Temporal profiles describing markers of inflammation and metabolism during the transition period of pasture-based, seasonal-calving dairy cows

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ABSTRACT

The physiology of the dairy cow while transitioning from pregnancy to lactation is complex, with multifactorial processes studied extensively for the role they play in manifestation of disease along with associated economic losses and compromised animal welfare. Manuscripts outlining associations among nutrition, production, physiology, and genetics variables and transition cow disorders are common in literature, with blood analytes that are central to energy metabolism (e.g., nonesterified fatty acids; NEFA, β -hydroxybutyrate; BHB) often reported. Immunity and inflammation have increasingly been explored in the pathogenesis and persistence of disorders, with cytokines and acute phase proteins well documented. However, most of these studies have involved cows fed total mixed rations, which may not always reflect profiles of blood analytes and other physiological indicators of transition cow health in grazing cows consuming fresh pasture. Considering the comparatively lesser characterization of these analytes and markers in pasture-based, seasonal-calving dairy cows, we compiled a database consisting of 2,610 cow lactations that span 20 yr of transition cow research in New Zealand. Using this database, analyte profiles from approximately 28 d precalving to 35 d postcalving were identified in dairy cows with a range of genetics, milk production potentials, and pasture-based farm management systems. These profiles characterize changes in energy reserves and metabolism (NEFA, BHB, glucose, insulin, growth hormone, insulin-like growth factor-1, leptin, body condition score, body weight), liver function (globulin, aspartate aminotransferase, glutamate dehydrogenase, gamma-glutamyl transpeptidase, bilirubin, cholesterol, liver triacylglycerides), protein metabolism (albumin, total protein, albumin:globulin ratio, creatinine, urea, creatine kinase), mineral balance (calcium,

magnesium, phosphate, potassium, sodium, chloride, bicarbonate), inflammation (IL-1 β , IL-6, haptoglobin, reactive oxygen species, total antioxidant capacity), and uterine health (polymorphonuclear cells, macrophage cells, vaginal discharge score). Temporal changes are generally consistent with previously characterized homeorhetic changes experienced by the dairy cow during the transition from pregnancy to lactation in both pastoral and housed systems. Some of the profiles had not previously been presented for pastoral systems, or in some cases, presented for either system. Our results indicate that moderate-yielding dairy cows undergo similar homeorhetic changes to high-yielding housed cows; however, differences in diet composition result in greater BHB concentrations than expected, based on their milk production and NEFA concentrations. In addition, most cows were able to transition to a state of higher energy requirement following calving, albeit with an increased metabolic challenge in the liver, and only a small percentage of cows were classified with severe hepatic lipidosis or severe hyperketonemia. Increases in metabolic function of the liver were accompanied by changes in indicators of the immune system and changes in mineral balance that, combined, probably reflect the innate response to the transition from gestation to lactation.

Key words: reference intervals, grazing, physiology, peripartum

INTRODUCTION

The transition period in dairy cows, often defined as 3 wk precalving to 3 wk postcalving, is characterized by marked physiological and immunological changes that facilitate the metabolically challenging shift from gestation to lactation (Bauman and Currie, 1980; Bell, 1995; Sordillo et al., 2009). Complex metabolic and neuroendocrine interactions facilitate the directing of nutrients and energy to lactogenic pathways; for example, increased lipolysis of adipose tissues associated with increased concentrations of growth hormone

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(GH), which is facilitated by the uncoupling of the somatotrophic axis (Ingvarsen, 2006; Lucy et al., 2009). These homeorhetic processes support the considerable increase in nutrient provision for lactogenesis, which typically surpasses nutrient intake during early lactation and results in a negative energy balance (NEB) as well as a negative protein and mineral balance. The NEB is commonly observed as a loss in BW and BCS, as adipose tissue reserves are mobilized to support lactation (Roche et al., 2009a, 2013a).

Physiological imbalances experienced during the periparturient period are central to the development of metabolic and infectious diseases (Ingvarsen, 2006). These disorders can have negative effects on reproduction and milk yield, with economic and animal welfare repercussions (Ospina et al., 2010a; McArt et al., 2013b). Therefore, much transition cow research has been devoted to exploring relationships between homeorhetic processes and metabolic or infectious diseases, with recent research focused more on inflammatory pathways activated during the establishment of these disorders (Heiser et al., 2015; Crookenden et al., 2019; Bradford and Swartz, 2020).

Blood analytes central to pathways of energy metabolism, liver function, protein metabolism, and inflammation are commonly measured and used to determine the effects of interventions on peripartum adaptation. Most transition cow research, however, has been undertaken in high-yielding housed dairy cows consuming a TMR. Fewer studies have focused on physiological changes in pasture-fed, moderate-yielding dairy cows managed in seasonal calving systems. Profiles of commonly measured analytes derived from studies of TMR-fed cows may not quantitatively or qualitatively reflect profiles in pasture-fed cows. Key differences in the metabolism of cows fed a TMR compared with pasture-fed cows have been identified, whereby the level of NFC consumed influences nutrient partitioning between body reserves and milk production (Kolver et al., 2006; Roche et al., 2006a). For example, diet manipulation shortened the time to recoupling of the somatotrophic axis, which was apparent in changes in plasma GH, insulin, and IGF-1 concentrations during early lactation, and resulted in a less severe loss of BCS following calving (Kolver et al., 2006; Roche et al., 2006a).

The primary objective of this study was to describe the temporal profiles of humoral metabolites, uterine health indicators, BCS, and BW of pasture-based, seasonally calving dairy cows. A database of detailed transition cow research conducted within pasture-based, seasonal calving systems was compiled to characterize the profile of change in humoral factors during the transition period. This enabled robust profiling of analytes measured during the transition period across a range of

cow genetics and milk production potentials, and under pasture-based farm management systems.

MATERIALS AND METHODS

Database Establishment

Previous experimental studies ($n = 20$) undertaken by DairyNZ that were conducted between 2000 and 2018, during the transition period, and included measurements of blood analytes were used for the pooled analysis. A brief description of the experimental objective and treatments, along with the number of cow records in each data set, is provided in Table 1. All experiments were designed to manipulate aspects of pasture-based seasonal-calving management systems including milking frequency, pre- and postcalving nutrition, and transition cow management, with a total of 85 experiment by treatment combinations included. A brief outline of experiment design elements including details of the treatments examined is provided in Supplementary Material Appendix I (<https://zenodo.org/record/5646637#.YZz1n9DMKUK>; Spaans et al., 2021).

Temporal measures of metabolites, minerals, cytokines, hormones, liver enzymes, acute phase proteins, BW, BCS, uterine health measures, and reproductive outcomes were collated into a large database of 2,610 complete cow lactations. Cow-lactation data were not included if there were missing blood analyte data for the entire experimental period in which they were enrolled ($n = 17$), missing treatment and reproduction data ($n = 1$), cows removed before the end of the experiment in which they were enrolled ($n = 5$), or incomplete or incorrect treatments during the experiment in which they were enrolled ($n = 3$). Breeds represented were Holstein-Friesian (HF; $n = 1,811$), Holstein-Friesian $\times$ Jersey crossbred (HF $\times$ J; $n = 350$), and Jersey (J; $n = 26$), with 423 cows of unknown breed. A summary of the number of observations for each variable by experiment is presented in Supplementary Table S1 (<https://zenodo.org/record/5646637#.YZz1n9DMKUK>; Spaans et al., 2021). Reproductive outcomes are not described in this study.

Experimental Design, Herd, and Pasture Management

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999) for all experiments included in the database. For each of the experiments, BCS were measured on a 1- to 10-point

Table 1. Year, description, treatment groups, number of cow records, and publication reference of experiments used in the collated database

	Year ¹	Description	Treatment ²	n	Publication references
1	2000/01	Effect of stocking rate on dairy farm efficiency	Stocking rate 2.2 cows/ha Stocking rate 3.1 cows/ha Stocking rate 4.3 cows/ha	26	Macdonald et al., 2001, 2008, 2011
2	2000/01	Overseas (OS) vs. New Zealand (NZ) Holstein-Friesian genetics under either pasture or TMR	NZ pasture: NZ Holstein-Friesian genetics pasture-fed NZ TMR: NZ Holstein-Friesian genetics TMR-fed OS pasture: OS Holstein-Friesian genetics pasture-fed OS TMR: OS Holstein-Friesian genetics TMR-fed	55	Kolver et al., 2002
3	2002/03	Overseas (OS) vs. NZ Holstein-Friesian genotype response to concentrate (yr 1)	NZ 0: NZ Holstein-Friesian genetics pasture-fed plus 0 kg of DM/cow per day of concentrate NZ 3: NZ Holstein-Friesian genetics pasture-fed plus 3 kg of DM/cow per day of concentrate NZ 6: NZ Holstein-Friesian genetics pasture-fed plus 6 kg of DM/cow per day of concentrate OS 0: OS Holstein-Friesian genetics pasture-fed plus 0 kg of DM/cow per day of concentrate OS 3: OS Holstein-Friesian genetics pasture-fed plus 3 kg of DM/cow per day of concentrate OS 6: OS Holstein-Friesian genetics pasture-fed plus 6 kg of DM/cow per day of concentrate	51	Kolver et al., 2005, 2006; Roche et al., 2006a
4	2003/04	Overseas (OS) vs. NZ Holstein-Friesian genotype response to concentrate (yr 2)	NZ 0: NZ Holstein-Friesian genetics pasture-fed plus 0 kg of DM/cow per day of concentrate NZ 3: NZ Holstein-Friesian genetics pasture-fed plus 3 kg of DM/cow per day of concentrate NZ 6: NZ Holstein-Friesian genetics pasture-fed plus 6 kg of DM/cow per day of concentrate OS 0: OS Holstein-Friesian genetics pasture-fed plus 0 kg of DM/cow per day of concentrate OS 3: OS Holstein-Friesian genetics pasture-fed plus 3 kg of DM/cow per day of concentrate OS 6: OS Holstein-Friesian genetics pasture-fed plus 6 kg of DM/cow per day of concentrate	56	Kolver et al., 2005, 2006; Roche et al., 2006a
5	2003/04	Effect of precalving pasture allowance	Very low: precalving intake 5.5 kg of DM/cow per day Low: precalving intake 8.0 kg of DM/cow per day Medium: precalving intake 10.5 kg of DM/cow per day High: precalving intake 13.0 kg of DM/cow per day CLA: pasture plus pre- and postcalving CLA supplementation Hypro: pasture plus pre- and postcalving Hypro supplementation	52	Roche et al., 2005
6	2003/04	Effect of lipid supplementation	Low precalving (4.8 kg of DM/cow per day) and high postcalving intake (13.6 kg of DM/cow per day) High precalving (11.9 kg of DM/cow per day) and low postcalving intake (8.5 kg of DM/cow per day)	26	Kay et al., 2006
7	2004/05	Effect of pre- and postcalving pasture DMI	Low precalving (4.8 kg of DM/cow per d) and low postcalving intake (8.5 kg of DM/cow per d) High precalving (11.9 kg of DM/cow per d) and high postcalving intake (13.6 kg of DM/cow per d)	66	Burke and Roche, 2007; Roche, 2007

Continued

Table 1 (Continued). Year, description, treatment groups, number of cow records, and publication reference of experiments used in the collated database

Year ¹	Description	Treatment ²	n	Publication references
8	2005/06	Altering proportion of structural carbohydrate and nonfiber carbohydrate pre- and postcalving	68	Burke et al., 2006; Roche et al., 2006b; Burke et al., 2010; Roche et al., 2010
9	2006/07	Effect of long-term infusion of ghrelin or obestatin	17	Roche et al., 2008; Grala et al., 2010
10	2009/10	Effect of milking frequency and duration	150	Phyn et al., 2011; Grala et al., 2014a; Grala et al., 2014b; Phyn et al., 2014
11	2011/12	Effect of BCS ³ at calving	57	Roche et al., 2013b
12	2011/12	Effect of postcalving nonsteroidal antiinflammatory (NSAID) treatment	213	Priest et al., 2013
13	2012/13	Effect of postcalving nonsteroidal antiinflammatory treatment	639	Meier et al., 2014; Heiser et al., 2015; Vallati Riboni et al., 2015
14	2012/13	Effect of late lactation once-daily milking	51	Grala et al., 2016
15	2013/14	Effect of precalving BCS ³ and feeding level	144	Roche et al., 2015; Crookenden et al., 2016a; Crookenden et al., 2016; Lange et al., 2016; Vallati-Riboni et al., 2016; Crookenden et al., 2019

Continued

Table 1 (Continued). Year, description, treatment groups, number of cow records, and publication reference of experiments used in the collated database

