissue are processed by the liver and either oxidized as fuel or esterified to triacylglycerol (TAG) before being released as very low density lipoproteins (VLDL). Because the release of VLDL from ruminant liver is much slower compared with other species, when the rate of esterification to TAG exceeds the rates of VLDL export and NEFA oxidation, cows are susceptible to fatty liver syndrome (Bobe et al., 2004). Fatty liver reduces liver function and decreases its ability to perform gluconeogenesis (Cadorniga-Valino et al., 1997; Strang et al., 1988; Bobe et al., 2003), to detoxify ammonia (Överton et al., 1999; Zhu et al., 2000), and to eliminate toxins (Rukkwamsuk et al., 1999a).
Activation of the immune system and the resultant inflammation may underlie several periparturient health problems (Drackley, 1999; Drackley et al., 2001). In rodents, fatty liver has been linked to inflammation (Cai et al., 2005; Reddy and Sambasiva Rao, 2006). In dairy cattle with naturally occurring fatty liver, increased production of positive acute phase proteins such as haptoglobin, serum amyloid A, and ceruloplasmin and reduction of negative acute phase proteins such as albumins, paraoxonase, and retinol binding protein in early lactation have been reported (Bertoni et al., 1997; Bertoni et al., 2001; Katoh, 2002; Turk et al., 2004; Ametaj, 2005). Activation of the immune system and its inflammatory sequelae have been postulated to be causative for a variety of metabolic disorders and infectious diseases during the transition period (Bertoni et al., 2008; Bradford et al., 2015; Horst et al., 2021). It is not known how plane of nutrition prepartum might affect the production of an inflammatory response and the associated acute phase proteins. A solid connection between liver lipid accumulation postpartum and inflammation postpartum has not been well established in dairy cattle, although Bertoni et al. (2006) showed that inflammation could have a role in liver lipodosis occurrence in dairy cows. If dry period plane of nutrition and inflammation are linked, more might be done nutritionally to ensure optimal liver function before parturition. Thus, identification of these mechanisms will help achieve better health of the cow during the periparturient period.

Our hypothesis was that overfeeding energy in the dry period would increase inflammation and be detrimental to liver function compared with feeding to requirements or moderate feed restriction. The objective of this study was to determine the impact of energy intake during the dry period on biomarkers in blood related to the acute phase response and liver function during the transition period. As part of this experiment, previously collected production, health, and metabolite data from Dann et al. (2006) and Litherland et al. (2011) were used to determine how metabolites analyzed in the present study related to prepartum energy intake, health, and liver functionality.

**MATERIALS AND METHODS**

**Animal Management**

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee. Complete details of experimental design and animal management can be found in Dann et al. (2006). Briefly, as depicted in Figure 1, dietary treatments were applied in a 3 × 2 factorial arrangement to encompass the far-off and close-up dry periods. During the far-off period (d −65 to −26 d before expected parturition), cows consumed 1 of 3 levels of energy (Table 1). Two diets were fed for ad libitum intake (ADLIB) to supply either 100% (100E) or >150% (150E) of NRC (2001) recommendations for NEI for mature cows in late gestation. For the third dietary far-off treatment, the 150E diet was fed at restricted intake (REST) to supply 80% (80E) of NRC (2001) recommendations for NEI. During the close-up period (d −25 relative to parturition until parturition), half of the cows in each group were fed a diet formulated to meet or exceed NRC (2001) nutrient recommendations either for ADLIB or at REST to supply 150% of NEI or 80% of NEI requirements, respectively. Starting after parturition, all cows were fed a lactation diet for ad libitum DMI through d 56 of lactation.

**Measurements, Sampling, and Analyses**

Serum samples from 73 multiparous Holstein cows from Dann et al. (2006) taken before the a.m. feeding on d −30, −21, −14, −7, −1, 1, 7, 14, 21, and 28 relative to parturition were analyzed for metabolites related to the acute phase response and liver health and function. Samples were obtained ± 1 d of the target days. Samples were stored at −20°C for approximately 2 yr before analysis. Complete details of the analytical methods have been described by Bionaz et al. (2007). Blood metabolites were analyzed at 37°C in a clinical autoanalyzer (ILAB 600, Instrumentation Laboratory). Total protein, albumin, total cholesterol, total bilirubin, creatinine, urea, Ca, P, Mg, aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT) were determined using commercial kits (Instrumentation Laboratory, IL Test). Globulin was calculated as the difference between total protein and albumin. Zinc was determined by a commercial kit (Wako Chemicals GmbH). Haptoglobin and ceruloplasmin were analyzed using methods described by Calamari et al. (2016). Vitamins A and E were analyzed by reverse phase HPLC with a UV detector at 325 nm for vitamin A or 290 nm for vitamin E.

Liver activity index (LAI) was used by Bertoni et al. (2001) to determine how well the liver is functioning postpartum relative to others in the same group of cows. A description of the LAI calculation is presented in Bertoni and Trevisi (2013). In this calculation, serum cholesterol, albumin, and vitamin A concentrations at 7, 14, 21, and 28 DIM are considered. To calculate the index, the mean (x) and standard deviation (σ) for each of these metabolites was calculated across all cows.
in the experiment at 7, 14, 21, and 28 DIM. Then, on an individual cow basis, LAI was calculated for each of the DIM above. Individual indexes for each DIM were then summed together for an overall LAI for each cow. An example for LAI calculation at 7 DIM (DIM7) for an individual cow follows:

\[
\text{LAI}_{\text{DIM7}} = \left( \frac{\text{VitA}_{\text{DIM7}} - \bar{x}_{\text{VitA}}}{\sigma_{\text{VitA}}} \right) + \left[ \frac{\text{Alb}_{\text{DIM7}} - \bar{x}_{\text{Alb}}}{\sigma_{\text{Alb}}} \right] + \left[ \frac{\text{Chol}_{\text{DIM7}} - \bar{x}_{\text{Chol}}}{\sigma_{\text{Chol}}} \right],
\]

where \( \bar{x} \) = the group average concentration for each blood metabolite; \( \sigma \) = the group standard deviation for each blood metabolite; VitA = vitamin A concentration for the individual at 7 DIM; Alb = albumin concentration for the individual at 7 DIM; Chol = cholesterol concentration for the individual at 7 DIM. This calculation is repeated using this cow’s metabolite values at 14, 21, and 28 DIM. The summative equation for an individual cow’s LAI (LAI_{cow}) can be represented by the following:

\[
\text{LAI}_{\text{cow}} = \text{LAI}_{\text{DIM7}} + \text{LAI}_{\text{DIM14}} + \text{LAI}_{\text{DIM21}} + \text{LAI}_{\text{DIM28}}.
\]

The present study used 73 of the original 74 cows used by Dann et al. (2006); therefore, DMI, milk yield, energy balance, BCS, BCS change, liver composition, and health data published by those authors was used to reference production characteristics. The cow included in the original study, but not in the current study, was from the group fed 150E during the far-off period and ADLIB during the close-up period and was excluded for missing sample values. Serum glucose, insulin, NEFA, and BHB from Dann et al. (2006) were also used; however, time points used for reporting blood metabolites were narrowed to time points used in the present analyses. Previously collected in vitro data for fatty acid metabolism of liver slices collected on d −30, −14, 1, 14, and 28 relative to parturition as described by Litherland et al. (2011) were used as an indication of liver function.

