



Metabolic and physiological adaptations to first and second lactation in Holstein dairy cows

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ABSTRACT

Huge differences exist between cow yields and body sizes during their first and second lactations. The transition period is the most critical and investigated phase of the lactation cycle. We compared metabolic and endocrine responses between cows at different parities during the transition period and early lactation. Eight Holstein dairy cows were monitored at their first and second calving during which they were reared under the same conditions. Milk yield, dry matter intake (DMI), and body weight (BW) were regularly measured, and energy balance, efficiency, and lactation curves were calculated. Blood samples were collected on scheduled days from −21 d relative to calving (DRC) to 120 DRC for the assessment of metabolic and hormonal profiles (biomarkers of metabolism, mineral status, inflammation, and liver function). Large variations in the period in question for almost all variables investigated were observed. Compared with their first lactation, cows during their second lactation had higher DMI (+15%) and BW (+13%), their milk yield was greater (+26%), lactation peak was higher and earlier (36.6 kg/d at 48.8 DRC vs. 45.0 kg/d at 62.9 DRC), but persistency was reduced. Milk fat, protein, and lactose contents were higher during the first lactation and coagulation properties were better (higher titratable acidity, faster and firmer curd formation). Postpartum negative energy balance was more severe during the second lactation (1.4-fold at 7 DRC) and plasma glucose was lower. Circulating insulin and insulin-like growth factor-1 were lower in second-calving cows during the transition period. At the same time, markers of body reserve mobilization (β -hydroxybutyrate and urea) increased. Moreover, albumin, cholesterol, and γ -glutamyl transferase were higher during second lactation, whereas

bilirubin and alkaline phosphatase were lower. The inflammatory response after calving was not different, as suggested by the similar haptoglobin concentrations and only transient differences in ceruloplasmin. Blood growth hormone did not differ during the transition period but was lower during the second lactation at 90 DRC, whereas circulating glucagon was higher. These results agree with the differences in milk yield and confirmed the hypothesis of a different metabolic and hormonal status between the first and second lactation partly related to different degrees of maturity.

Key words: metabolic profile, parity, hormone, insulin resistance

INTRODUCTION

Large differences exist between milk yield of dairy cows during their first and second lactations. In Italy, for instance, the average milk yield in Holstein cows is $9,349 \pm 1,864$ kg during the first lactation and $10,638 \pm 2,343$ kg during the second lactation (AIA, 2021). Potentially, several factors are involved, with mammary gland development being one of the main ones. In fact, secretory tissue volume and rate of tissue growth are greater during the second gestation than during the first (Knight and Wilde, 1993). Cell differentiation plays a pivotal role in this process (Capuco et al., 2001) but the mammary gland is also more metabolically active in multiparous than in primiparous cows (Miller et al., 2006). In primiparous cows, development of the whole body (in particular the digestive tract) and the higher level of DMI for the whole transition period can play a role in this process (Coffey et al., 2006).

The transition period is defined as the 3 wk before and after calving (Drackley, 1999) but a better understanding of this physiological phase requires extending it back to dry-off (Mezzetti et al., 2021). During this period, endocrine changes typical of the end of pregnancy occur: progesterone plasma concentration declines at calving, and estradiol, prostaglandins, and cortisol reach their peaks (Kindahl et al., 2004; Bruckmaier and

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Gross, 2017). Meanwhile, the galactopoietic hormone prolactin promotes initiation of lactation (Lacasse et al., 2016). Furthermore, sudden and dramatic physiological and metabolic changes happen in the period around calving (Trevisi and Minuti, 2018; Lopreiato et al., 2020), which can result in behavioral alterations (Calamari et al., 2014; Cattaneo et al., 2020). In fact, this phase is characterized by at least 5 main physiological processes that cows enact to adapt to the new lactation (Trevisi and Minuti, 2018): (1) reduction of immune competence, (2) negative energy balance, (3) hypocalcemia, (4) inflammatory responses, and (5) oxidative stress. Prolonged and exacerbated alteration of these processes can lead to the development of endocrine disturbances and metabolic and infectious diseases (Bauman, 2000; Sheldon et al., 2008; Sordillo and Raphael, 2013; Lacasse et al., 2018). In particular, insulin concentrations decrease during late gestation and at the beginning of lactation, paired with an attenuated sensitivity of many tissues to this hormone (i.e., insulin resistance; Vernon and Sasaki, 1991; De Koster and Opsomer, 2013), to prioritize mammary gland uptake. Moreover, at the onset of lactation, in a phase of dramatic negative energy balance, the somatotrophic axis is uncoupled, resulting in high circulating bST due to reduced negative feedback control elicited by the lower levels of IGF-1 (Lucy, 2008). High bST concentrations and low insulin lead to a decrease in lipogenic activity and promote lipid mobilization (Burton et al., 1994), with the adipose tissue becoming more sensitive to lipolytic stimuli (Bell, 1995; Liang et al., 2020). Therefore, lipolysis is enhanced, peripheral glucose uptake is reduced, and gluconeogenesis is increased to support lactose synthesis (Bell and Bauman, 1997). During negative energy balance, circulating leptin is also reduced to partition energy toward essential functions and suppress those functions, such as growth and reproduction, that are dispensable in the short term (Block et al., 2001; Gross et al., 2011).