Year ¹	Description	Treatment ²	n	Publication references
16	2014/15 Effect of strategies to gain BCS during the far-off and close-up nonlactating periods	BCS 4.25 at dry-off, overfed during the far-off nonlactating period, and fed 65% of estimated ME requirements ⁴ for 3 wk precalving BCS 5.0 at dry-off, control-fed during the far-off nonlactating period, and fed 65% of estimated ME requirements for 3 wk precalving BCS 4.25 at dry-off, overfed during the far-off nonlactating period, and fed 90% of estimated ME requirements for 3 wk precalving BCS 5.0 at dry-off, control-fed during the far-off nonlactating period, and fed 90% of estimated ME requirements for 3 wk precalving BCS 4.25 at dry-off, overfed during the far-off nonlactating period, and fed 120% of estimated ME requirements for 3 wk precalving BCS 5.0 at dry-off, control-fed during the far-off nonlactating period, and fed 120% of estimated ME requirements for 3 wk precalving Control: pasture-fed and corn silage supplement precalving Zeolite: pasture-fed and corn silage supplement mixed with 500 g/cow per d of synthetic zeolite A precalving	150	Roche et al., 2017a; Lange et al., 2019
17	2016/17 Effect of feeding zeolite precalving	Saline/saline: 2.7-mL saline injection precalving and 2.7-mL saline injection postcalving 0.25/0.25: 0.675-mL Imrester (3.75 mg) injection precalving and 0.675-mL saline injection postcalving 0.5/0.5: 1.35-mL Imrester (7.5 mg) injection precalving and 1.35-mL Imrester injection postcalving Full/full: 2.7-mL Imrester (15 mg) injection precalving and 2.7-mL Imrester injection postcalving Full/saline: 2.7-mL Imrester injection precalving and 2.7-mL saline injection postcalving Saline/full: 2.7-mL saline injection precalving and 2.7-mL Imrester injection postcalving Saline/0.5: 1.35-mL saline injection precalving and 1.35-mL Imrester injection postcalving High/positive FBV: +5% FBV Low/negative FBV: -5% FBV	47	Crookenden et al., 2020; C. V. C. Phyn, unpublished data (DairyNZ, Hamilton, New Zealand) Crookenden et al., 2021
18	2017/18 Effect of Imrester dose rate and timing	Pasture-fed plus corn silage supplement Pasture-fed plus corn silage supplement mixed with 250 g/cow per day of synthetic zeolite A for 10 d precalving Pasture-fed plus corn silage supplement mixed with 250 g/cow per day of synthetic zeolite A for 21 d precalving Pasture-fed plus corn silage supplement mixed with 500 g/cow per day of synthetic zeolite A for 10 d precalving Pasture-fed plus corn silage supplement mixed with 500 g/cow per day of synthetic zeolite A for 21 d precalving Pasture-fed plus corn silage supplement mixed with 750 g/cow per day of synthetic zeolite A for 10 d precalving Pasture-fed plus corn silage supplement mixed with 750 g/cow per day of synthetic zeolite A for 21 d precalving	173	
19	2017/18 Effect of positive or negative fertility breeding value (FBV)		480	Meier et al., 2017; Meier et al., 2021; Grala et al., 2022
20	2018/19 Effect of zeolite dose and duration precalving		91	C. V. C. Phyn, unpublished data (DairyNZ, Hamilton, New Zealand)

¹Year = season from June to May of the respective years the study was undertaken.

²CLA (RI-CLA; Bioproducts Inc.); Hypro (Hyprofat, a palm fatty acid distillate; Bonimex BV); carprofen (Carprive LA; 50 mg of carprofen/mL, Norbrook New Zealand Ltd.); synthetic zeolite A (Optimate MF+, Blue Pacific minerals); Imrester (pegbovigastim available as Imrester Elanco).

³BCS measured on a 10-point scale, where 1 = emaciated and 10 = obese (Roche et al., 2004).

⁴Estimated ME requirements during late gestation (1.05 MJ/kg of BW^{0.75}; Roche et al., 2005).

scale, where 1 is emaciated and 10 is obese (Roche et al., 2004). Scores can be converted to the 5-point scale using the regression equation: 5-point = $1.5 + 0.32 \times 10\text{-point}$ (Roche et al., 2004).

Unless otherwise stated, cows in all experiments were milked twice daily following calving and grazed predominantly ryegrass and white clover pasture, with a seasonal calving pattern. Cows were offered a generous pasture allowance and were managed to achieve a target BCS of 5.0 at calving, unless manipulated as part of the treatment design, in line with the pasture management decision rules described by Macdonald et al. (2010). Detailed descriptions of pasture and herd management, and pasture measurements are reported in the respective publications for each experiment (Table 1); briefly, cows were offered >35 kg of DM/cow per day to ground level (~20 kg of DM pasture available for consumption above grazing residual), had access to a fresh allocation of pasture at least once daily, and were rotationally grazed as one herd with grazing intervals ensuring they only returned to the same area when a minimum of 2 leaves had appeared on the majority (66%) of perennial ryegrass tillers (Roche et al., 2017b). Generally, treatment groups were grazed in the same paddock separated by an electric fence to manage the herd pasture allocation and, therefore, had the same pregrazing pasture mass. Postgrazing residuals of >1,800 kg of DM/ha were targeted during spring and autumn and >2,200 kg of DM/ha during summer (Macdonald et al., 2010).

Body weight and BCS were generally determined either weekly or fortnightly at the a.m. milking or at 0900 h during the nonlactating period. Blood sampling and laboratory analyses were performed according to the methods outlined in the respective publications and presented in summary in Supplementary Material Appendix I (Spaans et al., 2021).

Database Subset

Each of the measurements collated in the database was formatted as a day relative to calving, where the calving date was d 0. Calving date was defined as the day the calf was collected during daily calf retrieval (typically mid morning). The period between 28 d precalving and 35 d postcalving was chosen to select a subset of data to focus on the transition period. Because timing of blood sampling relative to calving date varied among the experiments, the days were grouped to the nearest week pre- or postcalving ± 3 d. The exceptions were the groupings: d -30 (30 d precalving ± 5 d), d -3 (4 to 2 d precalving, d 0 (1 d precalving and day of calving), d 1.5 (1 and 2 d postcalving), and d 3.5 (3 and 4 d postcalving). Multiple observations for a cow were averaged to give the best estimate for each time

point. Grouped time points were then clustered to give a precalving period of d -35 to -2 (i.e., grouped time points d -30 to -3 were clustered), a periparturient period of d -1 to 2 (i.e., grouped time points of d 0 and 1.5 were clustered), and a postcalving period of d 4 to 38 (i.e., grouped time points of d 3.5 to 35 were clustered).

Data Analyses

All data were analyzed using SAS Studio 3.8 (SAS release 9.04; SAS Institute Inc.). The mean, median, and standard deviation (**SD**) were calculated for each variable at each of the grouped time points (PROC MEANS). A repeated-measures mixed model (PROC MIXED) for effect of grouped day was fitted; fixed effects of parity, breed, and calving season day (**CSD**; number of days from the June 1 to actual calving date) were included as blocking factors or covariates; and treatment within experiment was included as a random effect. A compound symmetry covariance structure was included for all variables. A second mixed model included clustered period (precalving, periparturient, and postcalving) instead of grouped day as well as the same blocking factors and covariates. Data were $\log_{10}$ transformed to achieve homogeneity of variance for the following variables: urea, creatine kinase (**CK**), BHB, nonesterified fatty acids (**NEFA**), insulin, GH, IGF-1, aspartate aminotransferase (**AST**), glutamate dehydrogenase (**GDH**), cholesterol, γ -glutamyl transpeptidase (**GGT**), liver triacylglycerides (**TAG**), IL-1 β , IL-6, haptoglobin (**Hp**), reactive oxygen/nitrogen species (**ROS**), total antioxidant capacity (**TAC**), PMN, macrophage cells, and vaginal discharge score (0–5 scale; McDougall et al., 2020).

Parity was grouped as follows: group 1 = parity 1 animals; group 2.5 = parity 2 and 3 animals; group 5 = parity 4 to 6 animals, inclusive; and group 7 = parity 7+ animals. Breed was grouped as HF $\geq 12/16$ ths, J $\geq 12/16$ ths, and HF $\times$ J <12/16ths HF or J. The least squares means (**LSM**), maximum standard error of the difference (**SED**), mean, and upper SD for each variable at each grouped time point were plotted (R studio v4.0.1; <https://www.r-project.org/>). Maximum SED was the largest SED for any of the pairwise comparisons for LSM of different time points. For variables requiring $\log_{10}$ transformation, *P*-values presented are based on analysis of the transformed data, whereas LSM and SED are based on raw data.

To calculate prevalence of common transition cow disorders, established cut points from the literature were used as follows: (1) hyperketonemia (blood BHB): precalving ≥ 0.8 mmol/L (Chapinal et al., 2011; Ospina et al., 2013), moderate postcalving ≥ 1.2 mmol/L (LeB-

lanc et al., 2005; Ospina et al., 2010b; Chapinal et al., 2012), and severe postcalving ≥ 3.0 mmol/L (Oetzel, 2004); (2) hyperlipidemia (blood NEFA): precalving ≥ 0.5 mmol/L and postcalving ≥ 1.0 mmol/L (LeBlanc et al., 2005; Chapinal et al., 2011; Bogado Pascottini and LeBlanc, 2020); (3) hepatic lipidosis (liver TAG): mild 1–5% wet weight, moderate 5–10% wet weight, and severe $>10\%$ wet weight (Bobe et al., 2004); (4) hypocalcemia (blood total Ca): clinical pre- and postcalving <1.4 mmol/L (DeGaris and Lean, 2008), subclinical pre- and postcalving <2.0 mmol/L (Reinhardt et al., 2011) and ≤ 2.15 mmol/L (Neves et al., 2018; McArt and Neves, 2020), respectively; (5) hypomagnesemia (blood total Mg): clinical pre- and postcalving ≤ 0.5 mmol/L, subclinical pre- and postcalving ≤ 0.8 mmol/L (Goff, 2008); and (6) hypermagnesemia: pre- and postcalving >1.1 mmol/L (Goff, 2008). Prevalence was expressed as the percentage of cows in each cut point group out of all the cows with data for the same time period.

RESULTS

The mean, SD, median, minimum and maximum values, number of observations, and LSM at each grouped time point are presented in Tables 2, 3, 4, 5, 6, and 7 for energy metabolism, liver function, protein metabolism, mineral status, inflammatory markers, and uterine and animal health variables, respectively. Temporal profiles of the mean, upper SD, LSM, and maximum SED for each variable are also presented in Figures 1 to 6. The comparison between precalving, periparturient, and postcalving periods including the LSM and maximum SED are presented in Table 8. Overall, time significantly affected all variables, both by day ($P < 0.001$) and period ($P < 0.05$). Parity and breed also affect the concentration of many of these analytes (data not presented), but the shape of the profiles is not different.

Indicators of Energy Metabolism, BCS, and BW

Changes in the circulating concentrations of BHB, NEFA, glucose, insulin, GH, IGF-1, and leptin were used to monitor energy status and metabolism, with BCS and BW reflecting changes in body composition and energy reserves. Beta-hydroxybutyrate concentration gradually increased from a minimum LSM of 0.65 mmol/L at d -30 to 0.75 mmol/L at d -3 ($P < 0.01$), and then increased sharply around calving to a peak of 0.96 mmol/L by d 7 ($P < 0.001$; Figure 1A). On average, postcalving concentrations of BHB were greater than precalving and periparturient concentrations ($P < 0.001$; Table 8). During the period of d 7 to 35, the SD of BHB was noticeably increased, reflecting a greater

maximal range of BHB concentrations for individual cows. The median was less than the mean indicating a skewed distribution of the data (Table 2) including a few very high values. The prevalence of precalving hyperketonemia (≥ 0.80 mmol/L) increased from 5% at d -30 to 17% at d -14 , with a further increase to 25% at d -7 . Postcalving, the prevalence of moderate hyperketonemia (≥ 1.2 mmol/L) ranged from 2% at d 0 and d 1.5 to a maximum of 16% at d 7, remaining around that level for the remainder of the observational period. Severe hyperketonemia prevalence (≥ 3.0 mmol/L) only reached a maximum of 2% at d 28 and 35.

Concentrations of NEFA (Figure 1B) increased from 0.30 mmol/L at d -30 to 0.50 mmol/L at d -3 ($P < 0.001$), with a rapid increase following calving to peak at a LSM concentration of 0.86 mmol/L at d 7 ($P < 0.001$; Table 2). Nonesterified fatty acid concentration gradually declined thereafter but remained greater, on average, during the postcalving period than precalving ($P < 0.001$; Table 8). Prevalence of precalving hyperlipidemia (≥ 0.5 mmol/L) gradually increased from 23% at d -30 to 55% at d -3 . Postcalving prevalence of hyperlipidemia (≥ 1.0 mmol/L) increased at each time point from 17% at d 0 to a peak of 43% at d 7, declining again gradually thereafter to 19% at d 35.