Figure 1. Schematic of the 3 × 2 factorial arrangement of dietary treatments used by Dann et al. (2006). References to percent of NEL correspond to NEL intake relative to the NRC (2001) requirement for mature cows in late gestation.

Table 1. Experimental diets (% of DM unless otherwise indicated) fed to cows during the dry and lactation period

<table>
<thead>
<tr>
<th>Item</th>
<th>Far-off²</th>
<th>Close-up</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>100E</td>
<td>80E and 150E</td>
<td>100E</td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>41.68</td>
<td>26.24</td>
<td>25.14</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>—</td>
<td>13.97</td>
<td>13.40</td>
</tr>
<tr>
<td>Corn silage</td>
<td>21.10</td>
<td>25.52</td>
<td>24.49</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>26.15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cottoneed</td>
<td>11.07</td>
<td>29.88</td>
<td>32.79</td>
</tr>
<tr>
<td>Grain mix³</td>
<td>15.8</td>
<td>16.5</td>
<td>15.7</td>
</tr>
<tr>
<td>Chemical</td>
<td>31.8</td>
<td>26.1</td>
<td>25.6</td>
</tr>
<tr>
<td>CP</td>
<td>46.5</td>
<td>38.1</td>
<td>36.9</td>
</tr>
<tr>
<td>NE₄, Mcal/kg</td>
<td>1.30</td>
<td>1.59</td>
<td>1.61</td>
</tr>
</tbody>
</table>

¹Complete detail can be found in Dann et al. (2006). Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before expected parturition until parturition. The lactation diet was fed to all cows from parturition through 56 DIM.
²Far-off treatments: 100E = cows fed to meet NRC (2001) requirements for energy during the dry period; 80E = cows restricted to 80% of NRC (2001) requirement for energy; and 150E = cows fed to consume ≥150% of NRC (2001) requirement for energy.
³For complete composition, refer to Dann et al. (2006). Grain mixes based on ground corn grain, soybean meal, expeller soybean meal, soybean hulls, and vitamin and mineral mixes.
Statistical Analyses

Data for blood metabolites from Dann et al. (2006) and blood metabolites measured in the present study were analyzed separately for the pre- and postpartum periods. Cow was the experimental unit. To determine the effects of far-off and close-up treatments and their interaction, data were analyzed as a randomized design using the MIXED procedure of SAS (SAS Institute Inc.) with the following model:

\[ y_{ijkl} = \mu + D_i + F_j + DF_{ij} + C_k + DC_{ik} \]
\[ + FC_{jk} + DFC_{ijk} + A_{(ijkl)}, \]

where \( y_{ijkl} \) = an observation from the ith day relative to calving, jth far-off treatment, kth close-up treatment and lth cow; \( \mu \) = the grand mean; \( D_i \) = effect of the ith day; \( F_j \) = effect of the jth far-off treatment; \( DF_{ij} \) = effect of the day by far-off treatment interaction; \( C_k \) = effect of the kth close-up treatment; \( DC_{ik} \) = effect of the day by close-up treatment interaction; \( FC_{jk} \) = effect of the day by far-off treatment by close-up treatment interaction; \( DFC_{ijk} \) = effect of the day by far-off treatment by close-up treatment interaction by day; and \( A_{(ijkl)} \) = random experimental error from the ith cow nested within the ith day, jth far-off treatment, and kth close-up treatment. Model residuals were examined for non-normal distribution and heteroscedasticity. No variables required transformation.

The REPEATED statement was used for days. The random error term used for all mixed models was cow within far-off and close-up treatment, and the covariance structure yielding the lowest Akaike’s information criterion was used (Littell et al., 1998). Covariance structures tested included unstructured, compound symmetry, autoregressive order 1, and autoregressive heterogeneous order 1. Using this methodology, an autoregressive covariance structure best fit the data for all variables. Degrees of freedom were estimated by using the Satterthwaite option in the model statement. When significant interactions with treatment occurred, linear contrast statements were constructed to explore them. Liver activity index was analyzed as a randomized design using the MIXED procedure of SAS (SAS Institute Inc.); however, the REPEATED statement was not used as this was a single calculation for each cow. Therefore, only the main effects of far-off and close-up treatment and their interaction were evaluated. The CORR procedure in SAS was used to evaluate the relationship of inflammation and liver function variables with liver lipid accumulation postpartum. Because greatest lipid accumulation occurred on d 14 postpartum for all groups, this time point was used together with blood variables measured over the 14 d pre- and postpartum. Pearson correlations were reported in the discussion when significant \( (P < 0.05) \) relationships were found. Significance was declared at \( P \leq 0.05 \) and tendencies or trends in all data were declared at \( 0.05 < P \leq 0.10 \).

RESULTS

Intake, Energy Balance, BCS, Milk Yield, Liver, and Health

Data were reported in Dann et al. (2006) but are summarized here for context. As designed, cows consumed different amounts of DM and were in different states of energy balance during the far-off and close-up dry periods. Body condition score was greater for 150E cows compared with 100E or 80E cows in the far-off period, as these cows gained condition during this time. This difference in BCS was also found in the close-up period, regardless of close-up period intake, but no significant gain or loss of BCS was observed among far-off and close-up group combinations.

In the first 10 d postpartum, energy balance was highest for cows fed 80E and lowest for 150E. Energy balance for 100E was not different from either group during this time. Also during the first 10 DIM, DMI was numerically lower \((1.7 \, \text{kg/d})\) for 150E cows than for either 100E or 80E cows. Numerical differences were observed for milk yield during this time, and cows in the 100E group had nearly 3.5 kg/d greater milk yield than either 150E or 80E. The difference in milk yield carried through the 8 wk of lactation studied, whereas differences in DMI and energy balance were greatly diminished beyond 10 DIM. Despite lower milk yield \((P = 0.12)\), cows fed 150E, especially those fed ADLIB in the close-up period, produced milk with a higher percent fat as a result of their negative energy balance and mobilization of body stores in response to their lower DMI postpartum.