In this context, we hypothesized that Holstein dairy cows will have distinct hormonal and metabolic profiles in their first and second lactations. Heifers at their first calving require nutrients for both their growth and the final development of the calf (in late gestation) or milk synthesis (after calving), whereas cows during their second lactation have greater mammary gland size and activity. Thus, this observational study aimed at characterizing longitudinal physiological and metabolic adaptations to the first and second lactation in Holstein cows managed identically for 3 years, by measuring hematochemical parameters and metabolic hormones before the morning feeding starting in late gestation through 120 DIM.

MATERIALS AND METHODS

Animal Management and Experimental Design

The research was carried out at the Università Cattolica del Sacro Cuore research dairy barn (Experiment Station, San Bonico, Piacenza, Italy) from 2007 to 2010 in accordance with Italian laws on animal experimentation (DL n. 116, 27/01/1992) and ethics. Eight Holstein dairy cows with an expected first calving date ranging from October to February were housed in tiestalls in 4-place chambers under controlled environmental conditions (room temperature of 20°C, relative humidity of 65%, 14 h of light) from -55 ± 5 to 120 d relative to calving (**DRC**). Cows were kept in the same position throughout the study and calved in their stalls. After a voluntary waiting period of 65 d, cows were bred when natural estrus was detected. Cows were monitored and compared throughout 120 DRC in their first and second lactation.

The feeding strategy is displayed in Supplemental Figure S1 (<https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023). From -55 until -7 DRC, animals received hay-based feeding with corn silage (10 kg) combined with concentrate (1.5 kg) to satisfy NRC recommendations (NRC, 2001). Seven days before the expected day of calving, 1 kg of lactation concentrate was added to the diet and, just after calving, alfalfa-dehydrated hay was fixed to 3 kg and grass hay was gradually reduced to 2.0 kg/d. Moreover, after calving, corn silage was incremented at the rate of 2 kg/wk (to a maximum of 20 kg/d), and the concentrate was increased by 0.5 kg/d to satisfy the requirement of 1 kg of concentrate for every 3 kg of produced milk. The daily amount of forage was individually fed twice a day (0730 and 1930 h) and the daily amount of concentrate (delivered in pelleted form) was fed in 8 equal meals at 3-h intervals during the day using an automatic feeder, with 2 of the 8 meals programmed to deliver the concentrate 30 min before the forage meals. A representative sample was taken from each feed at every batch change and after DM determination, analyzed for CP, crude fiber, NDF, ether extract, ash, and starch contents (AOAC International, 2012). Feed and diet composition are shown in Supplemental Table S1 (<https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023).

Health Status, BW, BCS, Rectal Temperature, and DMI

Health status was checked daily by study personnel and veterinarian staff. Retained placenta was diagnosed

when fetal membranes were not expelled within 24 h after calving (Beagley et al., 2010), and mastitis was diagnosed by visual assessment of each quarter's milk and SCC analysis of suspicious cases. Ketosis was diagnosed when blood BHB was >1.4 mmol/L (Duffield, 2000). Treatment for a retained placenta consisted of administration of lysine acetylsalicylate and benzylpenicillin. Milk from mastitic quarters was weighed but not included in the samples for milk composition assessment. Body weight was measured every 2 wk and on the day after calving before the morning feeding. At -21, -7, 7, 21, 35, 49, 63, 90, and 120 DRC, BCS was determined by the same operator using a 1 to 4 scale (ADAS, 1986). After the first 120 DRC, BW and BCS were measured monthly. Daily individual feed intake was measured by weighing the amounts of administered feed and their refusals, and DMI was calculated using the DM content of each feed.

Milk Yield and Composition

During lactation, cows were milked twice a day at the stand (0600 and 1600 h), and milk yield was weighed after each milking. Starting from 120 DRC, milk yield was recorded monthly on milk test days. Milk samples were collected twice weekly for the first 120 DRC (every Monday and Thursday) at the morning milking, kept in a water bath at 28°C, and analyzed within 2 h for fat, protein, and lactose contents using a mid-infrared analyzer (Milko Scan 133B, Foss Electric), SCC using an automated counter (Fossomatic 180, Foss Electric), pH with a combined electrode, and titratable acidity measuring the milliliters of NaOH (0.25 *N*) necessary to maintain the pH of 50 mL of milk at 8.65 using an automatic titrator (Micro TT 2050; Crison). Fat and protein output were calculated by multiplying the relative content by the milk yield. The SCC was $\log_{10}$ transformed and reported as geometric mean. Urea was determined on skim milk using a spectrometric assay (IL Test Urea Nitrogen kit; Werfen) using the ILAB-600 clinical auto-analyzer (Werfen).

Ten milliliters of milk was used to measure coagulation properties (McMahon and Brown, 1982) after adding 0.2 mL of rennet [Chr. Hansen A/S, 1:15,000 in a 1.5% (vol/vol) solution in distilled water] and measuring the thromboelastogram at 35°C for a total time of 30 min using the Formagraph (Foss Electric). The lag time (min) before the beginning of coagulation was defined as the rennet clotting time (**RCT**), the time (min) necessary to reach 20 mm of width of the thrombus was defined as curd firming rate (**k₂₀**), and the width of the thrombus after 30 min (mm) was defined as curd firmness (**a₃₀**).