Concentrations of glucose (Figure 1C) and insulin (Figure 1D) followed generally similar patterns. However, glucose concentrations remained stable at approximately 3.7 mmol/L leading up to calving with no differences between time points from d -30 to -3 ($P > 0.42$); insulin concentrations, in comparison, decreased ($P < 0.05$) from 9.8 to 8.4 $\mu\text{U/mL}$ between d -30 and -21 , and remained stable to d -3 . Both glucose and insulin were markedly elevated around calving, increasing to 4.4 mmol/L and 10.9 $\mu\text{U/mL}$, respectively, between d -3 and 0 ($P < 0.001$). Thereafter, concentrations declined ($P < 0.001$) rapidly to reach 3.4 mmol/L for glucose and 5.9 $\mu\text{U/mL}$ for insulin by d 7. Both glucose and insulin subsequently remained relatively stable (Figure 1C and D), but lower during the postcalving period than the precalving period ($P < 0.001$; Table 8). Median insulin concentrations were consistently lower than the means throughout the observational period, indicating a right skewed distribution of data (Table 2).

Concentrations of GH were stable during the precalving period (Figure 1E), with a mean of 4.1 ng/mL (Table 8). However, GH concentration spiked between d -3 and d 0 ($P < 0.001$) to reach 30.9 ng/mL, albeit with a large variation around this d 0 mean. Growth hormone rapidly decreased thereafter to 13.5 ng/mL at d 1.5 ($P < 0.001$). Concentrations then remained stable between d 3.5 and 35 ($P > 0.79$); however, there was more variation around the means at each grouped time point (Table 2) and the mean GH concentration dur-

Table 2. Summary statistics for the indicators of energy metabolism variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED
BHB (mmol/L)														
Mean	0.41	0.47	0.55	0.61	0.60	0.62	0.65	0.71	0.89	0.80	0.71	0.83	0.80	
SD	0.214	0.277	0.341	0.327	0.282	0.225	0.211	0.28	0.547	0.517	0.536	0.701	0.653	
Median	0.36	0.40	0.47	0.55	0.53	0.60	0.60	0.65	0.77	0.68	0.60	0.60	0.63	
Minimum ³	0.14	0.10	0.10	0.10	0.18	0.10	0.20	0.20	0.10	0.16	0.10	0.10	0.10	
Maximum ³	2.09	3.70	3.87	3.10	2.45	2.50	2.50	3.30	8.15	5.60	5.84	7.88	6.50	
n	444	736	1,054	1,097	521	1,044	965	1,068	1,760	1,695	1,043	1,497	782	
LSM ⁴	0.65	0.67	0.71	0.73	0.75	0.84	0.88	0.92	0.96	0.93	0.91	0.95	0.87	0.024
Nonesterified fatty acids (mmol/L)														
Mean	0.37	0.42	0.48	0.54	0.58	0.62	0.73	0.88	0.97	0.87	0.81	0.74	0.72	
SD	0.29	0.33	0.338	0.364	0.355	0.378	0.399	0.459	0.439	0.415	0.378	0.47	0.554	
Median	0.30	0.34	0.40	0.47	0.52	0.53	0.66	0.80	0.90	0.80	0.76	0.66	0.60	
Minimum	0.03	0.06	0.07	0.07	0.07	0.04	0.00	0.08	0.10	0.07	0.09	0.04	0.05	
Maximum	2.11	3.00	2.55	2.53	2.29	2.14	2.46	2.60	2.63	2.61	2.26	3.80	3.80	
n	503	943	1,267	1,304	614	1,254	1,044	1,347	2,105	2,124	1,459	1,910	1,037	
LSM	0.30	0.33	0.38	0.46	0.50	0.52	0.63	0.77	0.86	0.75	0.68	0.63	0.61	0.023
Glucose (mmol/L)														
Mean	3.6	3.5	3.5	3.5	3.6	4.3	3.6	3.3	3.2	3.2	3.4	3.3	3.3	
SD	0.27	0.35	0.36	0.38	0.38	0.79	0.47	0.40	0.44	0.50	0.50	0.70	0.90	
Median	3.6	3.5	3.6	3.5	3.7	4.2	3.6	3.3	3.2	3.3	3.4	3.4	3.5	
Minimum	2.8	2.0	2.2	2.3	2.7	2.5	2.4	2.0	1.7	1.6	1.7	0.4	0.3	
Maximum	4.5	4.4	4.9	4.8	4.7	7.8	5.9	4.7	4.6	4.6	4.7	4.9	5.1	
n	115	152	248	238	145	308	382	375	471	574	439	587	282	
LSM	3.8	3.7	3.7	3.7	3.7	4.4	3.7	3.5	3.4	3.4	3.5	3.4	3.4	0.06
Insulin (μU/mL)														
Mean	7.1	5.1	4.9	4.2	4.6	7.6	5.4	3.5	3.0	3.2	2.9	3.2	3.3	
SD	5.53	3.75	4.28	3.23	4.31	7.35	5.39	3.60	2.66	3.25	2.22	2.77	2.87	
Median	5.6	4.0	3.4	3.2	3.2	4.6	3.3	2.1	2.0	2.1	2.1	2.3	2.3	
Minimum	1.3	0.8	0.8	0.5	1.2	0.9	0.1	0.3	0.3	0.4	0.5	0.4	0.6	
Maximum	29.2	24.3	29.4	24.0	25.8	39.3	32.8	29.0	17.1	32.2	16.6	24.7	23.0	
n	114	141	164	133	68	196	295	295	459	522	440	512	281	
LSM	9.8	8.4	8.7	8.3	8.6	10.9	8.5	6.7	5.9	6.2	6.2	6.1	6.5	0.47
Growth hormone (ng/mL)														
Mean	5.7	5.0	4.0	5.1	5.9	30.6	12.7	9.7	8.4	7.3	7.9	7.4	7.8	
SD	6.45	3.88	4.21	6.47	5.11	57.13	14.44	12.1	11.24	9.59	10.86	9.49	9.42	
Median	4.4	3.8	3.0	3.1	4.3	11.4	7.3	5.5	4.3	3.6	3.7	4.2	4.5	
Minimum	0.5	0.7	0.5	0.5	0.5	1.0	0.7	0.6	0.5	0.5	0.4	0.5	0.5	
Maximum	61.3	20.7	44.5	46.4	26.8	410.4	81.6	106.7	113.9	84.5	88.7	77.9	55.5	
n	114	141	164	133	68	196	277	288	419	474	389	462	253	
LSM	6.0	3.9	4.3	4.8	3.5	30.9	13.5	10.5	9.1	8.3	8.6	8.3	8.6	2.38
IGF-1 (ng/mL)														
Mean	29.9	24.1	20.9	18.5	16.8	8.3	8.0	7.1	7.6	7.7	8.4	8.4	9.7	
SD	12.69	10.93	10.34	9.20	9.09	6.00	4.75	3.99	4.67	5.18	5.45	5.70	6.11	
Median	27.8	22.0	20.7	17.6	14.9	6.8	6.8	6.2	6.8	6.6	7.2	7.2	8.2	
Minimum	3.4	4.1	2.6	2.7	4.9	2.0	1.2	1.0	1.2	1.0	1.0	1.0	1.6	
Maximum	80.0	64.2	54.8	43.0	47.4	36.8	33.4	29.1	39.0	44.6	42.6	65.6	39.6	

Continued

Table 2 (Continued). Summary statistics for the indicators of energy metabolism variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED
n	114	141	164	133	68	196	277	288	419	473	389	462	253	
LSM	32.1	26.4	22.9	20.1	18.3	10.2	9.9	9.2	9.1	9.3	9.7	10.0	11.0	0.82
Leptin (ng/mL)														
Mean	1.1	1.0	0.8	0.7	0.6	0.8	0.8	0.7	0.6	0.6	0.4	0.6	0.5	
SD	0.54	0.53	0.42	0.37	0.33	0.40	0.47	0.36	0.36	0.35	0.27	0.31	0.33	
Median	1.1	1.0	0.8	0.6	0.5	0.7	0.7	0.7	0.5	0.5	0.4	0.5	0.5	
Minimum	0.3	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	
Maximum	3.3	2.3	2.1	2.0	1.3	2.2	2.1	1.6	2.9	2.9	1.8	1.9	2.3	
n	70	94	89	63	30	120	160	152	184	205	116	191	100	
LSM	1.1	1.1	1.0	1.0	0.9	0.8	0.9	0.8	0.7	0.7	0.8	0.7	0.7	0.05
BCS														
(1–10 scale)														
Mean	4.9	5.0	5.0	5.0	5.0	4.8	4.8	4.8	4.5	4.4	4.3	4.2	4.2	
SD	0.6	0.58	0.6	0.61	0.59	0.62	0.57	0.67	0.56	0.56	0.55	0.51	0.55	
Median	5.0	5.0	5.0	5.0	5.0	5.0	4.9	5.0	4.5	4.5	4.4	4.0	4.0	
Minimum	3.1	3.1	3.0	3.2	3.0	3.0	3.1	3.0	3.0	2.5	2.5	2.5	2.5	
Maximum	8.5	8.5	9.0	9.0	7.0	7.0	6.5	8.5	7.5	8.0	8.0	7.5	8.5	
n	1,925	1,837	1,895	1,825	933	458	726	808	1,996	2,422	2,440	2,398	1,479	
LSM	4.9	5.0	5.0	5.0	5.0	4.9	4.8	4.8	4.6	4.5	4.4	4.3	4.2	0.02
BW (kg)														
Mean	520	531	538	545	546	520	483	489	468	462	457	455	457	
SD	66.8	66.8	67.8	67.9	68.4	71.9	60.7	75.3	60.1	64.5	64.4	63.9	66.5	
Median	513	522	530	540	540	518	478	475	463	454	450	449	449	
Minimum	247	308	325	329	343	320	325	342	288	281	268	251	273	
Maximum	882	888	894	900	804	770	762	808	700	782	760	746	738	
n	1,929	1,807	1,747	1,513	729	383	611	681	1,572	1,876	1,872	1,851	1,165	
LSM	486	494	500	506	509	479	448	446	431	421	415	414	413	1.4

¹-30 = 30 ± 5 d precalving; -21, -14, -7 = 21, 14, 7 $\pm$ 3 d precalving; -3 = 3 ± 1 d precalving; 0 = 1 d precalving and day of calving; 1.5 = 1 and 2 d postcalving; 3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 $\pm$ 3 d postcalving.

²Minimum concentration observed during time point; maximum concentration observed during time point.

³LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number). Effect of day was significant ($P < 0.001$) for all variables.

Table 3. Summary statistics for the indicators of liver function variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED ²
Globulin (g/L)														
Mean	43	41	38	35	33	34	34	36	36	38	39	38	37	
SD	6.0	7.2	7.9	7.4	6.7	6.4	5.9	6.0	6.2	6.1	6.0	5.9	5.9	
Median	43	41	38	34	32	34	34	35	35	37	38	38	37	
Minimum ³	25	22	18	17	5	16	12	20	21	16	21	26	22	
Maximum ³	61	64	68	67	62	66	59	62	59	63	67	79	61	
n	418	860	1,160	1,216	563	1,112	814	1,106	1,357	1,292	1,079	1,455	792	
LSM ⁴	39	37	35	32	30	30	29	31	33	34	35	35	35	0.26
Aspartate aminotransferase (IU/L)														
Mean	58	60	62	63	65	76	84	82	83	81	82	81	81	
SD	18.2	20.1	20.1	17.5	21.7	30.9	36.4	36.4	32.4	24.4	23.2	26.9	20.9	
Median	54	56	58	60	61	72	78	76	78	78	78	76	78	
Minimum	28	28	21	29	31	33	25	35	39	27	39	33	39	
Maximum	164	337	284	198	322	586	598	536	580	322	273	422	174	
n	423	841	1,132	1,162	530	1,102	839	1,137	1,486	1,422	1,126	1,615	819	
LSM	53	54	56	58	59	72	80	79	79	78	77	77	77	1.4
Glutamate dehydrogenase (IU/L)														
Mean	14	16	16	16	18	19	20	21	26	29	29	30	29	
SD	18.3	23.5	16.4	15.3	22.6	37.9	41.1	41.1	28.4	40.0	41.1	50.4	30.9	
Median	10	11	11	11	11	12	12	13	17	18	17	18	20	
Minimum	3	2	2	2	1	2	3	3	3	2	3	3	5	
Maximum	264	395	280	127	284	635	948	847	323	616	638	692	250	
n	285	697	987	1,041	473	994	695	1,004	1,425	1,278	982	1,471	737	
LSM	10	13	13	14	13	17	16	18	23	26	26	28	26	2.3
Cholesterol (mmol/L)														
Mean	2.3	2.7	3.0	2.7	2.5	2.4	2.2	2.1	2.4	2.8	3.1	3.0	3.6	
SD	0.84	0.73	0.76	0.85	0.68	0.55	0.53	0.73	1.08	0.94	0.91	1.17	0.35	
Median	2.2	2.7	2.8	2.6	2.5	2.4	2.1	2.1	2.3	2.8	3.1	2.9	3.6	
Minimum	1.3	0.8	1.8	1.3	0.8	1.1	0.8	0.1	0.2	0.9	1.3	0.1	3.3	
Maximum	3.8	4.3	5.7	7.0	4.8	4.1	3.5	5.0	10.2	7.4	6.5	6.9	4.0	
n	6	50	95	153	113	145	180	197	158	128	72	207	3	
LSM	2.3	2.3	2.6	2.6	2.2	2.1	1.9	2.0	2.2	2.5	2.8	2.8	3	0.44
Bilirubin (μmol/L)														
Mean	2.4	2.5	2.7	3.3	3.5	5.2	4.4	6.5	5.3	4.1	3.3	3.0	2.6	
SD	1.71	1.44	1.58	2.03	1.96	3.79	2.69	3.71	2.59	1.73	1.55	1.64	1.53	
Median	1.3	2.0	2.1	3.0	3.0	4.5	4.0	6.0	5.0	4.0	3.0	3.0	3.0	
Minimum	1.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	1.0	1.3	0.0	1.0	1.3	
Maximum	10.0	12.0	8.5	13.0	14.0	25.0	16.0	29.5	23.6	12.0	9.0	13.0	7.0	
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	1.1	1.2	1.7	2.3	2.5	4.3	3.6	5.5	4.4	3.1	2.4	2.0	1.4	0.38
Gamma-glutamyl transpeptidase (IU/L)														
Mean	14	16	16	16	18	19	20	21	26	29	29	30	29	
SD	18.3	23.5	16.4	15.3	22.6	37.9	41.1	41.1	28.4	40	41.1	50.4	30.9	
Median	21	19	20	20	22	24	25	23	23	23	23	22	21	