Differences in liver composition were not noted for the dry period, but cows in the 150E REST group tended to have greater total lipid and TAG contents and lower glycogen concentration in their liver compared with other treatment combinations on d −14 relative to parturition. Total liver lipid and TAG for cows in the 100E and 150E groups fed ADLIB in the close-up period tended to be greater on d 1 postpartum compared with other far-off and close-up dietary treatment combinations. No further differences beyond d 1 were noted; however, for all cows, liver lipid and TAG was greatest in samples collected at d 14 postpartum. With the exception of individual cows, TAG accumulation postpartum did not classify any of the groups as having severe fatty liver (Bobe et al., 2004).
Data for in vitro metabolism of palmitate by liver slices were reported by Litherland et al. (2011). Briefly, in vitro rate of palmitate conversion to CO₂ by liver slices was affected by the interaction of day by far-off diet \((P = 0.01)\) where CO₂ production was lower for 150E cows relative to 80E or 100E cows on d 1 postpartum \((P = 0.02)\). The rate of palmitate conversion to acid soluble products (ASP) tended to be affected by far-off diet \((P = 0.08)\). On d 1 postpartum, rate of palmitate conversion to ASP was lower for 150E cows compared with 100E cows \((P = 0.04)\) and tended to be lower than 80E cows \((P = 0.06)\). Conversion of palmitate to esterified products was not affected by prepartum diet, but was higher in all groups postpartum \((day, P < 0.001)\). Despite no significant interactions of diet and day, postpartum production of esterified products was lower for 80E cows compared with 150E cows postpartum \((P = 0.05)\), especially on d 14 \((P = 0.04)\).

Health issues were tabulated in Dann et al. (2006). The number of cows treated for subclinical ketosis in the 150E group was higher compared with 100E or 80E, however, the effect of far-off diet was not significant. The same was true for the incidence of retained fetal membranes and other diseases; nevertheless, the total number of health disorders recorded for 150E \((56)\) was greater \((P < 0.05)\) than either 80E \((37)\) or 100E \((29)\).

**Blood Serum Metabolites**

**Glucose and Insulin.** Glucose in serum prepartum was not affected by far-off diet, but cows fed ADLIB in the close-up period had greater serum glucose than those fed REST \((P = 0.01; Figure 2A)\). This was especially evident during the last week before parturition \((close-up \times day, P = 0.04)\). Glucose declined sharply postpartum \((day, P < 0.001)\), then increased again by d 14 postpartum for all groups. Cows fed ADLIB tended to have greater glucose compared with REST, but far-off diet did not affect glucose postpartum \((P = 0.71)\). Serum insulin was affected by far-off diet \((P = 0.05; Figure 2B)\) and close-up diet \((P = 0.002)\) prepartum as cows in the ADLIB group had greater insulin compared with REST. Additionally, cows in the 150E group had greater serum insulin compared with either 100E or 80E \((P = 0.04)\). These effects were most pronounced in the last 2 wk before parturition \((far-off \times close-up \times day, P = 0.01)\). Postpartum, dietary effects were largely absent. The effect of close-up diet \((P = 0.05)\) was driven by higher insulin on d 14 postpartum for 100E cows compared with other groups. The glucose to insulin ratio \((Figure 2C)\) was lower prepartum for cows fed 150E and was higher for cows fed REST during the close-up period. Postpartum, the ratio tended \((P = 0.07)\) to be greater for cows fed REST during the close-up period.

**Nonesterified Fatty Acids and BHB.** The effect of close-up diet on NEFA prepartum was clearly evident during the last 2 wk of gestation \((close-up \times day, P = 0.001; Figure 3A)\). Cows in the 150E group tended to have greater NEFA prepartum compared with 100E cows \((P = 0.06)\), especially those fed REST in the close-up period. Nonesterified fatty acids spiked for all groups on d 1 postpartum. Far-off diet affected NEFA postpartum \((P = 0.05)\), whereas the effect of close-up diet was no longer evident \((P = 0.75)\). During this time, 150E cows had greater NEFA compared with 80E cows \((P = 0.02)\), and 100E cows tended to have greater NEFA compared with 80E cows \((P = 0.08)\). The effect of prepartum diets on BHB was absent postpartum \((P \geq 0.10; Figure 3B)\). However, 150E REST cows had greater BHB during the last week before parturition compared with other groups \((P = 0.04)\). Postpartum, BHB was greater for 150E cows compared with 80E cows \((P = 0.03)\) but was not different from 100E cows \((P = 0.12)\). Cows in the 100E ADLIB group had a greater spike in BHB postpartum than those in the 100E REST group, whereas those in the 150E group had greater BHB and those in the 80E group had lower BHB immediately postpartum regardless of close-up diet \((far-off \times close-up \times day, P = 0.03)\).

**Calcium.** Both far-off and close-up period feeding interacted with day relative to parturition to affect serum Ca prepartum \((P = 0.03; Figure 4)\). Cows in the 80E group had higher serum Ca than cows fed 150E \((P = 0.04)\). On d −1 prepartum, serum Ca decreased for all groups, but the decrease was more pronounced for cows fed 150E and either ADLIB or REST in the close-up period or 100E in the far-off period followed by REST in the close-up period. Three of the cows in the 150E group developed clinical signs of hypocalcemia and were treated \((Dann et al., 2006)\); however, group means on d 1 postpartum were not indicative of Ca concentrations observed in clinical cases of hypocalcemia \((Goff and Stabel, 1990)\). A large drop in serum Ca was observed on d 1 postpartum, followed by an increase and more steady concentration by d 7 postpartum and thereafter \((day, P < 0.001)\). Cows in the 100E group had higher serum Ca postpartum compared with those fed 150E \((P = 0.03)\). Similarly, cows fed 80E tended to have higher serum Ca postpartum compared with 150E \((P = 0.08)\). Cows fed REST in the close-up period took longer to increase serum Ca postpartum and, with the exception of 150E REST cows, serum Ca remained lower over the period studied for REST-fed cows compared with ADLIB groups \((close-up \times day, P < 0.001)\).
Creatinine and Bilirubin. Far-off and close-up diets had large effects on serum creatinine prepartum ($P < 0.001$; Figure 5A) with no far-off by close-up interaction. These main effects interacted with day ($P \leq 0.002$). Cows fed 150E prepartum had lower serum creatinine compared with those fed 100E or 80E ($P \leq 0.05$), and cows fed 80E had greater serum creatinine than 100E cows ($P = 0.02$). As parturition approached, creatinine concentration converged on d $-7$ prepartum for all 3 groups, then slowly began to rise as parturition approached. Cows fed REST had higher creatinine prepartum, which steadily increased until parturition compared with ADLIB cows that showed only a small decrease in concentration through d $-7$ prepartum (Figure 5B). Postpartum, the effects of far-off and close-up diets were absent, but REST cows experienced
a more rapid decrease in creatinine during the first week postpartum than ADLIB cows (close-up × day, \( P = 0.01 \)). Bilirubin also tended to increase steadily prepartum for all far-off groups (Figure 5C). This was especially true for cows fed 150E in the far-off period; however, no significant differences were observed among far-off groups immediately prepartum (\( P \geq 0.11 \)). Close-up diet also had a large effect on serum bilirubin concentration prepartum, as cows fed REST had higher bilirubin than those fed ADLIB (\( P < 0.001 \); Figure 5D). In both groups, bilirubin increased as parturition approached, though this increase was more pronounced in cows fed REST (close-up × day, \( P < 0.001 \); Figure 5D). Postpartum, serum bilirubin peaked sharply on d 1 and then steadily declined for all groups (day,
Bilirubin was higher for 150E cows than for 80E cows postpartum \((P < 0.001)\). No effect of close-up diet was observed for serum bilirubin postpartum \((P = 0.96)\).