Blood Sample Collection and Analysis

At -21, -7, 7, 21, 35, 49, 63, 90, and 120 DRC immediately before the delivery of morning concentrate (0700 h), blood samples were harvested from the jugular vein. For the metabolic profile assessment, samples were collected into 10-mL heparinized vacuum tubes (Vacutainer, Becton Dickinson) and placed on ice until centrifugation. Within 1 h of collection, a small amount of blood was used for the determination of packed cell volume (Centrifugette 4203, ALC International Srl), and the remainder was centrifuged at $3,500 \times g$ for 16 min at 4°C. Aliquots of the plasma obtained were frozen at -20°C until further analysis. Blood metabolites were analyzed at 37°C using a clinical auto-analyzer (ILAB 600; Werfen). Commercial kits were used to determine plasma concentrations of glucose, urea, triglycerides, Mg, Ca, P, total protein, albumin, cholesterol, bilirubin, aspartate aminotransferase-glutamate oxaloacetate transaminase (**GOT**), γ -glutamyltransferase (**GGT**), and creatinine (Werfen), nonesterified fatty acids (**NEFA**), Zn (Wako Chemicals GmbH), and BHB (Randox Laboratories Ltd.); Na, K, and Cl were measured using a potentiometer method (ion selective electrode). Ceruloplasmin was determined following the method proposed by Sunderman and Nomoto (1970) and haptoglobin as proposed by Skinner et al. (1991). Globulin was calculated as the difference between total protein and albumin and albumin-to-globulin ratio as the ratio between albumin and globulin. Methods and kit catalog numbers for each biomarker are reported in Supplemental Table S2 (<https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023).

Plasma concentration of bST was quantified using a heterologous double-antibody RIA with materials and procedures obtained through the National Hormone and Peptide Program and the National Institute of Diabetes and Digestive and Kidney Diseases (Torrance, CA). Highly purified bST (reagent AFP-11182B/AFP-9884C) was used for standards (useful concentration range: 0–100 ng/mL) and iodination (by the iodogen method of Salacinski et al., 1981). Monkey anti-bovine bST serum (AFP-B55Bb) was used as the primary antibody (1:500,000 final tube dilution). Precipitation of the antigen-antibody complexes was done using a goat anti-human γ -globulin (Jackson ImmunoResearch Labs) as the second antibody (2.0% final dilution) together with normal human serum (0.2%) and diluted polyethylene glycol (PEG 6000, 3.0% final tube dilution). Spike, recovery, and linearity testing yielded results within the 85 to 120% range of expected concentrations. Inter- and intraassay coefficients of variation (**CV**) were 4.0 and

6.2%, respectively. For glucagon determination, 2.5 mL of blood was drawn into silicone-evacuated tubes, added to a tube containing 2 mg of Na₂-EDTA and 1,000 kallikrein inhibitory units of Trasylol (Bayer), and immediately centrifuged ($3,500 \times g$ for 16 min at 4°C); the resulting plasma was stored in 2 aliquots at -20°C. Glucagon was analyzed by a commercially available kit using a double-antibody RIA for human glucagon (Diagnostic Products Corp.) as human and bovine glucagon have identical AA sequences. Intraassay CV was 5.8% and interassay CV was 7.9%. Insulin concentration in plasma was assayed by a double-antibody RIA kit for human insulin (DSL 1600; Diagnostic Systems Laboratories Inc.) that used a polyclonal antibody with high cross-reactivity to bovine insulin. For bovine plasma samples, the kit was validated by performing linearity testing, in which the observed result was compared with expected results. Results were in the range of 85 to 115%. The intra- and interassay CV were 7.5 and 9.5%, respectively. The concentration of IGF-1 was determined by RIA as described by Ronge et al. (1988). The assay was modified using a monoclonal antibody and separation of bound and free fractions with an antibody against mouse γ -globulin raised in sheep. The intra- and interassay CV were 6.5 and 8.7%, respectively. Total circulating triiodothyronine (T₃) in plasma was measured using a commercially available solid-phase RIA (Coat-A-Count, Diagnostic Products Corp.). Both free and protein-bound T₃ were measured with the aid of blocking agents for thyroid-binding proteins with no effect on hormone measurement by variations in total protein concentration. Intraassay CV was 3.6% and interassay CV was 5.0%.

Calculations

Lactation curves were modeled with the function of Wood (Wood, 1967), using daily milk yield data until 120 DRC and monthly data afterward. The Wood model is

$$Y_t = at^be^{ct},$$

where Y_t is the milk test-day yield at time t , t represents the days in milk, a represents the initial yield, and b and c are the parameters associated with the inclining and declining slope of the lactation curves, respectively, and e is the Euler's number. The following statistics were also calculated from the Wood curve. Persistency was calculated as $-(b + 1) \times \ln(c)$. The peak milk yield (Y_{max}) was calculated as $a \times (b \div c)^b \times e^{-b}$, and the time of maximum milk yield (T_{max}) as $b \div c$. The milk

yield at 300 DRC was calculated as $a \times 300^b \times e^{(c \times 300)}$, whereas the total milk yield in 305 d was calculated from the estimated curve.