Continued

Table 3 (Continued). Summary statistics for the indicators of liver function variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED ²
Minimum	3	2	2	2	1	2	3	3	3	2	3	3	5	
Maximum	264	395	280	127	284	635	948	847	323	616	638	692	250	
n	285	697	987	1,041	473	994	695	1,004	1,425	1,278	982	1,471	737	
LSM	22	20	20	20	21	28	26	24	24	24	24	24	23	1.7
Liver triacylglyceride (% wet weight)														
Mean	1.4	1.3	1.3	1.5	1.4	1.7	2.9	3.9	3.2	3.7	4.0	4.2		
SD	0.15	0.17	0.17	0.32	0.15	1.33	1.44	2.1	2.23	2.5	2.97	3.35		
Median	1.4	1.3	1.3	1.3	1.3	1.4	2.1	2.2	2.5	2.2	1.9	1.7		
Minimum	1.1	1.0	1.0	1.0	1.2	1.2	1.3	1.3	0.3	1.1	1.2	1.2		
Maximum	1.5	1.8	1.6	2.7	1.8	8.6	8.7	8.7	16.9	11.7	12.7	14.6		
n	6	31	9	29	35	31	52	22	484	92	32	56		
LSM	1.7	1.3	1.7	0.6	0.4	0.6	2.6	2.6	3.1	3.2	2.9	3.2		1.00

¹-30 = 30 ± 5 d precalving; -21, -14, -7 = 21, 14, 7 ± 3 d precalving; -3 = 3 ± 1 d precalving; 0 = 1 d precalving and day of calving; 1.5 = 1 and 2 d postcalving; 3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 ± 3 d postcalving.

²SED = standard error of the difference.

³Minimum concentration observed during time point; maximum concentration observed during time point.

⁴LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number). Effect of day was significant ($P < 0.001$) for all variables.

ing the postcalving period (10.0 ng/mL) was greater than precalving ($P < 0.001$; Table 8). The distribution of GH data was right skewed, indicated by median concentrations being consistently lower than the means throughout the observational period (Table 2).

Concentrations of IGF-1 decreased ($P < 0.001$) gradually from a peak concentration of 32.1 ng/mL at d -30 to 18.3 ng/mL at d -3, with a rapid decrease at d 0 to 10.2 ng/mL ($P < 0.001$; Figure 1F). Overall, IGF-1 concentrations remained lower through the postcalving than precalving period ($P < 0.001$; Table 8). At most time points, median IGF-1 concentrations were lower than means, reflecting a right skewed distribution of data (Table 2).

As with the profile of IGF-1, leptin concentrations were greatest at d -30, decreasing gradually from 1.1 to 0.9 ng/mL at d -3 ($P < 0.001$; Figure 1G). Leptin concentrations were lower during the periparturient ($P < 0.001$) than precalving period, averaging 0.9 ng/mL, and then decreased ($P < 0.001$) further to stabilize at about 0.8 ng/mL during the postcalving period (Table 8).

The LSM profile of BCS (Figure 1H) remained steady at about 4.9 BCS units precalving; however, there was large variation around the means (Table 2). The BCS decreased after calving from 5.0 BCS units at d -3 to 4.2 units at d 35 ($P < 0.001$; Table 8). In comparison, BW (Figure 1I) increased ($P < 0.001$) leading up to calving, from a LSM of 486 kg at d -30 to 509 kg at d -3, before decreasing rapidly immediately after calving to 448 kg at d 1.5 ($P < 0.001$). Body weight gradually declined thereafter to reach 415 kg at d 21 ($P < 0.001$; Table 2). For both BCS and BW, precalving values were greater than the periparturient period ($P < 0.001$), with the postcalving period lower than both previous periods ($P < 0.001$; Table 8).

Indicators of Liver Function

Variations in the concentrations of globulin, AST, GDH, bilirubin, cholesterol, GGT, and liver TAG were examined as indicators of change in liver function. Plasma globulin concentration slowly decreased in the weeks preceding calving: from 39 to 30 g/L between d -30 and d -3 ($P < 0.001$; Figure 2A), and was lower during the periparturient period than either the pre- or postcalving periods ($P < 0.001$; Table 8). Concentrations of globulin increased by d 3.5 postcalving and stabilized around 35 g/L between d 21 and d 35 ($P > 0.17$). In contrast, mean AST activities (Figure 2B) gradually increased from 53 to 59 IU/L between d -30 and -3 ($P < 0.01$), followed by a rapid increase in AST to 72–80 IU/L at d 0 to 1.5 ($P < 0.001$). A greater variation among cows was indicated by larger SD val-

Table 4. Summary statistics for the indicators of protein metabolism variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED ²
Albumin (g/L)														
Mean	34.6	34.4	34.8	35.1	35.1	35.8	35.1	34.5	34.8	35.1	35.7	35.6	35.5	
SD	2.36	2.45	2.66	2.65	2.58	2.59	3.04	2.83	3.06	3.17	2.79	2.98	3.17	
Median	35.0	34.5	35.0	35.0	35.0	36.0	35.0	35.0	35.0	35.5	36.0	36.0	36.0	
Minimum ³	26.0	24.0	24.0	24.0	25.0	24.0	14.0	21.0	21.0	17.0	22.0	19.0	20.0	
Maximum ³	41.0	42.0	44.0	43.0	43.0	44.0	43.5	43.0	43.0	43.0	44.0	44.0	43.0	
n	448	895	1,227	1,276	592	1,177	882	1,174	1,425	1,360	1,147	1,523	1,176	
LSM ⁴	35.5	35.4	35.9	36.4	36.4	37.0	36.4	35.8	36.0	36.3	36.8	36.8	36.7	0.13
Total protein (g/L)														
Mean	77.2	75.3	73.2	69.8	68.4	70.1	69.4	70.1	70.6	72.9	74.8	73.7	72.9	
SD	5.41	6.78	7.22	6.86	6.32	6.23	6.02	5.78	6.21	5.89	5.45	5.35	5.75	
Median	77.5	75.0	73.0	69.0	68.0	70.0	69.5	70.0	70.0	73.0	74.0	74.0	73.0	
Minimum	61.5	52.0	50.0	49.0	43.0	47.0	26.0	45.0	48.0	39.0	49.0	52.0	49.0	
Maximum	94.0	94.0	98.0	98.0	94.0	103.0	90.0	95.0	91.0	92.0	93.0	98.0	90.0	
n	418	860	1,160	1,216	563	1,112	814	1,106	1,357	1,292	1,079	1,455	792	
LSM	74.5	72.9	71.0	68.0	66.3	67.2	65.8	67.1	69.2	70.7	72.0	71.8	72.2	0.28
Albumin:globulin ratio														
Mean	0.8	0.9	1.0	1.1	1.1	1.1	1.1	1.0	1.0	1.0	0.9	1.0	1.0	
SD	0.16	0.19	0.25	0.27	0.37	0.23	0.21	0.2	0.21	0.19	0.18	0.18	0.19	
Median	0.8	0.8	0.9	1.0	1.1	1.1	1.1	1.0	1.0	1.0	0.9	1.0	1.0	
Minimum	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.2	0.4	
Maximum	1.6	1.9	2.2	2.4	7.6	2.5	1.7	1.7	1.7	1.8	1.6	1.6	1.6	
n	418	860	1,160	1,216	563	1,112	814	1,106	1,357	1,292	1,079	1,455	792	
LSM	1.0	1.0	1.1	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1	0.01
Creatinine (μmol/L)														
Mean	63	67	67	69	66	70	59	57	56	52	50	50	49	
SD	12.7	13.8	12.4	12.6	10.9	9.8	8.4	8.1	7.1	6.4	6.4	6	6.6	
Median	63	66	66	68	66	70	59	57	56	52	50	49	48	
Minimum	42	37	30	37	40	43	22	36	34	35	33	34	34	
Maximum	105	108	106	109	96	98	89	115	79	72	71	67	67	
n	47	241	365	349	138	451	328	425	453	460	462	459	141	
LSM	64	66	66	68	67	70	60	56	55	51	50	49	47	1.1
Urea (mmol/L)														
Mean	4.9	4.9	5.0	4.9	5.1	5.8	5.1	4.5	4.5	4.4	4.3	4.9	5.1	
SD	1.08	1.2	1.34	1.36	1.3	1.56	1.49	1.55	1.35	1.44	1.31	1.69	1.75	
Median	4.9	4.7	4.8	4.8	4.9	5.8	5.1	4.5	4.3	4.3	4.2	4.7	4.8	
Minimum	1.5	2.3	2.4	2.0	1.7	1.7	2.1	1.5	1.6	1.1	1.4	1.2	1.2	
Maximum	8.1	10.4	10.8	10.1	8.6	10.6	18.1	14.4	10.3	11.8	9.1	11	11.6	
n	164	400	584	555	279	727	641	736	759	813	695	842	316	
LSM	4.8	4.9	5.1	5.1	5.1	5.9	5.1	4.6	4.5	4.5	4.5	4.9	5.1	0.12
Creatine kinase (IU/L)														
Mean	156	143	153	124	126	290	337	213	174	163	165	163	168	
SD	54	76.9	124.3	63.3	120.1	419.7	786.7	498.7	162.2	69.1	78.8	110.2	86.8	
Median	141	126	123	110	105	199	185	138	140	148	147	138	149	
Minimum	79	59	61	44	44	74	57	58	59	70	60	45	88	
Maximum	368	909	1,048	660	1,320	4,665	10,499	7,215	2,145	805	838	1,335	816	
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	187	163	173	146	158	310	351	231	192	181	183	181	199	51.9

¹-30 = 30 ± 5 d precalving; -21, -14, -7 = 21, 14, 7 ± 3 d precalving; -3 = 3 ± 1 d precalving; 0 = 1 d precalving and day of calving; 1.5 = 1 and 2 d postcalving; 3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 ± 3 d postcalving.

²SED = standard error of the difference.

³Minimum concentration observed during time point; maximum concentration observed during time point.

⁴LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number). Effect of day was significant ($P < 0.001$) for all variables.

ues during the first week following calving (Table 3). Generally, postcalving AST activity remained greater than precalving ($P < 0.001$; Table 8) and stabilized around 77 to 79 IU/L, with no differences between d 3.5 and 35 ($P > 0.26$; Table 3).

Mean GDH activity (Figure 2C) was stable precalving, ranging between 10 and 14 IU/L during d -30 and -3 ($P > 0.87$). Glutamate dehydrogenase activity increased following calving and reached 23 IU/L by d 7 ($P < 0.01$ relative to d 1.5 or 3.5). Thereafter, GDH activity remained greater during the postcalving period than the periparturient and precalving periods ($P < 0.001$; Table 8) and stabilized around 26 IU/L with no significant differences between d 14 and 35 ($P > 0.93$). However, greater variation in GDH was evident (i.e., larger SD values) during the periparturient and postcalving periods (Table 3). In addition, median GDH activity was lower than means from d 7 to 35, indicating a right-skewed distribution of data at these time points (Table 3).

Cholesterol decreased steadily from 2.6 mmol/L at d -14 and -7 to 1.9 mmol/L at d 1.5 ($P < 0.001$; Figure 2D), before increasing over the first few weeks of lactation to reach 2.8 mmol/L by d 21. Bilirubin increased from 1.1 to 2.5 $\mu\text{mol/L}$ between d -30 and -3 ($P < 0.05$), which was followed by a rapid increase at calving to a peak mean concentration of 5.5 $\mu\text{mol/L}$ at d 3.5 ($P < 0.001$ relative to d -3; Figure 2E). Thereafter, bilirubin concentrations decreased to 1.4 $\mu\text{mol/L}$ at d 35 ($P < 0.001$); however, the postcalving LSM remained higher than the precalving LSM ($P < 0.001$; Table 8). An increased degree of variation in bilirubin was indicated by larger SD values during the periparturient period and the first week postcalving (Table 3).