*Paraoxanase and Vitamin A.* Serum paraoxonase was affected by far-off and close-up feeding prepartum \((P \leq 0.05; \text{Figure 6A})\). Cows fed 150E had lower paraoxonase compared with either 100E or 80E prepartum \((P \leq 0.02)\). Cows fed ADLIB had higher paraoxonase compared with REST prepartum \((P = 0.05)\). Serum paraoxonase was lowest for all groups on d 1 postpartum and began to increase as lactation progressed \((day, P < 0.001)\). Dry period feeding did not affect paraoxonase concentration postpartum \((P \geq 0.49)\).

Serum vitamin A concentration was higher for cows fed ADLIB during the close-up period compared with cows fed REST \((P = 0.03; \text{Figure 6B})\). All groups tended to have lower serum vitamin A as parturition approached, but these changes interacted with dry period feeding strategy. Serum vitamin A tended to be lower for cows fed 80E than for those fed 100E prepartum \((P = 0.10)\). Prepartum, cows in the 80E and 100E groups had a smaller change in vitamin A than 150E cows \((far-off \times day, P = 0.01)\). Cows fed REST in the close-up period had lower serum vitamin A, which tended to decrease more rapidly prepartum compared with ADLIB \((close-up \times day, P = 0.03)\). Postpartum serum vitamin A was not affected by prepartum feeding strategy \((P \geq 0.50)\) and tended to
increase steadily as lactation progressed for all groups (day, $P < 0.001$).

**Ceruloplasmin and Haptoglobin.** A 3-way interaction affected ceruloplasmin prepartum ($P = 0.02$; Figure 7A), where from d $-30$ to $-14$ prepartum, ADLIB cows tended to have decreased ceruloplasmin compared with REST. With the exception of 80E REST cows, which were higher, groups were very similar in the 7 d before parturition. Postpartum, a gradual increase in ceruloplasmin was observed for all groups (day, $P = 0.01$), but no significant dietary treatment effects were observed.

Serum haptoglobin tended to be higher for cows fed 100E than for those fed 150E prepartum ($P = 0.06$; Figure 7B). An interaction between far-off and close-up diet tended to occur prepartum and was a result of a larger difference between 100E ADLIB and 100E REST cows on d $-14$ compared with the other groups, as well as a similar trend observed between 150E ADLIB and 150E REST on d $-1$ prepartum ($P = 0.07$). Serum haptoglobin increased until the 2 wk before parturition for all groups, then decreased immediately before parturition ($P < 0.001$). Cows fed REST tended to have higher serum haptoglobin postpartum than cows fed ADLIB ($P = 0.08$). A 3-way interaction occurred postpartum because of the changes observed in the first week postpartum. While 150E and 100E cows fed REST prepartum had higher peaks in serum haptoglobin, their respective ADLIB groups were much lower. For 80E cows, ADLIB and REST groups were similar the first week postpartum. Beyond 14 d postpartum, all groups had similar and low concentration of haptoglobin in serum.

**Total Protein, Minerals, Vitamins, and Liver Enzymes.** These biomarkers had modest variations and are reported as mean values of the pre- and postpartum periods in Tables 2 and 3, respectively. The average values observed were within the reference range reported by Bertoni and Trevisi (2013). Total protein and globulin were higher for cows fed 150E compared with those fed 100E prepartum ($P = 0.02$; Table 2), but albumin was not affected by diet ($P \geq 0.41$). The albumin to globulin ratio was affected by a far-off by close-up interaction (Figure 8), where the highest values were observed for 100E and the lowest for 150E, whether fed ADLIB or REST in the close-up period.

Serum P was higher for cows fed REST during the close-up period compared with cows fed ADLIB ($P = 0.001$), and serum Zn was higher for cows fed 150E than for those fed 80E prepartum ($P = 0.01$). For serum Mg, the interaction between far-off diet and day ($P = 0.05$) occurred primarily because of a small increase in Mg on d $-21$ and $-14$ for 100E and 80E cows that did not occur in 150E, in which Mg concentration steadily decreased as parturition approached. A similar trend occurred for β-carotene, but the rise was observed for 150E instead of 100E and 80E, which helped explain the interaction between far-off diet and

| Figure 6. Least squares means of serum paraoxanase (A) and vitamin A (B) in multiparous cows fed different levels of energy prepartum. Pooled SE bars are shown. 150E ADLIB = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E ADLIB = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E ADLIB = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 150E REST on d −1 prepartum ($P = 0.06$; Figure 7B). An interaction between far-off and close-up diet tended to occur prepartum and was a result of a larger difference between 100E ADLIB and 100E REST cows on d $-14$ compared with the other groups, as well as a similar trend observed between 150E ADLIB and 150E REST on d $-1$ prepartum ($P = 0.07$). Serum haptoglobin increased until the 2 wk before parturition for all groups, then decreased immediately before parturition ($P < 0.001$). Cows fed REST tended to have higher serum haptoglobin postpartum than cows fed ADLIB ($P = 0.08$). A 3-way interaction occurred postpartum because of the changes observed in the first week postpartum. While 150E and 100E cows fed REST prepartum had higher peaks in serum haptoglobin, their respective ADLIB groups were much lower. For 80E cows, ADLIB and REST groups were similar the first week postpartum. Beyond 14 d postpartum, all groups had similar and low concentration of haptoglobin in serum.

**Total Protein, Minerals, Vitamins, and Liver Enzymes.** These biomarkers had modest variations and are reported as mean values of the pre- and postpartum periods in Tables 2 and 3, respectively. The average values observed were within the reference range reported by Bertoni and Trevisi (2013). Total protein and globulin were higher for cows fed 150E compared with those fed 100E prepartum ($P = 0.02$; Table 2), but albumin was not affected by diet ($P \geq 0.41$). The albumin to globulin ratio was affected by a far-off by close-up interaction (Figure 8), where the highest values were observed for 100E and the lowest for 150E, whether fed ADLIB or REST in the close-up period.