At 7, 21, 35, 49, 63, 90, and 120 DRC, fat- and protein-corrected milk (FPCM) was calculated as milk (kg/d) $\times [0.337 + 0.116 \times \text{fat content (g/100 g)}] + 0.06 \times \text{protein content (g/100 g)}$; Kok et al., 2016). Dairy efficiency was calculated as FPCM (kg/d) divided by DMI (kg/d). Calculations on energy and nitrogen balance were carried out according to NRC (2001). The net energy content in milk (NE_L) was calculated as NE_L (Mcal/kg) = $[0.0929 \times \text{fat (g/100 g)}] + [0.0547 \times \text{protein (g/100 g)}] + [0.0395 \times \text{lactose (g/100 g)}]$ and multiplied by milk yield to calculate the daily amount of produced NE_L. Conceptus weight (CW) was considered for energy requirements of pregnant cows as CW (kg) = $18 + (\text{days of pregnancy} - 190) \times 0.665$. Net energy required for maintenance (NE_M) was calculated as NE_M (Mcal/d) = $(\text{BW} - \text{CW})^{0.75} \times 0.08$. Net energy required for pregnancy (NE_P) was calculated as NE_L for maintenance (Mcal/d) = $[(2 \times 0.00159 \times \text{days of pregnancy} - 0.0352) / 0.33] \times 0.64$. Energy content of the diets was calculated from feed batch analysis and multiplied by observed daily DMI to obtain the daily intake of NE_L (NRC, 2001). Then, the net energy balance was calculated as follows: net energy balance (Mcal/d) = intake of NE_L - (NE_L for milk synthesis + NE_M + NE_P). The potential contributions of growth and changes in tissue energy were not considered in our analysis. Nitrogen intake was calculated after determining the daily CP intake from DMI and CP content of feed and then dividing by 6.25 to return the values to an N basis. Milk N content was calculated from the milk protein content divided by 6.38. The N efficiency was calculated as the percentage of dietary N intake recovered as N in milk and body tissues: N efficiency (%) = milk N yield/N intake.

Statistical Analysis

Sample size was calculated to achieve a power >0.80 with an $\alpha = 0.05$ using the G*Power package (Faul et al., 2007). The effect size (f) was calculated using the variance of blood parameters (mainly glucose and insulin) observed in our previous studies. Statistical analyses were performed using SAS software (release 9.4, SAS Institute Inc.). The lactation curves of each cow during each lactation were estimated according to the Wood equation (NLIN procedure of SAS), and curve indices were compared between lactations using the one-way ANOVA (GLM procedure). Data were subject to the repeated-measures mixed models (GLIMMIX procedure). Distributions of residuals

were visually assessed. The model included the fixed effect of lactation (**LACT**; first and second), the effect of time (**T**), and the interaction lactation $\times$ time (**LACT** $\times$ **T**); cow was included as the random effect. The covariance structure (compound symmetry, autoregressive order, Toeplitz, or spatial power) with the lowest Akaike information criterion (Littell et al., 1998) was included in the model. Pair-wise comparisons were performed using the least significant difference test using Tukey adjustment. Statistical significance was set at $P \leq 0.05$, and differences among means with $0.05 < P \leq 0.10$ were considered in the context of tendencies.

RESULTS

Health Status, DMI, Milk Yield, BW, BCS, and Energy Balance

During the period considered, 6 cases of mastitis occurred (average detection on 52.1 ± 35.0 DRC, mean $\pm$ SD), equally split between lactations, and 3 mild cases of retained placenta (all during the second lactation). Diseases were mild, affected animals did not reduce DMI, and their lactation curve was comparable to those that remained healthy. Dry period DMI was lower than DMI during the lactation period (T ; $P < 0.01$; Figure 1A). The DMI was higher in second-lactation cows in the period investigated (18.5 vs. 21.2 ± 0.34 kg/d; $P < 0.01$), except immediately after calving (1–3 DRC; $P > 0.05$). Similarly, milk yield was higher during the second lactation in the first 120 d in milk (33.4 vs. 42.1 ± 1.1 kg/d; $P < 0.01$; Figure 1B). Regardless of parity, milk yield had a sharp increase during the first weeks after calving, reaching a peak at around 55 DRC (41.4 ± 1.38 kg/d; T ; $P < 0.01$). The main statistics of Wood lactation curves are reported in Table 1 (and shown in Supplemental Figure S2; <https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023). Cows during their first lactation had lower starting milk yield (i.e., lower a parameter; $P = 0.01$), but slopes of the curve were not influenced by parity. Moreover, these cows had a lower peak milk yield ($P = 0.02$) and reached it almost 18 d later ($P = 0.05$). Nevertheless, persistency was higher during the first lactation ($P = 0.05$), leading to a similar milk yield at 300 DRC. Despite a lack of significance, a numerically lower 305-d milk yield was observed during the first lactation.