Mean activities of GGT (Figure 2F) were stable, averaging 20 IU/L, during the 4 wk leading up to calving ($P > 0.99$); there was a rapid increase in activity to 28 IU/L on the day of calving ($P < 0.01$ relative to each time point precalving). During the first week postcalving, GGT activity decreased ($P < 0.001$) to 24 IU/L by d 3.5 and remained at this level to d 35 ($P > 0.93$). Accordingly, postcalving GGT activity was greater, on average, than precalving activity ($P < 0.001$; Table 8). Large variation was also apparent around the means for each time point, especially in the periparturient and postcalving periods (Table 3).

During the precalving period, liver TAG content (Figure 2G) was stable ($P > 0.75$), averaging 1.6% wet weight, with little variation at each time point; however, both the LSM, mean, and SD of TAG content increased at d 1.5 and remained greater throughout the postcalving period, averaging 3.3% wet weight ($P < 0.001$; Table 3). Precalving prevalence of mild hepatic lipodosis (1–5% wet weight) ranged from 100% at d -30

to 75% at d -3, but there was no moderate or severe hepatic lipodosis prevalent. Following calving, mild hepatic lipodosis prevalence gradually decreased from 93% at d 0 to 57% at d 35, and moderate hepatic lipodosis (5–10% wet weight) prevalence increased from 2% at d 0 to 13% at d 28. Severe hepatic lipodosis (>10% wet weight) was only prevalent from d 14, ranging from 3% at this time to 5% at d 28. Overall, most cows with TAG data had some degree of increased hepatic lipodosis postcalving. Furthermore, the median of TAG content was lower than the mean from d 3.5 to 35, indicating a skew in the distribution of TAG data at each of these time points (Table 3).

Indicators of Protein Metabolism

Variations in the mean concentrations of albumin, total protein, albumin:globulin ratio (AGR), creatinine, urea, and CK were examined to provide an indication of protein metabolism. Mean concentrations of albumin (Figure 3A) varied within a 2 g/L range during the observation period (Table 4). However, there was large variation around the means at each time point. Albumin concentrations were lowest at 35.5 and 35.4 g/L at d -30 and -21, respectively, and increased ($P < 0.001$) thereafter to peak at 37.0 g/L on the day of calving. Following calving, albumin immediately decreased ($P < 0.001$) to a nadir of 35.8 g/L at d 3.5, before gradually increasing again to 36.8 at d 21 (Table 4). There was no difference between pre- and postcalving average plasma albumin concentrations, although albumin was greater during the periparturient period ($P < 0.001$; Table 8).

Total protein decreased from a maximum of 74.5 to 66.3 g/L between d -30 and -3 ($P < 0.001$; Figure 3B) and remained lower during the periparturient period ($P < 0.001$). Circulating concentrations of total protein increased from 67.1 g/L at d 3.5 to 72.0 g/L at d 21 ($P < 0.001$) and remained stable thereafter ($P > 0.52$). The AGR increased from 1.0 to 1.2 between d -30 and -7 ($P < 0.001$; Figure 3C) and remained at this ratio during the periparturient period until d 3.5 when the AGR decreased ($P < 0.001$) to a plateau at 1.1 between d 7 and 35 (Table 4).

Creatinine concentrations were greatest and had a higher SD during the precalving period (Figure 3D; Table 4). Creatinine gradually increased from 64 $\mu\text{mol/L}$ at d -30 to 68 $\mu\text{mol/L}$ at d -7 ($P < 0.01$). Immediately following calving, creatinine decreased from a maximum of 70 $\mu\text{mol/L}$ at d 0 to 60 $\mu\text{mol/L}$ by d 1.5 ($P < 0.001$), with a steady decline thereafter to 47 $\mu\text{mol/L}$ at d 35. Urea concentrations remained stable throughout the precalving period ($P > 0.19$; Figure 3E; Table 4), averaging 5.1 mmol/L. Urea con-

Table 5. Summary statistics for the indicators of mineral status variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED ²
Calcium														
(mmol/L)														
Mean	2.38	2.32	2.32	2.30	2.32	2.03	2.09	2.26	2.27	2.31	2.31	2.31	2.28	
SD	0.18	0.19	0.16	0.17	0.16	0.33	0.27	0.2	0.16	0.17	0.15	0.17	0.18	
Median	2.39	2.32	2.33	2.32	2.33	2.09	2.15	2.28	2.27	2.32	2.31	2.31	2.29	
Minimum ³	1.65	1.47	1.56	1.65	1.78	0.1	0.77	1.18	1.56	1.28	1.73	1.39	1.43	
Maximum ³	3.02	2.77	2.83	2.8	2.72	3.44	3.1	3.08	2.93	2.93	2.84	2.81	2.72	
n	174	442	757	1,009	515	1,245	964	1,281	1,468	1,233	737	1,190	605	
LSM ⁴	2.32	2.29	2.29	2.28	2.29	2.00	2.06	2.23	2.25	2.28	2.28	2.27	2.25	0.016
Magnesium														
(mmol/L)														
Mean	0.83	0.84	0.82	0.79	0.77	0.87	0.81	0.75	0.77	0.82	0.87	0.86	0.85	
SD	0.15	0.129	0.137	0.143	0.153	0.197	0.182	0.142	0.162	0.169	0.154	0.181	0.177	
Median	0.84	0.85	0.83	0.82	0.80	0.88	0.83	0.76	0.78	0.84	0.88	0.88	0.87	
Minimum	0.27	0.38	0.28	0.13	0.18	0.11	0.15	0.17	0.15	0.22	0.28	0.13	0.22	
Maximum	1.34	1.27	1.4	1.32	1.17	1.63	1.4	1.23	1.21	1.34	1.33	1.79	1.95	
n	233	649	970	1,200	606	1,236	981	1,282	1,643	1,446	949	1,403	750	
LSM	0.79	0.79	0.78	0.76	0.75	0.83	0.78	0.71	0.73	0.78	0.82	0.81	0.82	0.010
Phosphate														
(mmol/L)														
Mean	1.8	1.9	1.8	1.7	1.6	1.3	1.2	1.3	1.4	1.5	1.5	1.5	1.6	
SD	0.47	0.41	0.48	0.49	0.57	0.5	0.47	0.46	0.35	0.34	0.36	0.4	0.42	
Median	1.9	1.9	1.8	1.8	1.7	1.3	1.2	1.2	1.4	1.5	1.5	1.5	1.6	
Minimum	0.6	0.5	0.5	0.4	0.4	0.2	0.3	0.2	0.3	0.3	0.5	0.4	0.7	
Maximum	2.7	3	3.5	3	3.2	2.5	2.7	3.5	2.7	2.9	3.1	3.4	2.7	
n	51	261	422	421	209	530	408	505	466	511	465	533	144	
LSM	1.9	1.9	1.8	1.7	1.6	1.4	1.3	1.3	1.5	1.5	1.6	1.6	1.6	0.06
Potassium														
(mmol/L)														
Mean	4.4	4.4	4.3	4.3	4.3	4.4	4.8	4.7	4.7	4.7	4.7	4.7	4.8	
SD	0.35	0.34	0.34	0.33	0.33	0.41	0.46	0.41	0.35	0.42	0.5	0.46	0.39	
Median	4.4	4.4	4.3	4.3	4.2	4.4	4.9	4.8	4.8	4.7	4.8	4.8	4.8	
Minimum	3.8	3.6	3.4	3	3.6	3	2.1	3.8	3.9	3.6	3	3.7	3.9	
Maximum	5.2	5.2	5.4	5.2	5.1	5.5	6	6	5.9	6	6	6	5.7	
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	4.4	4.3	4.3	4.3	4.2	4.4	4.8	4.7	4.7	4.6	4.7	4.7	4.7	0.07
Sodium														
(mmol/L)														
Mean	140.2	140.2	140.2	141.2	141.1	144.3	139.3	140.2	138.7	138.1	138.4	138.2	140.4	
SD	3.73	4.87	5.77	4.56	4.46	3.68	7.79	3.34	2.93	2.74	3.76	3.38	3.27	
Median	140.5	141.0	141.0	141.0	142.0	145.0	141.0	140.0	138.4	138.0	138.0	138.0	140.0	
Minimum	122.5	108.0	78.5	113.5	121.0	127.0	58.0	128.0	114.0	118.5	113.0	127.0	134.0	
Maximum	145.0	150.0	148.0	150.0	147.7	158.0	149.0	150.0	146.0	146.5	149.0	146.0	149.0	
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	139.4	139.2	139.6	140.5	140.7	143.7	139.3	139.7	138.1	137.6	137.9	137.7	139.0	0.73
Chloride														
(mmol/L)														
Mean	97.1	97.3	97.8	99.4	98.8	101.5	98.9	99.3	97.9	97.1	97.0	96.6	96.6	
SD	3.05	3.66	4.34	4.01	3.75	3.57	6.18	3.34	2.22	2.65	2.99	2.59	3.40	
Median	97.0	98.0	98.0	99.5	99.0	102.0	100.0	99.0	98.0	97.0	97.0	97.0	96.0	
Minimum	84.5	72.0	55.5	80.5	84.0	91.0	39.0	84.0	83.0	86.5	82.0	89.0	88.0	
Maximum	103.0	106.0	105.0	108.0	105.0	111.0	107.0	110.0	104.5	104.0	105.0	104.0	105.0	

Continued

Table 5 (Continued). Summary statistics for the indicators of mineral status variables by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	35	SED ²
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	96.1	96.3	97.1	98.8	98.2	100.9	98.5	98.7	97.3	96.5	96.4	95.9	95.5	0.61
Bicarbonate														
(mmol/L)														
Mean	24.2	22.7	23.3	23.8	26.6	24.9	23.7	23.7	24.1	25.2	25.4	25.6	23.6	
SD	4.08	3.79	3.78	3.49	2.27	3.24	2.29	3.20	3.09	2.86	3.06	3.22	3.65	
Median	25.0	23.5	24.0	25.0	27.0	25.0	24.0	24.0	25.0	26.0	26.0	26.0	23.0	
Minimum	16.0	17.0	15.5	16.0	17.0	17.0	8.0	15.0	16.5	17.5	16.0	17.0	17.0	
Maximum	30.0	31.5	31.0	30.5	30.3	37.0	31.0	33.0	31.0	32.0	32.0	35.0	33.0	
n	44	241	335	310	126	411	311	415	413	415	417	414	117	
LSM	24.4	23.9	23.9	24.2	25.6	25.3	22.7	24.1	24.4	25.5	25.8	25.9	25.7	0.37

¹-30 = 30 ± 5 d precalving; -21, -14, -7 = $21, 14, 7 \pm 3$ d precalving; -3 = 3 ± 1 d precalving; 0 = 1 d precalving and day of calving; 1.5 = 1 and 2 d postcalving; 3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 ± 3 d postcalving.

²SED = standard error of the difference.

³Minimum concentration observed during time point; maximum concentration observed during time point.

⁴LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect; treatment within experiment; repeated variable = animal number). Effect of day was significant ($P < 0.0001$) for all variables.

centration increased to 5.9 mmol/L at calving ($P < 0.001$ relative to all time points), but decreased ($P < 0.001$) back to 5.1 mmol/L at d 1.5 and further decreased ($P < 0.001$) to 4.6 mmol/L at d 3.5 (Figure 3E). Concentrations of urea remained constant at approximately 4.5 mmol/L between d 3.5 and 21 ($P > 0.81$) and increased ($P < 0.001$) thereafter to reach 5.1 mmol/L at d 35. Urea concentrations were lower postcalving than both the precalving and periparturient periods ($P < 0.001$), with the periparturient greater than the precalving period ($P < 0.001$; Table 8). During the pre- and postcalving periods, mean CK activity (Figure 3F) remained relatively stable between 146 and 231 IU/L and did not differ ($P = 0.11$; Table 8). However, CK activities doubled to 351 IU/L at d 1.5 ($P < 0.05$ relative to each time point in the pre- and postcalving periods) and were highly variable through the periparturient period (Table 4). Median CK activity was consistently lower than the mean activity throughout the observational period, and by a considerable amount at some time points, thereby suggesting a large skew in the distribution of CK data (Table 4).

Indicators of Mineral Balance

Minerals that were measured as indicators of transition cow health were Ca, Mg, phosphate (PO_4), K, Na, Cl, and bicarbonate. Mean Ca concentration during the precalving period was approximately 2.3 mmol/L, with a rapid decrease in circulating concentration from 2.29 mmol/L to a nadir of 2.00 mmol/L between d -3 and d 0 ($P < 0.001$). Following calving, mean Ca concentration increased, remaining at approximately 2.2 mmol/L from d 3.5 to 35 (Table 5). Prevalence of subclinical hypocalcemia using a cut point of <2.0 mmol/L was 35% and 28% on d 0 and 1.5, respectively. Using a greater cut point of ≤ 2.15 mmol/L, it increased to 60% and 50% on d 0 and 1.5, respectively. The prevalence of clinical hypocalcemia (≤ 1.4 mmol/L) was 6% and 3% on d 0 and 1.5, respectively.