Serum P was higher for cows fed REST during the close-up period compared with cows fed ADLIB ($P = 0.001$), and serum Zn was higher for cows fed 150E than for those fed 80E prepartum ($P = 0.01$). For serum Mg, the interaction between far-off diet and day ($P = 0.05$) occurred primarily because of a small increase in Mg on d $-21$ and $-14$ for 100E and 80E cows that did not occur in 150E, in which Mg concentration steadily decreased as parturition approached. A similar trend occurred for β-carotene, but the rise was observed for 150E instead of 100E and 80E, which helped explain the interaction between far-off diet and

| Figure 7B. Least squares means of serum albumin and total protein (A) and serum P, Mg, Zn, Vitamin A, and β-carotene (B) in multiparous cows fed different levels of energy prepartum. Pooled SE bars are shown. Prepartum: all cows were fed to meet or exceed NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period. Far-off and close-up dry period. Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before estimated parturition until parturition. (A) A lactation diet was fed to all cows from parturition through 56 DIM. Paraoxonase, prepartum: far-off, $P = 0.03$; close-up, $P = 0.05$; day, $P < 0.001$; far-off × day, $P = 0.07$; other interactions of main effects $P \geq 0.16$. Paraoxonase, postpartum: far-off, $P = 0.72$; close-up, $P = 0.49$; day, $P < 0.001$; interactions of main effects, $P \geq 0.51$. (B) Vitamin A, prepartum: far-off, $P = 0.23$; close-up, $P = 0.03$; day, $P < 0.001$; far-off × day, $P = 0.01$; close-up × day, $P = 0.03$; other interactions of main effects, $P \geq 0.43$. Vitamin A, postpartum: far-off, $P = 0.50$; close-up, $P = 0.73$; day, $P < 0.001$; interactions of main effects, $P \geq 0.21$. |
An interaction of far-off diet and day affected vitamin E concentration \( (P = 0.003) \), as larger differences among groups occurred between d −30 and d −21, likely because of differences in DMI. By d −14 until parturition, all groups were similar. Activities of AST and GGT, enzymes associated with liver function, were not affected by prepartum diets before parturition \( (P \geq 0.22) \).

Postpartum, few differences in these blood variables were observed (Table 3). Serum albumin was higher postpartum for cows fed 100E than for those fed 150E \( (P = 0.02) \) but was not different from cows fed 80E \( (P = 0.14) \). Cows in the REST group prepartum had higher serum P postpartum than cows fed ADLIB \( (P = 0.001) \). An interaction between close-up diet and day occurred primarily because of a very sharp rise in P the first week after parturition for ADLIB cows compared with very little change for REST. The liver enzyme AST was higher for ADLIB cows compared with REST cows postpartum \( (P = 0.04) \).

Liver Activity Index. The serum metabolites used for calculation of LAI are shown by Figure 9A-C. For these time points, all variables were affected by day \( (P < 0.001) \), as all increased as lactation progressed. Serum vitamin A was not affected by prepartum diet, but cholesterol tended to be higher for cows fed ADLIB compared with cows fed REST \( (P = 0.06) \), and serum albumin tended to be higher for cows fed 100E compared with those fed 150E or 80E \( (P = 0.09) \) at these time points postpartum. Far-off diet overall tended to affect LAI \( (P = 0.07; \text{Figure } 10) \). Within the far-off treatments, 150E cows had lower LAI than 100E cows \( (P = 0.05) \), and the strongest difference among groups tended to be between the groups fed 150E REST and 100E ADLIB \( (P = 0.06) \). The distribution of cows among treatments stratified by LAI quartiles is shown in Table 4. In the lowest LAI quartile were clustered about 1/3 of the cows of the 150E groups, while in the highest LAI quartile were clustered about 1/3 of the cows of the 100E groups. The LAI was positively correlated with postpartum DMI during wk 1 to 4 \( (r = 0.54) \) and with wk 1 DMI \( (r = 0.69) \). Although milk yield did not significantly differ among LAI quartiles, the LAI was correlated \( (r = 0.62, P < 0.001) \) with average milk yield.

**DISCUSSION**

The importance of inflammation in adaptations of cows during the periparturient period is well recognized (Bertoni et al., 2008; Bradford et al., 2015; Horst et al., 2021). In this experiment, we compared...
**Table 2. Least squares means for proteins, minerals, vitamins, and enzymes in serum related to liver function measured weekly prepartum in cows fed different levels of energy prepartum**

<table>
<thead>
<tr>
<th>Variable</th>
<th>150E ADLIB</th>
<th>150E REST</th>
<th>100E ADLIB</th>
<th>100E REST</th>
<th>80E ADLIB</th>
<th>80E REST</th>
<th>SEM</th>
<th>Statistics</th>
<th>FO</th>
<th>CU</th>
<th>FO × CU</th>
<th>FO × d</th>
<th>CU × d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein, mg/L</td>
<td>78.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>77.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.32</td>
<td>0.005</td>
<td>0.82</td>
<td>0.51</td>
<td>0.06</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Albumin, g/L</td>
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<td>33.9</td>
<td>34.6</td>
<td>34.4</td>
<td>34.9</td>
<td>34.6</td>
<td>0.53</td>
<td>0.41</td>
<td>0.64</td>
<td>0.76</td>
<td>0.68</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>44.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>43.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.46</td>
<td>0.03</td>
<td>0.97</td>
<td>0.71</td>
<td>0.03</td>
<td>0.15</td>
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</tr>
<tr>
<td>P, mmol/L</td>
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<td>2.07</td>
<td>2.27</td>
<td>2.07</td>
<td>2.34</td>
<td>0.072</td>
<td>0.86</td>
<td>0.001</td>
<td>0.87</td>
<td>0.15</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Mg, mmol/L</td>
<td>0.99</td>
<td>0.99</td>
<td>1.01</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
<td>0.011</td>
<td>0.53</td>
<td>0.42</td>
<td>0.75</td>
<td>0.05</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Zn, μg/mL</td>
<td>13.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.48</td>
<td>0.04</td>
<td>0.13</td>
<td>0.47</td>
<td>0.23</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Vitamin E&lt;sup&gt;-1&lt;/sup&gt;, mg/mL</td>
<td>3.07</td>
<td>2.43</td>
<td>2.22</td>
<td>2.22</td>
<td>2.56</td>
<td>2.14</td>
<td>0.270</td>
<td>0.11</td>
<td>0.10</td>
<td>0.46</td>
<td>0.003</td>
<td>0.68</td>
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</tr>
<tr>
<td>β-Carotene, mg/100 mL</td>
<td>0.17</td>
<td>0.17</td>
<td>0.15</td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>0.015</td>
<td>0.60</td>
<td>0.77</td>
<td>0.53</td>
<td>0.04</td>
<td>0.66</td>
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</tr>
<tr>
<td>AST, U/L</td>
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<td>57.7</td>
<td>56.1</td>
<td>46.0</td>
<td>54.1</td>
<td>56.0</td>
<td>4.17</td>
<td>0.32</td>
<td>0.48</td>
<td>0.27</td>
<td>0.68</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>GGT, U/L</td>
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<td>23.2</td>
<td>23.0</td>
<td>20.4</td>
<td>22.9</td>
<td>25.4</td>
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<td>0.22</td>
<td>0.89</td>
<td>0.19</td>
<td>0.84</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