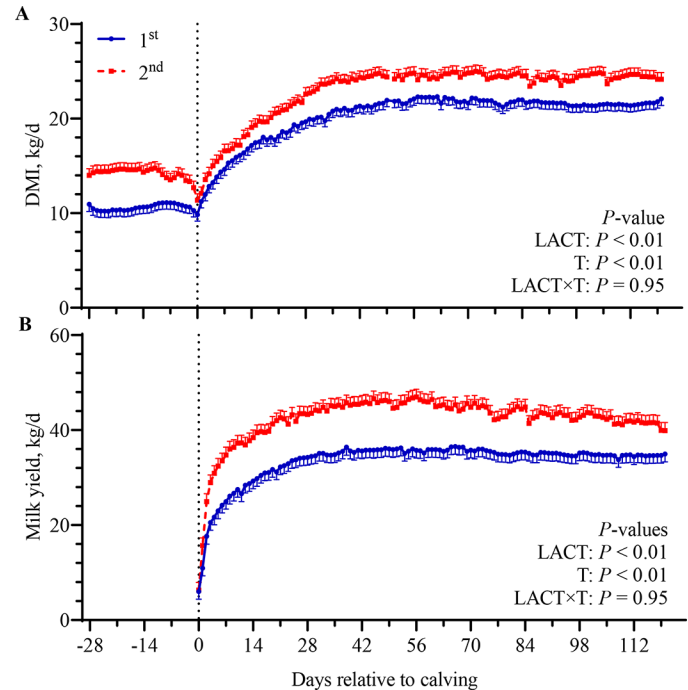


Figure 1. Daily DMI (A) and milk yield (B; LSM $\pm$ SEM) from -21 to 120 d relative to calving in Holstein dairy cows during their first (solid line) and second lactation. Vertical dotted line indicates calving day. Significance levels of the effects of parity (LACT), time (T), and their interaction (LACT $\times$ T) are reported.

Table 1. Least squares means of Wood's curve statistics in dairy cows during their first and second lactations

Item	Lactation		SEM	P -value ¹
	First	Second		
a^2	14.9	20.9	1.32	0.01
b^2	0.29	0.27	0.02	0.66
c^2	-0.005	-0.006	0.001	0.13
Time at peak milk yield, d	66.6	48.8	4.95	0.02
Peak milk yield, kg/d	36.6	45.0	1.25	<0.01
Persistency	3.03	2.86	0.06	0.05
Milk yield at 300 DRC, ³ kg/d	19.9	18.2	2.14	0.58
305-d milk yield, kg	8,914	9,959	509	0.17

¹ P -values of the lactation effect.

²Lactation curve parameters, with a representing starting milk yield, b the inclining slope, and c the declining slope (Wood, 1967).

³DRC = days relative to calving.

Regardless of parity, BW and BCS had a similar trend, with higher values during the dry period and a sharp decline afterward (T; $P < 0.01$; Figure 2A,B). Parity influenced BW, which was 12.6% higher during the second lactation (609 vs. 686 ± 16 kg; $P < 0.01$). Body condition score values did not differ between lactations but had different trends (LACT $\times$ T; $P < 0.01$), with higher scores until the second month after calving during the first lactation and lower in late lactation. Energy balance showed large variations over time (T; $P < 0.01$; Figure 2C), being positive before calving (3.19 and 4.53 ± 1.19 Mcal/d at -21 and -7 DRC, respectively), and plunging at the beginning of lactation (-9.60 ± 1.19 Mcal/d at 7 DRC). Afterward, it slowly improved until breaking even for the first time at 63 DRC (0.37 ± 1.21 Mcal/d). Cows during the first and second lactation had similar overall energy balance (-1.00 vs. -1.45 ± 0.98 Mcal/d; $P = 0.40$) but differed over time (LACT $\times$ T; $P < 0.01$), with secondiparous cows having a higher positive balance during dry period (5.33 vs. 1.05 ± 1.42 Mcal/d at -21 DRC; $P < 0.01$) and a more severe drop at 7 DRC (-13.55 vs. -5.64 ± 1.42 Mcal/d; $P < 0.01$).

Milk Composition and Coagulation Properties

Least squares means of milk composition are shown in Table 2. All investigated parameters varied with time (T; $P < 0.05$). Fat yield was maximum in the second week after calving (1.48 ± 0.08 kg/d) due to the sharp increase that occurred during the second lactation and then decreased (T; $P < 0.01$), and protein yield reached its peak in week 8 (1.27 ± 0.04 kg/d; T; $P < 0.01$). Both were higher during the second lactation (1.16 vs. 1.41 ± 0.07 and 1.08 vs. 1.35 ± 0.03 kg/d, respectively, during the first and second lactation; $P < 0.01$) with different trends over time. In fact, fat output was stable during the first lactation, whereas in the second lactation, it was higher until wk 10 after calving (LACT $\times$ T; $P < 0.01$). Except for the first week after calving, protein output was constantly greater during the second lactation (LACT $\times$ T; $P = 0.02$).

Milk fat, protein, and SCC had their maximum values during the first week of lactation, which then decreased, whereas lactose sharply increased during the first weeks. Milk fat and lactose contents were higher during the first lactation (LACT; $P < 0.01$; Supplemental Figure S3; <https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023), whereas no differences between lactations were observed for milk protein. Somatic cell count showed a different trend between lactations, with greater content in the first 2 wk after calving during the first lactation, resulting in

an interaction LACT $\times$ T effect ($P < 0.01$). After the colostral phase, SCC was always $<300 \times 10^3$ cells/mL.

Regardless of parity, milk pH, RCT, and k_{20} gradually increased after calving, whereas titratable acidity and a_{30} showed the opposite trend (T; $P < 0.01$). At the same time, pH, RCT, and k_{20} were lower during the first lactation than in the second (LACT; $P < 0.05$), whereas titratable acidity and a_{30} were higher (LACT; $P < 0.01$).