The profile of mean Mg concentration (Figure 4B) was inverse to Ca, with an increase in concentration from 0.75 to 0.83 mmol/L between d -3 and 0 ($P < 0.001$; Table 5). There were no differences between pre- and postcalving Mg concentration, with a LSM of 0.81 mmol/L for both periods ($P = 0.75$; Table 8). The prevalence of subclinical hypomagnesemia (≤ 0.8 mmol/L) and clinical hypomagnesemia (≤ 0.5 mmol/L) consistently ranged from 30% to 46% and 2% to 6%, respectively, throughout the observational period. Subclinical hypomagnesemia peaked during the periparturient period at 64% at d 3.5. Prevalence of hypomagnesemia (≥ 1.1 mmol/L) on d 0 was 9%, which

Table 6. Summary statistics for the inflammatory state markers by day relative to calving for grouped time points 30 ± 5 d precalving to 35 ± 3 d postcalving

Day ¹	-30	-21	-14	-7	-3	0	1.5	3.5	7	14	21	28	SED ²
IL-1β (pg/mL)													
Mean	20.3	22.7	15.0	47.1	17.6	20.4	21.0	25.4	18.5	18.7	14.3	16.4	
SD	2.09	43.47	11.03	83.43	8.47	11.93	11.98	26.72	13.24	10.62	14.33	28.17	
Median	20.3	12.5	11.1	20.2	15.9	16.0	18.4	20.4	12.7	18.5	11.2	10.0	
Minimum ³	18.8	4.7	5.5	5.9	6.3	7.6	7.0	3.3	3.3	4.8	4.3	4.0	
Maximum ³	21.8	248.4	52.3	449.7	44.2	66.2	91.3	250.4	90.6	64.3	114.3	306.1	
n	2	30	38	81	42	66	100	117	145	77	69	133	
LSM ⁴	32.0	32.0	24.0	48.9	21.7	25.8	23.9	26.4	21.7	23.5	18.9	20.5	6.68
IL-6 (pg/mL)													
Mean	108.9	158.0	182.4	1,315.1	474.6	510.4	1,228.0	2,382.0	730.6	330.0	307.0	968.2	
SD	5.60	112.54	221.13	1,632.00	492.93	1,092.58	2,019.80	2,585.83	1,173.53	460.15	411.42	2,004.30	
Median	108.9	112.8	93.4	687.2	340.3	224.3	416.2	1,233.3	307.8	172.4	137.6	218.2	
Minimum	105.0	66.1	6.2	36.4	6.2	0.8	0.8	138.3	0.8	5.4	21.9	24.8	
Maximum	112.9	603.2	881.8	10,000.0	2,165.7	8,384.7	10,000.0	10,000.0	10,000.0	3,603.6	1,882.5	10,000.0	
n	2	30	38	81	42	66	100	117	145	77	69	133	
LSM	650.5	650.5	703.0	968.8	647.3	658.8	1,250.0	2,081.2	690.5	420.3	544.6	895.4	323.13
Haptoglobin													
(μ g/mL)													
Mean	1.5	4.1	3.4	8.8	2.7	12.3	55.7	26.4	11.9	11.2	9.3	18.1	
SD	0.25	3.13	4.85	36.92	3.21	17.76	102.79	57	28.55	27.66	21.91	54.41	
Median	1.5	3.3	1.6	5.5	1.4	6.5	16.2	5.7	6.2	5.4	4.0	5.9	
Minimum	1.3	0.6	0.1	0.1	0.1	0.7	1.9	0.8	1.3	0.7	0.1	0.5	
Maximum	1.7	13.4	26.1	336.2	15.6	87.6	688.8	346.8	216.8	216.8	132.5	405.4	
n	2	30	38	81	42	66	100	115	144	77	69	133	
LSM	0	0	0	0	0	0	45.2	17.8	0.4	0	0	6.0	35.50
Reactive oxygen species													
(μ mol/L)													
Mean	1.63	1.70	1.39	0.32	0.68	0.96	0.77	0.49	0.99	1.10	1.22	0.98	
SD	0.100	0.248	0.106	0.266	0.668	0.620	0.547	0.275	0.585	0.546	0.668	0.592	
Median	1.63	1.66	1.37	0.30	0.35	1.26	0.72	0.43	0.87	1.31	1.42	0.82	
Minimum	1.56	1.31	1.26	0.01	0.01	0.01	0.01	0.04	0.03	0.30	0.16	0.16	
Maximum	1.70	2.37	1.79	1.39	1.50	2.12	1.61	1.68	3.81	1.94	2.34	2.24	
n	2	30	38	81	42	66	100	116	145	77	69	133	
LSM	0.49	0.49	0.20	0.03	0.05	0.16	0.19	0.26	0.49	0.39	0.44	0.44	0.08
Total antioxidant capacity													
(mmol/L)													
Mean	2.7	3.2	1.9	1.8	1.6	1.8	1.4	1.9	2.0	1.3	2.7	2.8	
SD	0.16	0.60	0.55	1.13	1.00	1.55	1.78	1.60	1.95	0.85	1.33	1.18	
Median	2.7	3.3	1.8	1.8	1.4	1.5	1.0	1.6	1.6	1.3	3.0	2.6	
Minimum	2.6	1.7	0.1	0.0	0.1	0.1	0.3	0.0	0.0	0.0	0.1	0.0	
Maximum	2.8	4.3	3.4	4.6	4.2	11.3	11.7	11.1	11.3	3.3	4.7	5.1	
n	2	30	38	81	41	66	100	117	143	77	69	133	
LSM	4.0	4.0	2.6	2.5	2.3	2.5	2.1	2.5	2.6	2.0	3.3	3.5	0.36

¹-30 = 30 $\pm$ 5 d precalving; -21, -14, -7 = 21, 14, 7 $\pm$ 3 d precalving; -3 = 3 $\pm$ 1 d precalving; 0 = 1 d precalving and day of calving; 1.5 = 1 and 2 d postcalving; 3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 $\pm$ 3 d postcalving.

²SED = standard error of the difference.

³Minimum concentration observed during time point; maximum concentration observed during time point.

⁴LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number). Effect of day was significant ($P < 0.001$) for all variables.

Table 7. Summary statistics for indicators of uterine health markers by day relative to calving for grouped time points 3 ± 1 d postcalving to 35 ± 3 d postcalving

Day ¹	3.5	7	14	21	28	35	SED ²	Day
PMN (%)								
Mean		48	35	35	21	11		
SD		37	31.1	33.4	28.3	16.5		
Median		53	26	24	5	4		
Minimum ³		0.2	0	0	0	0		
Maximum ³		90	100	99	99	100		
n		10	1,097	696	288	494		
LSM ⁴		47	39	35	23	14	8.9	<0.001
Macrophage (%)								
Mean			2	3	0.6	0.6		
SD			3.7	6.1	2.1	1.4		
Median			0.49	1.0	0.00	0.18		
Minimum			0	0	0	0		
Maximum			44	41	16	16		
n			763	242	73	269		
LSM			2	4	2	1	0.5	<0.001
Vaginal discharge score (0–5 scale)								
Mean	2.2	2.2	1.5	1.5	1.3	1.1		
SD	1.36	1.42	1.21	1.29	0.73	0.77		
Median	2.0	2.0	1.0	1.0	1.0	1.0		
Minimum	1	0	0	0	0	0		
Maximum	5	5	5	5	5	5		
n	308	17	1,118	700	306	499		
LSM	1.5	1.8	1.4	1.2	1.1	1.1	0.27	<0.001

¹3.5 = 3 and 4 d postcalving; 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 $\pm$ 3 d postcalving.

²SED = standard error of the difference.

³Minimum concentration observed during time point; maximum concentration observed during time point.

⁴LSM for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number).

decreased immediately to 5% at d 1.5 and further to 1% at d 7.

Mean PO_4 concentrations decreased through the precalving period, from 1.9 mmol/L at d -30 to 1.6 mmol/L at d -3 ($P < 0.001$; Figure 4C). Concentrations of PO_4 rapidly decreased on the day of calving to 1.4 mmol/L, followed by a further decrease to a nadir of 1.3 mmol/L at d 1.5 (Table 5). By d 7, concentrations of PO_4 had increased to 1.5 mmol/L, but they remained lower postcalving than precalving ($P < 0.001$; Table 8).

Concentrations of K remained relatively stable around 4.3 mmol/L through the precalving period (Figure 4D; $P > 0.90$ relative to all precalving time points), before increasing on the day of calving to 4.8 mmol/L at d 1.5 ($P < 0.001$; Table 5). Mean K concentrations remained around 4.7 mmol/L thereafter, which resulted in greater concentrations postcalving compared with precalving ($P < 0.001$; Table 8).

Mean concentrations of Na (Figure 4E) also remained stable around 139 mmol/L precalving, but increased ($P < 0.001$; Table 5) on the day of calving to 144 mmol/L. By d 1.5, Na concentrations had decreased and, on average, remained lower than the precalving and periparturient periods at a concentration of approximately 137 mmol/L from d 7 to 28 ($P < 0.001$; Table 5).

The profile of mean Cl concentration (Figure 4F) was similar to that of Na, although there was a slight increase in Cl concentration through the precalving period ($P < 0.001$; Table 5). Peak LSM Cl concentration of 101 mmol/L occurred on the day of calving, with an immediate decrease to 98.5 mmol/L at d 1.5, and a gradual decrease back to 96 mmol/L by d 14 (Table 5).

Overall, bicarbonate concentrations during the postcalving period were greater than those during the periparturient period, which, in turn, were greater than the precalving period ($P < 0.05$; Table 8). There was a sharp decrease at calving from 25.3 mmol/L on d 0 to 22.7 mmol/L on d 1.5 ($P < 0.001$; Table 6).

Indicators of Inflammation

Changes in concentrations of IL-1 β , IL-6, Hp, ROS, and TAC were examined as indicators of inflammation. Mean IL-1 β concentration (Figure 5A) peaked on d -7 at 48.9 pg/mL ($P < 0.01$), but with a large SD, before decreasing to a relatively stable postcalving concentration of approximately 20 to 25 pg/mL by d 0 ($P > 0.86$ relative to all postcalving time points; Table 6). Consequently, the precalving LSM tended to be greater than the LSM for the periparturient ($P = 0.06$) and was greater than the postcalving ($P < 0.001$)

Table 8. Least squares means of repeated measures model¹ for each of the indicators of energy metabolism, liver function, protein metabolism, mineral status, acute phase, and uterine and animal health for the precalving (Pre; d -35 to -2), periparturient (Peri; d -1 to 2), and postcalving (Post; d 4 to 38) periods

Item ²	Pre	Peri	Post	SED ³	Period
Energy metabolism					
BHB (mmol/L)	0.60	0.75	0.84	0.012	<0.001
NEFA (mmol/L)	0.47	0.62	0.77	0.011	<0.001
Glucose (mmol/L)	3.66	3.99	3.41	0.025	<0.001
Insulin (μU/mL)	6.77	7.29	4.27	0.271	<0.001
Growth hormone (ng/mL)	4.12	18.98	10.02	1.210	<0.001
IGF (ng/mL)	23.7	9.4	8.8	0.45	<0.001
Leptin (ng/mL)	1.1	0.9	0.8	0.02	<0.001
BCS (1–10)	4.9	4.7	4.4	0.02	<0.001
BW (kg)	503	466	427	0.86	<0.001
Liver function					
Globulin (g/L)	37	32	37	0.1	<0.001
AST (IU/L)	58	77	78	0.9	<0.001
GDH (IU/L)	18	22	30	1.0	<0.001
Cholesterol (mmol/L)	2.7	2.2	2.6	0.06	<0.001
Bilirubin (μmol/L)	2.9	5.1	4.3	0.14	<0.001
GGT (IU/L)	20	28	24	0.9	<0.001
Liver TAG (% of wet weight)	1.6	2.6	3.3	0.23	<0.001
Protein metabolism					
Albumin (g/L)	35	36	35	0.1	<0.001
Total protein (g/L)	72	68	72	0.1	<0.001
Albumin:globulin ratio	1.0	1.2	1.0	0.01	<0.001
Creatinine (μmol/L)	66	65	51	0.4	<0.001
Urea (mmol/L)	5.1	5.5	4.7	0.05	<0.001
Creatine kinase (IU/L)	137	306	175	19	<0.001
Mineral balance					
Sodium (mmol/L)	140.8	142.7	139.0	0.21	<0.001
Potassium (mmol/L)	4.3	4.6	4.7	0.02	<0.001
Chloride (mmol/L)	98.1	100.9	97.7	0.19	<0.001
Bicarbonate (mmol/L)	24.4	24.7	25.5	0.11	<0.001
Phosphate (mmol/L)	1.8	1.3	1.5	0.02	<0.001
Calcium (mmol/L)	2.32	2.06	2.28	0.007	<0.001
Magnesium (mmol/L)	0.81	0.84	0.81	0.005	<0.001
Inflammation					
IL-1β (pg/mL)	35.0	24.3	19.0	4.68	<0.01
IL-6 (pg/mL)	602.2	956.2	784.4	120.57	0.32
Haptoglobin (μg/mL)	0.0	31.9	10.5	4.68	<0.001
ROS (μmol/L)	0.35	0.53	0.63	0.072	<0.001
TAC (mmol/L)	2.9	2.6	3.1	0.16	<0.01
Uterine health					
PMN (%)			29	3.9 ⁴	
Macrophage (%)			2	0.8 ⁴	
Vaginal discharge score (0–5)			1.2	0.14 ⁴	

¹Least squares means for repeated measures model, where variable = breed + parity group + calving season day + period (random effect: treatment within experiment; repeated variable = animal number).

²NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GDH = glutamate dehydrogenase; GGT = gamma-glutamyl transpeptidase; TAG = triacylglyceride; ROS = reactive oxygen species; TAC = total antioxidant capacity; BCS (1–10 scale; Roche et al., 2004).

³SED = standard error of the difference.

⁴SEM for variables with only postcalving measurements.

periods, which did not differ from each other ($P = 0.48$; Table 8). Mean concentrations of IL-6 (Figure 5B) were greatest during the first 4 d after calving, trebling from 647.3 pg/mL at d -3 to 2,081.2 pg/mL at d 3.5 ($P < 0.001$), before declining again. A large SD and range of concentrations for IL-6 were also apparent during this time (Table 6). Overall, there was an increase in IL-6 concentration from the precalving to periparturi-

ent period ($P < 0.01$); however, there was no difference between the pre- and postcalving concentrations or between periparturient and postcalving concentrations ($P > 0.17$; Table 8).

Mean Hp concentration (Figure 5C) was lowest during the precalving period, with an increase in LSM, mean, and SD at calving, to a peak of 45.2 μg/mL at d 1.5 ($P < 0.001$; Table 6). Haptoglobin was lower

during the postcalving period than the periparturient period ($P < 0.001$) but remained greater than precalving ($P = 0.02$; Table 8). The LSM concentration of ROS decreased through the precalving period from 0.49 $\mu\text{mol/L}$ at d -21 to 0.03 $\mu\text{mol/L}$ at d -3 ($P < 0.001$; Figure 5D). By d 7, concentrations had increased back to 0.49 $\mu\text{mol/L}$ and remained stable thereafter ($P > 0.60$ relative to all postcalving time points; Table 6).

Overall, postcalving ROS concentrations were greater than precalving concentrations ($P < 0.001$), with periparturient concentrations intermediary (Table 8).

For IL-1 β , IL-6, Hp, and ROS, median concentrations were lower than means at most of the time points, indicating a skewed distribution for each of these indicators of inflammation during most of the observational period (Table 6).

Figure 1. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for concentrations of energy indicator analytes. (A) β -Hydroxybutyrate (mmol/L), (B) nonesterified fatty acids (mmol/L), (C) glucose (mmol/L), (D) insulin ($\mu\text{U/mL}$), (E) growth hormone (ng/mL), (F) IGF-1 (ng/mL), (G) leptin (ng/mL), and animal health measures (H) BCS (1–10 scale) and (I) BW (kg) for the population of grazing dairy cows in the collated database with selected grouped time points by days relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving, d $-30 = 30 \pm 5$ d precalving; d $-21, -14, -7 = 21, 14, 7 \pm 3$ d precalving; d $-3 = 3 \pm 1$ d precalving; d 0 = d 1 precalving and day of calving; d 1.5 = d 1 and 2 postcalving; d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, and 35 d ± 3 d postcalving.

Mean TAC concentration was greatest at 4.0 mmol/L at d -21, decreasing to a relatively stable concentration of around 2.5 mmol/L from d -14 to 7 ($P > 0.88$ relative to any time point between; Figure 5E). Concentrations reached a nadir of 2.0 mmol/L at d 14 before increasing to 3.3 mmol/L at d 21 ($P < 0.001$; Table 6). As a result, TAC concentrations during the periparturient period tended to be lower than the pre-

calving period ($P = 0.07$), with no difference between the pre- and postcalving periods ($P > 0.49$; Table 8).

Uterine Health

Together, PMN %, macrophage %, and vaginal discharge score provide an indication of uterine health. From d 7 to 35, PMN % (Figure 6A) decreased from a

Figure 2. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for concentrations of liver function analytes. (A) Globulin (g/L), (D) cholesterol (mmol/L), (E) bilirubin (mmol/L), and (G) liver triacylglyceride (% of wet weight) and activities of liver enzymes, (B) aspartate aminotransferase (IU/L), (C) glutamate dehydrogenase (IU/L), and (F) γ -glutamyl transpeptidase (IU/L) for the population of grazing dairy cows in the collated database with selected grouped time points by day relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, and group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving. d -30 = 30 d $\pm$ 5 d precalving; d -21, -14, -7 = 21, 14, 7 d $\pm$ 3 d precalving; d -3 = d 3 $\pm$ 1 d precalving; d 0 = d 1 precalving and day of calving; d 1.5 = d 1 and 2 postcalving; d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 d $\pm$ 3 d postcalving.

Figure 3. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for concentrations of protein metabolism analytes. (A) Albumin (g/L), (B) total protein (g/L), (C) albumin:globulin ratio, (D) creatinine ($\mu\text{mol/L}$), (E) urea (mmol/L) and protein metabolism enzyme activities (F) creatine kinase (IU/L) for the population of grazing dairy cows in the collated database with selected grouped time points by days relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, and group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving. d -30 = 30 d $\pm$ 5 d precalving; d -21, -14, -7 = 21, 14, 7 d $\pm$ 3 d precalving; d -3 = d 3 $\pm$ 1 d precalving; d 0 = d 1 precalving and day of calving; d 1.5 = d 1 and 2 postcalving; d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 d $\pm$ 3 d postcalving.

LSM of 47% to 14% in a linear fashion ($P < 0.001$). In contrast, macrophage % (Figure 6B) increased 2-fold from 2% to 4% between d 14 and 21 ($P < 0.001$) but decreased thereafter to 1% by d 35. Vaginal discharge score (Figure 6C) decreased gradually ($P < 0.001$) over the first 5 wk of lactation, from a mean score of about 2 at d 3.5 to a score of about 1 at d 35 (Table 7).

DISCUSSION

We present a compilation from 20 experiments spanning almost 20 years, as a comprehensive research data set for temporal profiles of blood analytes for pasture-fed transition dairy cows in New Zealand. The data set provides a valuable reference base for various animal health markers of relevance to transition cows in pasture-fed, seasonal-calving dairy systems. Here, we discuss the key temporal trends and range in values observed for various energy, protein, mineral, liver function, inflammation, and uterine and animal health variables in New Zealand pasture-based cows.

Profiles of Energy Metabolism Are Consistent with Uncoupling of the Somatotrophic Axis

The presented profiles of plasma GH and IGF-1, and the associated changes in insulin, NEFA, BHB, BW, and BCS, are consistent with a peripartum uncoupling of the somatotrophic axis in anticipation of the energetic demands of lactogenesis and an associated increase in hepatic gluconeogenesis. Postcalving, plasma GH concentrations were greater than precalving, with a concurrent decrease in insulin, glucose, and IGF-1 concentrations in the days immediately following calving. Furthermore, the greater plasma NEFA concentrations represent greater utilization of adipose tissue reserves immediately postcalving, which is reflected in the decrease in BCS and BW (Roche et al., 2009a). The release of GH is pulsatile, and hence, collecting a single blood sample for GH measurement on a particular day may not produce an accurate assessment of the GH status for a particular animal, but in our data set this is mitigated by the large population of animals that had

Figure 4. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for concentrations of mineral balance analytes. (A) Calcium (mmol/L), (B) magnesium (mmol/L), (C) phosphate (mmol/L), (D) potassium (mmol/L), (E) sodium (mmol/L), (F) chloride (mmol/L), and (G) bicarbonate (mmol/L) for the population of grazing dairy cows in the collated database with selected grouped time points by days relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, and group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving. d -30 = 30 d $\pm$ 5 d precalving; d -21, -14, -7 = 21, 14, 7 d $\pm$ 3 d precalving; d 0 = d 1 precalving and day of calving; d 1.5 = d 1 and 2 postcalving; d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 d $\pm$ 3 d postcalving.

blood samples collected on the same day. The periparturient changes to blood analytes representative of changes in energy balance are well established in dairy cows (Bauman and Currie, 1980; Lucy et al., 2009).

The profile of IGF-1 presented is consistent with other experiments investigating pasture-based cows (Lucy et al., 2009), but the concentration of total IGF-1 in circulation is less than many other studies. We would expect the concentrations to be less because of the lack of NFC in the diet (Kolwer et al., 2006) and

the older age structure of the cows in this study relative to many other studies (Kerr et al., 1991). Plasma concentrations of IGF-1 increase when pasture-fed cows are supplemented with corn silage (Lucy et al., 2009) or a grain-based concentrate feed (Kolwer et al., 2006). Some of the difference in reported values, however, may also be a result of differences in laboratory assays and caution is recommended in cross-study comparisons of analyte concentrations. For example, Chanson et al. (2016) reported that different laboratory methods can

result in concentration differences of >50%, even when IGF-1 kit manufacturers follow consensus guidelines. Despite the different concentrations produced, however, pairwise concordances were moderate to good. The profile of IGF-1 presented here is consistent with the reported changes in expression of hepatic growth hormone-receptor-1A and IGF-1 in grazing dairy cows (Lucy et al., 2009).

Peripartum Plasma NEFA and BHB Profiles Are Consistent with Profile of BCS Change, But BHB Concentrations Are Greater Than Expected

Concentrations of NEFA and BHB peaked between d 7 and 14 postcalving, consistent with the maximum rate of BCS loss reported by Roche et al. (2007) from the first derivative of the BCS profile in pasture-based cows. Circulating NEFA concentrations subsequently declined, reflecting a less severe NEB state after wk 2, which, again, is consistent with the first derivative of the BCS curve presented by Roche et al. (2007).

The BHB concentrations were greater than would be anticipated for low to moderate milk production cows consuming TMR with similar NEFA profiles. This is not unusual in cows predominantly consuming pasture and should not be confused with metabolically induced hyperketonemia. Roche et al. (2010) reported that circulating BHB concentrations in early-lactation pasture-fed dairy cows were double those of cows fed an isoenergetic equivalent diet, but with 33% of dietary ME coming from a barley-corn grain concentrate during early lactation; the difference in BHB was despite similar circulating NEFA concentrations and BCS- and BW-loss profiles. Low to moderate NFC content of the pasture diet (<15% DM in spring; Roche et al., 2009b) limits the amount of propionate resulting from microbial fermentation, decreasing the amount of acetyl-CoA entering the tricarboxylic acid cycle (Roche et al., 2009a, 2010). As a result, cellular export of ketone bodies is expected to be increased, thus increasing circulating BHB concentrations in pasture-fed cows.

Figure 5. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for the change in cytokine concentration. (A) Interleukin-1B (pg/mL) and (B) IL-6 (pg/mL), and change in indicators of inflammation analytes, (C) haptoglobin (μg/mL), (D) reactive oxygen species (μmol/L), and (E) total antioxidant capacity (mmol/L) for the population of grazing dairy cows in the collated database with selected grouped time points by days relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, and group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving. d -30 = 30 d $\pm$ 5 d precalving; d -21, -14, -7 = 21, 14, 7 d $\pm$ 3 d precalving; d -3 = d 3 $\pm$ 1 d precalving; d 0 = d 1 precalving and day of calving; d 1.5 = d 1 and 2 postcalving; d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 d $\pm$ 3 d postcalving.

Figure 6. Mean (blue triangles) and upper SD, LSM (red circles), and maximum standard error of the difference (Max SED) for uterine health measures. (A) Polymorphonuclear cells (%), (B) macrophage (%), and (C) vaginal discharge (VD) score (0–5 scale) for the population of grazing dairy cows in the collated database with selected grouped time points by days relative to calving (d 0). Least squares means for repeated measures model, where variable = breed + parity group + calving season day + grouped day relative to calving (random effect: treatment within experiment; repeated variable = animal number); breed: Holstein-Friesian ($\geq 12/16$ ths Holstein-Friesian genetics), Holstein-Friesian $\times$ Jersey crossbred, and Jersey ($\geq 12/16$ ths Jersey genetics); parity: group 1 = parity 1 animals, group 2.5 = parity 2 and 3 animals, group 5 = parity 4 to 6 animals, and group 7 = parity 7+ animals; calving season day: number of days between June 1 and calving date in the year of calving. d 3.5 = d 3 and 4 postcalving; d 7, 14, 21, 28, 35 = 7, 14, 21, 28, 35 $\pm$ 3 d postcalving.