1<sup>a</sup> Main effects of far-off (FO) diet in the same row with different superscripts differ (P ≤ 0.05).
1<sup>b</sup> 150E ADLIB = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E ADLIB = cows fed to meet NRC (2001) requirements for energy during the far-off dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the close-up dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E ADLIB = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the close-up dry period; 100E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period.
2<sup>a</sup> The most notable changes for variables related to inflammation and liver function were observed for cows that were allowed to overconsume energy in the far-off period and had REST during the close-up dry period. Results indicated that both overfeeding and modest underfeeding of energy and other nutrients (coupled with the lack of feed available for an appreciable amount of time daily for 80E and REST cows) during the dry period resulted in greater evidence for inflammation compared with feeding cows to NRC (2001) requirements. Cows fed to requirements tended to produce more milk and had fewer health disorders (Dann et al., 2006).
Table 3. Least squares means for proteins, minerals, vitamins, and enzymes in serum related to liver function measured weekly postpartum in cows fed different levels of energy prepartum

<table>
<thead>
<tr>
<th>Variable</th>
<th>150E ADLIB</th>
<th>150E REST</th>
<th>100E ADLIB</th>
<th>100E REST</th>
<th>80E ADLIB</th>
<th>80E REST</th>
<th>SEM</th>
<th>FO</th>
<th>CU</th>
<th>FO × CU</th>
<th>FO × d</th>
<th>CU × d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein, g/L</td>
<td>78.0</td>
<td>77.5</td>
<td>78.8</td>
<td>75.2</td>
<td>77.3</td>
<td>77.6</td>
<td>1.07</td>
<td>0.77</td>
<td>0.14</td>
<td>0.17</td>
<td>0.13</td>
<td>0.81</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>32.8a</td>
<td>32.2a</td>
<td>34.5b</td>
<td>33.6b</td>
<td>33.1b</td>
<td>33.1b</td>
<td>0.05</td>
<td>0.06</td>
<td>0.39</td>
<td>0.09</td>
<td>0.61</td>
<td>0.43</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>45.2</td>
<td>45.3</td>
<td>44.3</td>
<td>41.6</td>
<td>44.2</td>
<td>44.4</td>
<td>1.16</td>
<td>0.14</td>
<td>0.39</td>
<td>0.39</td>
<td>0.10</td>
<td>0.93</td>
</tr>
<tr>
<td>P, mmol/L</td>
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<td>2.29</td>
<td>2.03</td>
<td>2.17</td>
<td>2.01</td>
<td>2.16</td>
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<td>0.46</td>
<td>0.001</td>
<td>0.60</td>
<td>0.79</td>
<td>0.01</td>
</tr>
<tr>
<td>Mg, mmol/L</td>
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<td>1.00</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.015</td>
<td>0.15</td>
<td>0.10</td>
<td>0.81</td>
<td>0.60</td>
<td>0.68</td>
<td>0.11</td>
</tr>
<tr>
<td>Zn, μmol/L</td>
<td>11.3</td>
<td>10.4</td>
<td>11.5</td>
<td>11.6</td>
<td>10.8</td>
<td>10.4</td>
<td>0.55</td>
<td>0.21</td>
<td>0.36</td>
<td>0.69</td>
<td>0.74</td>
<td>0.71</td>
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<tr>
<td>Vitamin E, μg/mL</td>
<td>2.13</td>
<td>1.86</td>
<td>2.22</td>
<td>2.25</td>
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<td>2.18</td>
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<td>0.71</td>
<td>0.80</td>
<td>0.65</td>
<td>0.58</td>
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<tr>
<td>β-Carotene, mg/100 mL</td>
<td>0.11</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.011</td>
<td>0.87</td>
<td>0.35</td>
<td>0.91</td>
<td>0.92</td>
<td>0.28</td>
</tr>
<tr>
<td>AST, U/L</td>
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<td>7.75</td>
<td>79.1</td>
<td>74.2</td>
<td>81.6</td>
<td>74.4</td>
<td>3.80</td>
<td>0.47</td>
<td>0.04</td>
<td>0.94</td>
<td>0.48</td>
<td>0.91</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>28.1</td>
<td>24.9</td>
<td>24.6</td>
<td>23.3</td>
<td>24.2</td>
<td>24.7</td>
<td>1.46</td>
<td>0.18</td>
<td>0.26</td>
<td>0.47</td>
<td>0.22</td>
<td>0.40</td>
</tr>
</tbody>
</table>

a,bMain effects of far-off (FO) diet in the same row with different superscripts differ (P ≤ 0.05).

150E ADLIB = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E ADLIB = cows fed to meet NRC (2001) requirements for energy during the far-off and close-up dry period; 80E ADLIB = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off and close-up dry period. Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before expected parturition until parturition. A lactation diet was fed to all cows from parturition through 56 DIM.

1Unless otherwise noted, day relative to parturition affected blood variables, P < 0.01. FO = far-off dry period diet; CU = close-up dry period diet; FO × CU = interaction of far-off and close-up dry period diets; FO × d = interaction between far-off dry period diet and day relative to parturition; and CU × d = interaction between close-up dry period diet and day relative to parturition.

2AST = aspartate transaminase.

3GGT = γ-glutamyl transpeptidase.

Cows with higher BCS at parturition are prone to greater mobilization of adipose tissue TAG at parturition and are more susceptible to metabolic problems postpartum (Morrow, 1976; Gearhart et al., 1990; Waltner et al., 1993; Chilliard et al., 2000). It is important to note, however, that cows fed 150E were not obese and there was not a large disparity among groups in BCS lost postpartum. An alternate explanation for liver lipodosis occurring in these situations is the reduction of lipoproteins involved in TAG removal from liver cells. This would be indicated by lower vitamin A concentrations (resulting from less retinol binding protein) and lower cholesterol (indicating less apo-lipoprotein B synthesis; Mezzetti et al., 2019).

Calcium concentration in blood is decreased during inflammation (Bertoni et al., 1989; Waldron et al., 2003; Trevisi and Minuti, 2018) and the larger decreases of Ca with overfeeding likely reflect the effects of inflammation. In general, serum Ca is not affected by Ca intake; in fact, cows in the 80E group had rather small changes of serum Ca prepartum regardless of close-up energy intake. Furthermore, the body maintains calcium homeostasis and can mobilize calcium from bone when needed (Goff, 2006), so the magnitude of difference in DMI among the dietary treatments was likely not large enough to cause differences related to intake alone.