Dairy Efficiency

Regardless of parity, cows reached maximal dairy efficiency immediately after calving (2.23 ± 0.09 kg of milk/kg of DMI at 7 DRC; Figure 2D), after which they steadily declined during the first month of lactation until reaching stationary values (1.61 ± 0.09 at 49 DRC). Dairy efficiency was higher during the second lactation (1.58 vs. 1.86 ± 0.08 kg of FPCM/kg of DMI, respectively, during the first and second lactation; $P < 0.01$), with the differences concentrated in the first month after calving, resulting in a significant interaction effect ($P < 0.01$). The greatest difference was observed at 7 DRC (1.84 vs. 2.62 ± 0.11 during the first and second lactation; $P < 0.01$) and at 21 DRC (1.70 vs. 2.08 ± 0.11 ; $P < 0.01$), whereas no differences were recorded afterward. Nitrogen efficiency had a similar trend (Figure 2E), with the greatest value at 7 DRC (0.42 ± 0.01) and a decline afterward. Cows during the second lactation had better overall N conversion rate (0.33 vs. 0.35 ± 0.01 during the first and second lactation; $P < 0.01$), with significant differences only at 7 DRC (0.37 vs. 0.47 ± 0.02 ; $P < 0.01$), leading to an interaction effect ($P < 0.01$).

Metabolic and Inflammatory Biomarkers

Least squares means of plasma analytes are shown in Supplemental Table S3 (<https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023). Except for K, all parameters investigated varied with time (T; $P < 0.05$) and Na tended to be affected ($P = 0.10$). Hematocrit increased around calving (T; $P < 0.01$) and was higher during the first lactation (0.30 vs. 0.28 ± 0.004 L/L; LACT; $P < 0.01$). Plasma glucose concentration was higher before calving, reached its nadir at 7 DRC (3.49 ± 0.10 mmol/L; Figure 3A), and then increased again, fluctuating around values of 4 mmol/L. Parity influenced glucose concentrations over the whole period (LACT; $P < 0.01$), with higher values during the first lactation (3.94 vs. 3.79 ± 0.08 mmol/L, $P < 0.05$), in particular around calving (-7 and 7 DRC) when second-lactation cows had a more marked decrease (LACT $\times$ T; $P = 0.01$).