Despite the greater herd-average BHB concentrations, however, prevalence (9%; range 2% at d 0 to 16% at d 7) of postpartum moderate hyperketonemia (BHB ≥ 1.2 mmol/L) in the current study was lower than reported for TMR-fed cows (20% to 58%; Chapinal et al., 2012; McArt et al., 2013a) and similar to the minimum prevalence reported in pasture-fed dairy cows in commercial herds by Compton et al. (2015). The results highlight that cows grazing fresh pasture have greater BHB concentrations than cows consuming TMR, but the effect is likely due to differences in the products of rumen fermentation and incomplete hepatic oxidation of NEFA rather than an indicator of severe NEB.

Liver Activity Is Increased During Early Lactation

The profiles of circulating liver enzymes and liver TAG indicate increased liver activity and metabolic load during the week immediately following calving. During the transition period, the liver is challenged by the requirement for increased hepatic gluconeogenesis and NEFA oxidation in support of the new lactational state, while at the same time overcoming some degree of hepatic lipidosis as a consequence of lipid mobilization (Lean et al., 2013). In the current study, increases in NEFA concentration began 1 wk precalving, suggesting a change in balance between energy demand and supply, a result of changes in DMI, a gravid uterus at maximum energy and protein requirements (Bell, 1995), mammary development, and colostragenesis. This is important because the ruminant liver has a limited capacity to export TAG, so hepatic concentrations can become excessive during periods of high NEFA concentration (Grummer, 1993). Decreased circulating

bilirubin concentrations, increased AST activity, and increased liver TAG content were also evident during wk 1 following calving, confirming an increased burden on the liver associated with greater lipolysis or a failure to completely oxidize mobilized NEFA, even in moderate-yielding cows.

Inflammation Is Initiated Following Calving

Peripheral markers of inflammation indicated enhanced activation of the immune system in the days following calving, even in cows displaying no clinical signs of metabolic stress. Inflammation is activated by components of the adaptive and innate immune system in response to stimuli associated with bacterial infection and tissue injury and that trigger coordinated responses (i.e., the acute phase response; **APR**) to restore homeostasis and destroy pathogens (Bradford et al., 2015; Carneiro et al., 2016). The APR is characterized by increases in positive acute phase proteins (**APP**), cytokines, and other markers of inflammation, and decreases in negative APP (Bertoni et al., 2008; Trevisi et al., 2010, 2015). Haptoglobin, a commonly measured APP, and IL-6, a less commonly measured cytokine, both increased in circulation in the days immediately following calving, consistent with profiles characterizing the APR in housed cows fed TMR (Humblet et al., 2006; Huzzey et al., 2009; Bossaert et al., 2012). Furthermore, albumin is one of the most commonly measured negative APP, which decreases in concentration due to downregulation of hepatic synthesis, increasing availability of amino acids for the production of positive APP and ensuring osmotic pressure is maintained between the blood vessels and tissues

(Ceciliani et al., 2012; Trevisi et al., 2015). Presented profiles of albumin were also consistent with an APR in the days immediately following calving, wherein circulating albumin concentrations decreased.

Protein Metabolism and the Potential Role of IL-6 as a Myokine

The profiles of protein metabolism analytes are indicative of a NEB and concurrent muscle catabolism following calving. Skeletal muscle is affected by homeorhetic changes, whereby protein anabolism is suppressed and catabolism is upregulated; this contributes to a net mobilization of amino acids and decreased muscle fiber diameter in early lactation (Reid et al., 1980; Bell et al., 2000). Skeletal muscle is also considered an endocrine organ, which secretes myokines that include growth factors, cytokines, and peptides that regulate cell proliferation, cell differentiation, and inflammatory pathways (Belizário et al., 2016). For example, IL-6 has been reported as a myokine integral to the APR to exercise in humans, which includes the upregulation of antioxidant defenses in response to oxidative stress (Belizário et al., 2016; Hennigar et al., 2017). We hypothesize that the increase in circulating IL-6 in the days postcalving may be, at least in part, involved in skeletal muscle proteolysis or perhaps uterine involution in the bovine.

The transient increase in CK activity around parturition may be associated with reproductive tract damage that occurs during calving. The activity of CK returns to precalving levels with less variation by d 7, and this is consistent with CK activity presenting as an intermediate indicator of muscular damage that peaks 6 to 12 h later (Pavlata et al., 2001). Generally, CK activity returns to normal values within 24 to 48 h, but persisting muscular damage can result in an extended increase (Pavlata et al., 2001). As there are relatively few studies on CK in cows during the transition period, the profile presented here provides a guide of expected CK concentrations in pasture-fed transition dairy cows.

Postcalving decreases in creatinine concentration in conjunction with decreasing BCS and BW throughout the postcalving period are probably indicative of proteolytic processes (Pires et al., 2013; Roche et al., 2013b). Bruckmaier et al. (1998) reported a parallel decrease in plasma creatinine concentration and the diameter of the longissimus dorsi muscle (a measure of muscle mass) following calving. However, the concentration of creatinine in pasture-fed dairy cows presented in the current study was considerably less than reported for TMR-fed cows (Bruckmaier et al., 1998; Bertoni et al., 2008; Turk et al., 2013; Osorio et al., 2014; Megahed et al., 2019). Body weights were not always reported

in these previous studies, although mean BW ranged between 675 and 768 kg precalving and between 659 and 680 kg postcalving in TMR-fed cows (Bruckmaier et al., 1998; Osorio et al., 2013; Hiew et al., 2016). This is markedly heavier than the 520 to 546 kg precalving and 455 to 489 kg postcalving for the cows in the current study. It is plausible that differences in reported creatinine concentrations reflect a lower muscle mass in the cows in the current study compared with heavier TMR-fed cows in other studies.

Mineral Balance Is Affected at Parturition

Despite the usual tight regulation of plasma mineral concentrations by the kidneys, the peripartum period is characterized by sharp changes in mineral concentrations and critical imbalances in some minerals. Initiation of lactogenesis is the driving factor for changes in Ca, PO₄, and Mg at calving (DeGaris and Lean, 2008). Prevalence of clinical hypomagnesemia and hypocalcemia was minimal in the current study; however, the prevalence of subclinical states was considerably greater.

Several factors are likely to contribute to hypomagnesemia in the grazing dairy cow: ingestion of pastures rich in N and K from applied fertilizers, and high circulating NEFA concentration during early lactation, all of which have an antagonistic effect on circulating Mg; furthermore, inclement weather often reduces DMI, thereby reducing blood Mg as it is primarily maintained from the diet (Satter and Roche, 2011). Therefore, a common requirement for grazing systems is supplementation of Mg (Satter and Roche, 2011). Prevalence of subclinical hypomagnesemia (≤ 0.8 mmol/L) ranged between 30.6% and 63.7% and for clinical hypomagnesemia (≤ 0.5 mmol/L) between 1.8% and 6.2% during the transition period. The prevalence of subclinical hypomagnesemia is not well characterized in the literature, potentially because it is not a common risk factor in TMR-fed, housed dairy cows. In a retrospective study, Harris et al. (1983) reported annual suspected cases of grass tetany in dairy cows in Victoria, Australia, to be 2.1%; however, no measurements of circulating Mg were reported, and the data relied on farmer information. Reported mean prevalence of subclinical hypomagnesemia in grazing dairy cows in Brazil was 9.5% (da Silva et al., 2020) and at calving was 12% (Masoero et al., 2003). In TMR-fed dairy cows, prevalence has been reported as 7.4% between calving and d 30 postcalving (Fiorentin et al., 2018) and 4% between 1 wk pre- to 2 d postcalving (Neves et al., 2017). All of these reported values are considerably lower than in our study, but the previous studies did not use improved temperate pastures accelerated with nitrogen fertilizer; therefore,

this may be the first study to present the prevalence of subclinical hypomagnesemia in the transition period in a temperate grazing system.

The prevalence of subclinical hypocalcemia in the current study was also considerable (i.e., 35% of cows had ≤ 2.0 mmol/L and 60% had ≤ 2.15 mmol/L circulating Ca on the day of calving). A cut point of 2.0 mmol/L to designate subclinical hypocalcemia indicated it was only materially prevalent at calving, ranging from 2% to 8% during the rest of the observational period; this compared with using a cut point of 2.15 mmol/L, which indicated a prevalence of 8% to 24% throughout the measurement period. Although the prevalence of subclinical hypocalcemia is more common in the literature, there is still limited information for moderate-yielding dairy cows grazing temperate pastures. In TMR-fed cows, using cut points between 2.0 and 2.4 mmol/L, prevalence ranges from 5 to 66% (Reinhardt et al., 2011; Neves et al., 2017). In Irish grazing dairy herds, prevalence of milk fever was reported to be 30 to 75%, although the definition of milk fever or the plasma Ca “cut point” used to define the condition were not reported (Mulligan and Doherty, 2008). The prevalence of subclinical hypocalcemia reported here is within the range of previously reported estimates.

Hypomagnesemia has been associated with an increased risk of hypocalcemia, which in turn has been associated with suppressed immune function and increased risk of other production diseases, such as hyperketonemia (Goff, 2008; Reinhardt et al., 2011; Crookenden et al., 2020). In response to lower circulating Ca, parathyroid hormone (PTH) is released from the parathyroid gland, which mobilizes Ca from bone and increases reabsorption in the kidney (Goff, 2008). Magnesium is critical for both PTH release and PTH regulation of the hydroxylation of $1,25-(\text{OH})_2\text{D}_3$, the active metabolite of vitamin D that increases intestinal absorption and resorption from bone (DeGaris and Lean, 2008). Therefore, considering the importance of blood Mg to Ca homeostasis, the seemingly contradictory increase in circulating Mg concentration at calving when blood Ca nadirs, as in the presented profile, is likely a natural response to the onset of peripartum hypocalcemia. A lack of response to PTH or $1,25-(\text{OH})_2\text{D}_3$ has been suggested to be important for the pathogenesis of hypocalcemia, as cows with clinical milk fever have greater concentrations of PTH and $1,25-(\text{OH})_2\text{D}_3$ than normocalcemic cows (Horst et al., 1978; DeGaris and Lean, 2008). This response is also consistent with the PO_4 profiles presented; circulating PO_4 concentrations are directly regulated by $1,25-(\text{OH})_2\text{D}_3$ and indirectly regulated by the PTH–Ca negative feedback loop (Goff, 1999). Therefore, the decrease in circulating PO_4 at calving, with no change in phosphorus intake,

may reflect a decreased intestinal tissue sensitivity to $1,25-(\text{OH})_2\text{D}_3$. Circulating concentrations of Na, K, and Cl are tightly regulated as the main electrolytes of body fluids (Skrzypczak et al., 2014). The increase in circulating K concentrations following calving is likely due to the increase in DMI of pasture, as pasture is high in K content (Roche et al., 2009b). On the day of calving, there was a rapid increase in circulating concentrations of Na and Cl, which rapidly decreased again at the next sampling point. The profiles of Na and Cl are very similar, most likely resulting from the electrical neutrality that a 1:1 ratio of $\text{Na}^+:\text{Cl}^-$ provides as blood osmolytes (Skrzypczak et al., 2014). An increase in blood plasma osmotic pressure (i.e., an increase in Na and Cl) has been associated with increased vasopressin and oxytocin on the day of parturition. Vasopressin is secreted from the hypothalamus and aids in water regulation, through action on the kidneys, and elevates blood pressure, through capillary constriction and raising peripheral resistance. Vasopressin secretion is stimulated as blood osmolarity rises. Oxytocin is structurally related to vasopressin and is also involved in the regulation of osmotic pressure. Circulating oxytocin decreases following the expulsion of the placenta, leading to a decrease in the osmotic pressure again (Skrzypczak et al., 2014). Furthermore, data indicate that cows are dehydrated around calving, with electrolyte imbalances (i.e., elevated circulating Na and Cl) exacerbated by a loss of up to 60 L of uterine fluid at calving (Enemark et al., 2009). Urea was also elevated in circulation on the day of calving in the presented profiles; this might also reflect a reduction in urine output associated with the peripartum dehydration condition (Watford, 2003).

CONCLUSIONS

Temporal profiles of humoral indicators of metabolic state provide a reference for the expected range in concentration of blood factors associated with energy metabolism, liver function, protein metabolism, mineral status, inflammation, and uterine and animal health during the transition period in pasture-fed dairy cows. The profiles were consistent with expected homeorhetic changes, which reflected a peripartum uncoupling of the somatotropic axis to support lactation, and is accompanied by increases in liver metabolic function, activation of the immune system, and changes in mineral balance. Importantly, however, this is the first time that these profiles have been presented for low- to moderate-yielding grazing dairy cows. The overall general consistency in profile change when compared with higher yielding housed cows fed TMR reflects the “naturalness” or hard-wired nature of the metabolic stress that cows encounter during parturition and is an

important benchmark for understanding animal physiology in a production setting.

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