In cows with healthy kidney function, creatinine is either an indicator of absolute muscle mass in the body or of muscle breakdown, and thus is related to BCS (Valadares et al., 1998). Creatinine depends largely on muscle mass and hepatic synthesis of its precursor, creatine (Skluzacek et al., 2003). However, hepatic creatine synthesis can be decreased in liver disease (Cocchetto et al., 1983). Increased creatinine in cows from the REST group prepartum likely means that those cows had greater rates of proteolysis in skeletal muscle to provide AA as a substrate for gluconeogenesis in the liver.

Clearance of bilirubin by the liver is an indicator of liver function in domestic animal species (Tennant, 1997). Hyperbilirubinemia is associated with cirrhosis in cattle (Tennant, 1997) and has been observed in cattle with fatty liver (Reid et al., 1983; Tennant, 1997; Bobe et al., 2004) or impaired liver function (Bertoni and Trevisi, 2013). The concentration of serum bilirubin is an indirect index of liver enzymes synthesized for bilirubin clearance that are decreased by inflammation (Trevisi et al., 2012; Bertoni and Trevisi, 2013).
In a previous study (Jahan et al., 2015), there was a strong positive correlation between bilirubin and IL-1β, indicating an association between an inflammatory event and serum bilirubin. In our study, the differences observed in bilirubin pre- and postpartum presented evidence for inflammation and liver dysfunction related to energy excess and lipid accumulation before parturition, as bilirubin was associated with liver lipid accumulation postpartum \((r = 0.34; \ P = 0.001)\). Restricting intake in the close-up period also was detrimental to liver function, perhaps related to stressful conditions caused by the rapid consumption of limited DM, distributed only twice daily. Overfeeding followed by restriction of energy intake is perhaps most hazardous. Cows at high planes of intake prepartum experience larger decreases in DMI around parturition (Grummer et al., 2004), which often can be attributed to inflammatory conditions (Zhou et al., 2016, 2017).

Perhaps the high plane of intake produced similar effects in our study.

Paraoxonase is a high-density lipoprotein (HDL)-associated acute phase protein that helps protect low-density lipoprotein from oxidative damage (Khovidhunkit et al., 2000, 2004; Chait et al., 2005). Paraoxonase is a negative acute phase protein because concentrations decrease during infection or inflammation (Khovidhunkit et al., 2000). In dairy cows, the activity of paraoxonase has been shown to be lower in the first 2 wk postpartum compared with 12 to 16 wk into lactation (Turk et al., 2004). Bionaz et al. (2007) found that classifying cows by paraoxonase activity revealed relationships between inflammation and subsequent liver function during the periparturient period.

Paraoxonase was negatively associated with the acute phase protein haptoglobin and positively associated with albumin, vitamin A (proxy for retinol binding protein), and cholesterol (proxy for apolipoproteins). When cows were grouped retrospectively according to paraoxonase activity, those with high paraoxonase activity had no reported incidence of retained fetal membranes, metritis, foot and leg injuries, or serious inflammation compared with other groups (Bionaz et al., 2007). Liver lipids were not measured in the study of Bionaz et al. (2007), but cows with lower paraoxonase activity may have had more accumulation of lipid in liver postpartum, because in the present study serum paraoxonase was negatively associated with liver lipid accumulation postpartum \((r = -0.27, \ \ P = 0.01)\).

The trend for vitamin A (retinol) to decrease as parturition approaches and then increase again postpartum has been well described by many authors (e.g., Goff and Stabel, 1990). Retinol binding protein is considered to be a negative acute phase protein produced by the liver, which decreases in response to tissue injury (Fleck, 1989). Bionaz et al. (2007) used vitamin A as an indicator for retinol binding protein and reported that cows experiencing inflammatory phenomena had lower vitamin A in blood postpartum and low LAI as well (Bertoni et al., 2008). In the present study, cows fed ad libitum in the far-off period and then restricted in the close-up period had lower serum vitamin A concentration, suggesting a detrimental effect to liver function as previously shown by serum bilirubin results. These data confirm the hypothesis that dietary strategies used in the dry period could have substantial consequences in the following lactation, but differences at blood level are not always evident after calving (Trevisi and Minuti, 2018).

Despite no postpartum differences among dietary treatment groups in our study, a positive relationship was found between total lipid accumulation and ceruloplasmin concentration postpartum \((r = 0.21; \ P = 0.03)\).
Ceruloplasmin is an HDL-associated positive acute phase protein (Khovidhunkit et al., 2000). Its Cu-containing free radical scavenging properties are important during infection and inflammation (Gruys et al., 1998). On the other hand, ceruloplasmin exerts a potent oxidant activity in the blood and thus could be associated with oxidation of lipoproteins, as demonstrated in humans (Boero et al., 2010). In cattle, ceruloplasmin is considered to be a moderate indicator of the acute phase response (Gruys et al., 1998) compared with domestic nonruminants (Eckersall, 2007). Neither Bionaz et al. (2007) nor Bertoni et al. (2008) observed a significant relationship between ceruloplasmin and either paraoxonase activity or LAI as an indicator of inflammation.

The relationship between haptoglobin and inflammation has been well characterized in ruminants (Petersen et al., 2004; Eckersall, 2007; Huzzy et al., 2011). Haptoglobin is considered one of the major positive acute phase proteins produced by the liver, increasing in circulation with insult or injury. Haptoglobin binds to free hemoglobin in the blood to conserve Fe and prevent it from becoming free in the blood where it would be available to potential pathogenic microbes (Petersen et al., 2004). Like paraoxonase and ceruloplasmin, haptoglobin is associated with the HDL in the blood of

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**Figure 9.** Least squares means of serum cholesterol, albumin, and vitamin A for time points used to calculate a liver activity index (LAI) postpartum in multiparous cows fed different levels of energy prepartum. Pooled SE bars are shown. 150E ADLIB = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume ≥150% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off and close-up dry period. Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before expected parturition until parturition. A lactation diet was fed to all cows from parturition through 56 DIM. (A) Cholesterol: far-off, \( P = 0.33 \); close-up, \( P = 0.06 \); day, \( P < 0.001 \); interactions of main effects, \( P \geq 0.44 \). (B) Albumin: far-off, \( P = 0.09 \); close-up, \( P = 0.42 \); day, \( P < 0.001 \); interactions of main effects, \( P \geq 0.34 \). (C) Vitamin A: far-off, \( P = 0.44 \); close-up, \( P = 0.48 \); day, \( P < 0.001 \); far-off \times close-up \times day, \( P = 0.06 \); other interactions of main effects, \( P \geq 0.26 \).
Figure 10. Liver activity index (LAI) for multiparous cows fed different levels of energy prepartum. Pooled SE bars are shown. This index considered serum cholesterol, albumin, and vitamin A on d 7, 14, 21, and 28 postpartum for individual cows compared with the average of all cows in the study. 150E ADLIB = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E ADLIB = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E ADLIB = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 150E REST = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period. Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before expected parturition until parturition. A lactation diet was fed to all cows from parturition through 56 DIM.