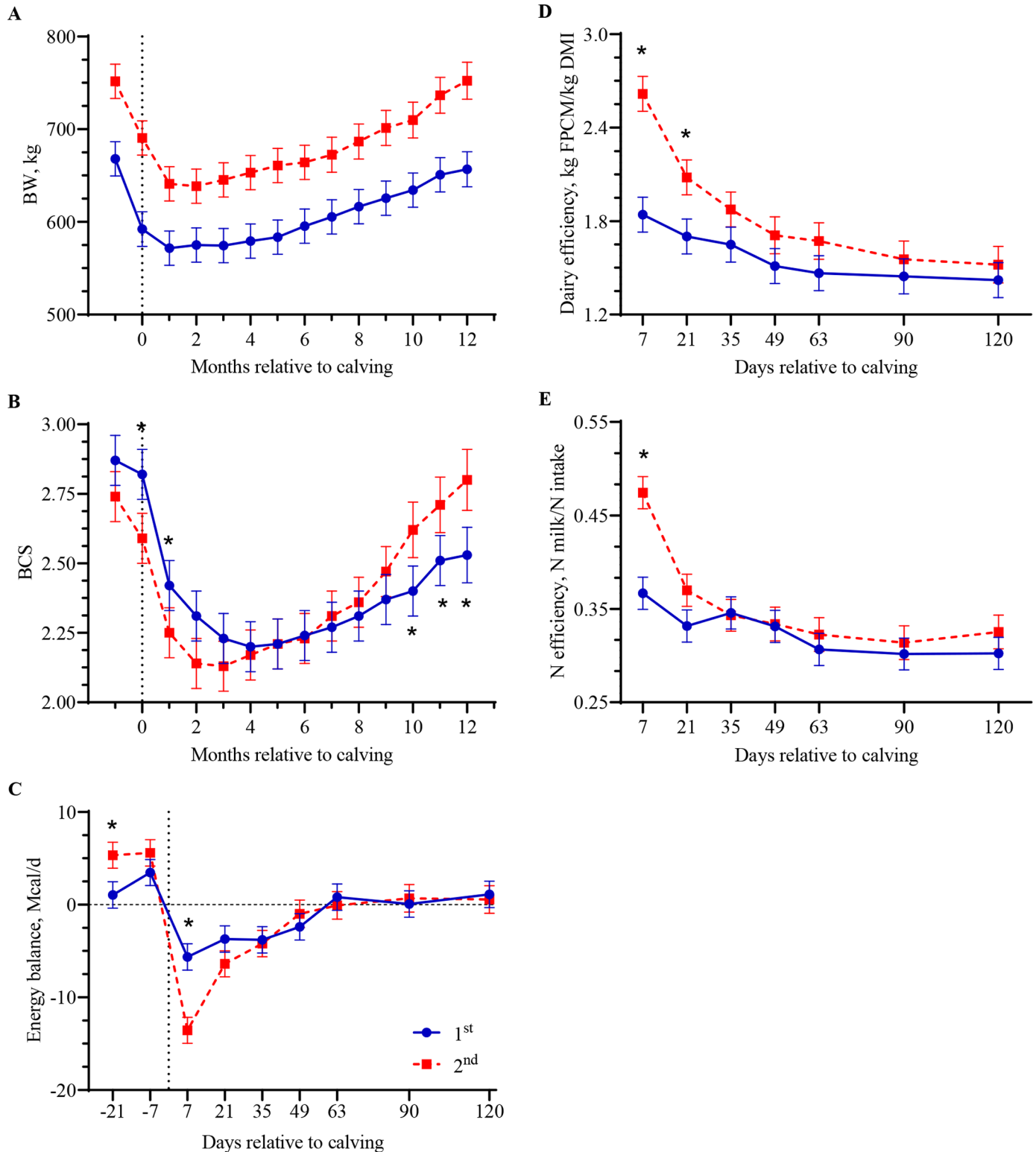


Figure 2. Average BW (A), BCS (B), energy balance (C), dairy efficiency (D), and nitrogen ratio (N in milk/N intake) (E) in Holstein dairy cows during their first and second lactations. FPCM = fat- and protein-corrected milk. Vertical dotted line indicates calving day. Significant differences ($P < 0.05$) between first and second lactation at each time point are denoted with an asterisk (*).

Table 2. Least squares means of milk yield, composition, pH, SCC, and coagulation properties in the first 120 d relative to calving in dairy cows during their first and second lactations

Item	Lactation		SEM	<i>P</i> -value ¹		
	First	Second		LACT	T	LACT × T
Yield, kg/d						
Milk	33.4	42.1	1.19	<0.01	<0.01	0.95
FPCM ²	31.0	40.1	1.12	<0.01	0.03	<0.01
Fat	1.16	1.41	0.07	<0.01	<0.01	<0.01
Protein	1.08	1.35	0.03	<0.01	<0.01	0.02
Composition, mg/100 mL						
Fat	3.39	3.25	0.11	<0.01	<0.01	0.13
Protein	3.35	3.28	0.08	0.26	<0.01	0.62
Lactose	5.29	5.21	0.11	<0.01	<0.01	0.22
Fat:protein	1.07	1.04	0.04	0.09	<0.01	0.09
pH	6.68	6.69	0.01	0.03	<0.01	0.17
SCC, ³ ×10 ³ cells/mL	34.8	31.8	1.31	0.28	<0.01	<0.01
Titrate acidity, °SH/50 mL ⁴	3.73	3.60	0.08	<0.01	<0.01	0.52
Rennet clotting time, min	15.7	16.3	0.87	0.05	<0.01	0.05
Curd firming rate, min	10.7	12.4	1.30	<0.01	<0.01	0.09
Curd firmness, mm	28.7	25.6	2.65	<0.01	<0.01	<0.01

¹*P*-values of the main effects: lactation (LACT), time (T), and their interaction (LACT × T).

²FPCM = fat- and protein-corrected milk production.

³Expressed as geometric mean.

⁴°SH = Soxhlet-Henkel degree.

In contrast, average urea (4.77 vs. 5.40 ± 0.19 mmol/L; *P* < 0.01) and BHB concentrations (0.48 vs. 0.58 ± 0.05 mmol/L; *P* = 0.01) were lower during the first lactation. Regardless of parity, urea constantly increased after calving (Figure 3B), whereas BHB concentrations were stable during the period investigated (~0.50 mmol/L; Figure 3C) but peaked the week after calving (1.03 ± 0.07 mmol/L). Despite the lack of differences between lactations, NEFA started to rise at −7, peaked at 7 DRC (0.72 ± 0.04 mmol/L; Figure 3D), and decreased after the first month of lactation. Triglycerides declined rapidly at calving (from 0.21 to 0.10 ± 0.01 mmol/L).

Among plasma minerals, regardless of parity, after calving, Ca tended to decrease (2.44 ± 0.05 mmol/L at 7 DRC; *P* = 0.10), whereas P and Zn had a marked decline (1.44 ± 0.09 mmol/L and 10.68 ± 0.75 μmol/L, respectively; *P* < 0.05). Considering the parity effect, Ca and Mg were higher during the second lactation (2.50 vs. 2.60 ± 0.03 mmol/L and 1.05 vs. 1.11 ± 0.03 mmol/L, respectively; *P* < 0.01; Figure 3E,F), whereas P tended to be higher during the first lactation (1.74 vs. 1.64 ± 0.05 mmol/L; *P* = 0.07; Figure 3E,G). Chloride showed a different trend between the 2 lactations (LACT × T; *P* = 0.01; Figure 3E,H) with higher concentrations at −21 DRC (*P* = 0.05) but lower at 21 and 49 DRC during the first lactation (*P* = 0.07 and *P* = 0.03, respectively). No differences in Na, K, and Zn concentrations were observed (Supplemental Figure S4; <https://doi.org/10.6084/m9.figshare.21679712.v2>; Cattaneo et al., 2023).