Table 4. Number of cows per prepartum diet that were in each quartile of liver activity index (LAI)

<table>
<thead>
<tr>
<th>LAI quartile</th>
<th>Prepartum diet1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100E ADLIB</td>
</tr>
<tr>
<td>Low</td>
<td>2</td>
</tr>
<tr>
<td>Intermediate-low</td>
<td>3</td>
</tr>
<tr>
<td>Intermediate-high</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
</tr>
</tbody>
</table>

150E ADLIB = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off and close-up dry period; 150E REST = cows fed to consume \( \geq 150\% \) of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 100E ADLIB = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 100E REST = cows fed to meet NRC (2001) requirements for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period; 80E ADLIB = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then ad libitum to meet or exceed energy requirements during the close-up dry period; 80E REST = cows restricted to 80% of NRC (2001) requirement for energy during the far-off dry period, then restricted to 80% of NRC (2001) requirement for energy during the close-up dry period. Far-off diets were fed from dry-off through 26 d before parturition and close-up diets were fed from 25 d before expected parturition until parturition. A lactation diet was fed to all cows from parturition through 56 DIM.
and haptoglobin are unclear, perhaps because the phenomena were not evolved at the same rate in all cows. It is difficult to compare the pattern of changes of ceruloplasmin and haptoglobin with those of the negative acute phase proteins (albumin, retinol bindin protein, paraoxonase, and apo-lipoproteins) because the rate of changes and their duration often are different.

Also relevant is the relationship between albumin (negative acute phase protein) and globulin (a proxy of inflammatory processes). Indeed, total protein and globulin were affected prepartum by far-off diet, and albumin was affected postpartum by far-off diet (namely by 150E decreasing albumin as a result of more frequent inflammatory conditions). According to Bobe et al. (2004), both globulin and albumin are negatively associated with fatty liver. More precisely, albumin was found to be significantly lower in cows with impaired liver function compared with cows with normal liver function (Bionaz et al., 2007; Bertoni et al., 2008). This confirms our calculated albumin to globulin ratio that was lowest both pre- and postpartum for cows overfed energy in the far-off period. Cattaneo et al. (2021) found an inverse association between the albumin to globulin ratio at dry-off and transition success.

Several biomarkers changed around parturition but were not associated with dry period diets. Although not greatly different in our study, Mg and vitamin E are known to be negatively associated with fatty liver in dairy cows (Bobe et al., 2004), and Zn has been shown to decrease around parturition to a greater degree in cows with hypocalcemia at parturition (Goff and Stabel, 1990). Interestingly, the sharp reduction of Zn and Ca in blood are both caused by inflammatory conditions (Bertoni et al., 1989). Phosphorus in all groups was within normal limits pre- and postpartum, and close-up effects may have been attributable to differences in intake amount and absorption efficiency with different DMI (NRC, 2001). Inadequate concentrations of β-carotene have been associated with nonalcoholic fatty liver disease in humans (Villaca et al., 2008). These biomarkers are perhaps not as important when looking at the effect of prepartum dietary energy effects on inflammatory responses in dairy cows.

Enzymes such as AST and GGT are commonly used to evaluate liver function (Tennant, 1997) and are elevated in cows with fatty liver (Bobe et al., 2004), indicating tissue damage. The elevation in AST that was observed for cows overfed energy prepartum in the present study corresponded to the greater accumulation of lipid in liver postpartum, which can cause cytolytic damage. However, the mobilization of muscle tissue can also have a role in increasing AST.

A further index that can be useful for evaluating the inflammatory status of cows in the transition period is the LAI. It includes the trend of 3 biomarkers during the first month of lactation, which are direct or indirect proxies of negative acute phase proteins (albumin, cholesterol, and vitamin A). Therefore, a lower or more negative LAI suggests poorer liver function. At calving, the values for the components of LAI tend to be low because inflammatory phenomena, usually small and short lived, occur. For lipoproteins (represented by cholesterol), further reasons for low values exist. For instance, the fat intake during the dry period is quite low and so intestinal production of VLDL, which contains cholesterol, is limited. Moreover, the liver esterification of TAG and consequent VLDL delivery in the blood is slowed. In this context, higher values of the components of LAI at calving or their quicker increase in the first weeks of lactation can indicate absence of inflammation or less severe inflammation and thus a better liver functionality.

Allowing cows to grossly overconsume energy during the far-off dry period, either with or without feed restriction during the close-up period, had negative effects on the calculated postpartum LAI in this study. Restricting intake over the entire dry period (80E REST) also resulted in negative LAI, much like the effect of overfeeding energy in the 150E groups. Indeed, even when fed ADLIB in the close-up period, LAI remained near 0 in the 80E cows. Positive LAI values were observed in cows fed 100E. These data are confirmed by Table 4 where the frequency of cows with high LAI is close to 38% when fed to their requirements or when fed 80E ADLIB. On the other hand, the frequency of cows with low LAI was 33% in the 150E and 80E REST groups. These data agree with the frequency of health problems observed by Dann et al. (2006) in the same experiment; subclinical ketosis was observed more frequently in 150E groups, and REST groups in the close-up period had more frequent metritis. Further, the performance data of Dann et al. (2006) confirm the association with LAI data, particularly for the first 10 DIM. Interestingly, the difference in milk yield carried through the 8 wk of lactation studied and remained numerically higher in 100E groups (2.5 kg/d), whereas differences in DMI and energy balance gradually diminished beyond 10 DIM.

Our results confirm previous observations by Bertoni et al. (2008). In particular, our data support that cows in the transition period can undergo inflammatory phenomena, which although not always clinically evident, compromise DMI and milk yield and, consequently, prolong and worsen negative energy balance (Dann et al., 2006). Many factors can contribute to the origin of these inflammatory processes. In this experiment, we found that an excess of energy or REST during the dry period can contribute to induce metainflammation.
CONCLUSIONS

Prepartum diet affected several of the temporal metabolite profiles during the transition period in this experiment. Changes for other variables related to inflammation and liver function were observed, especially when allowing cows to overconsume energy during the far-off dry period, either with or without feed restriction during the close-up period. In particular, the diet changes during the dry period impaired the concentration of negative acute phase proteins. Many of the described changes occurred prepartum, but the major effects, namely on health and lactation performance, were observed in the very early stage of lactation. The effects of prepartum diet did not consistently carry over to the more prolonged postpartum period; nevertheless, influencing liver function in a negative way by prepartum diets does not favor adaptation to lactation. Nutritional management strategies that help to reduce inflammation or improve liver function will be expected to enhance postpartum health and lactation performance.

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