All biomarkers directly or indirectly related to inflammation increased after calving, although in different ways. Haptoglobin, ceruloplasmin, and bilirubin peaked immediately after calving (7 DRC) and quickly decreased thereafter (T; *P* < 0.01). These parameters reached maximum concentrations at 7 DRC: 0.40 ± 0.04 g/L, 3.58 ± 0.20 mmol/L, and 7.04 ± 0.52 μmol/L, respectively. Otherwise, total protein, globulin, albumin, and cholesterol increased during the first month of lactation and then stabilized (T; *P* < 0.01). Parity influenced inflammatory conditions with ceruloplasmin (2.65 vs. 2.98 ± 0.16 μmol/L; Figure 4A) and total protein (70.2 vs. 71.4 ± 0.85 g/L; Supplemental Figure S4), which were lower during the first lactation (LACT; *P* < 0.01), whereas bilirubin concentrations were higher in first lactation (4.11 vs. 3.45 ± 0.38 μmol/L; LACT; *P* = 0.02; Figure 4F). Globulin showed a different trend between lactations (LACT × T; *P* = 0.03; Figure 4C) with lower values in the first during prepartum (29.8 vs. 34.3 ± 1.4 g/L at −21 DRC; *P* = 0.01) and a tendency toward higher values postpartum (34.2 vs. 31.5 ± 1.4 g/L at 7 DRC; *P* = 0.10). Albumin-to-globulin ratio mirrored this trend (LACT × T; *P* < 0.01; Supplemental Figure S4), with higher values during the first lactation at −21 DRC (1.20 vs. 1.03 ± 0.04 g/L; *P* < 0.01) and the opposite after calving. Haptoglobin did not show any difference between lactations (Figure 4B). Considering negative acute-phase proteins, albumin (35.3 vs. 36.4 ± 0.28 g/L; Figure 4D) and cholesterol (4.85 vs. 5.16 ± 0.15 mmol/L; Figure 4E) were lower during the first lactation (LACT; *P* < 0.01).

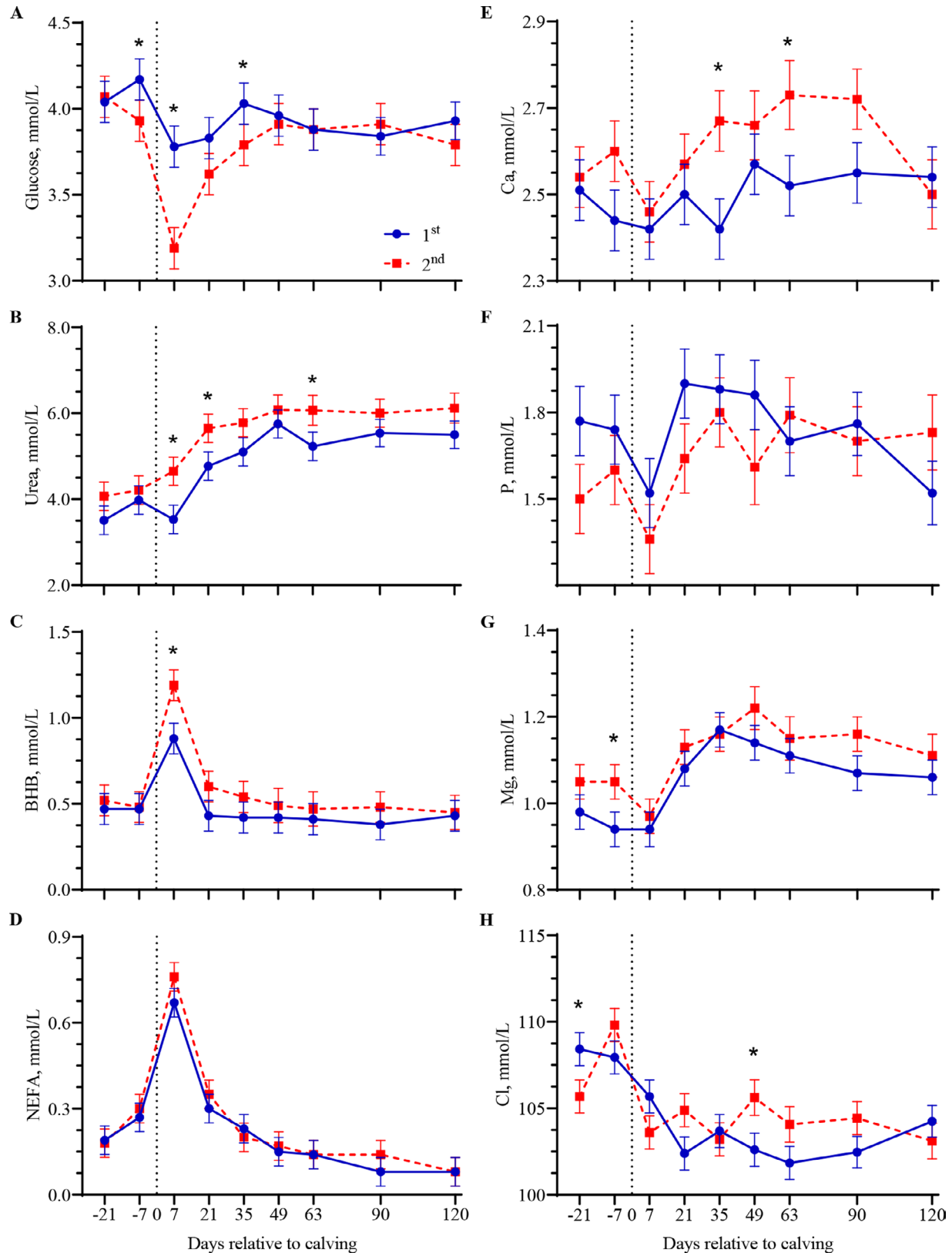


Figure 3. Plasma concentrations of glucose, urea, BHB, nonesterified fatty acids (NEFA), Ca, Mg, P, and Cl from -21 to 120 d relative to calving in Holstein dairy cows during their first and second lactations. Vertical dotted line indicates calving day. Significant differences ($P < 0.05$) between first and second lactation at each time point are denoted with an asterisk (*).

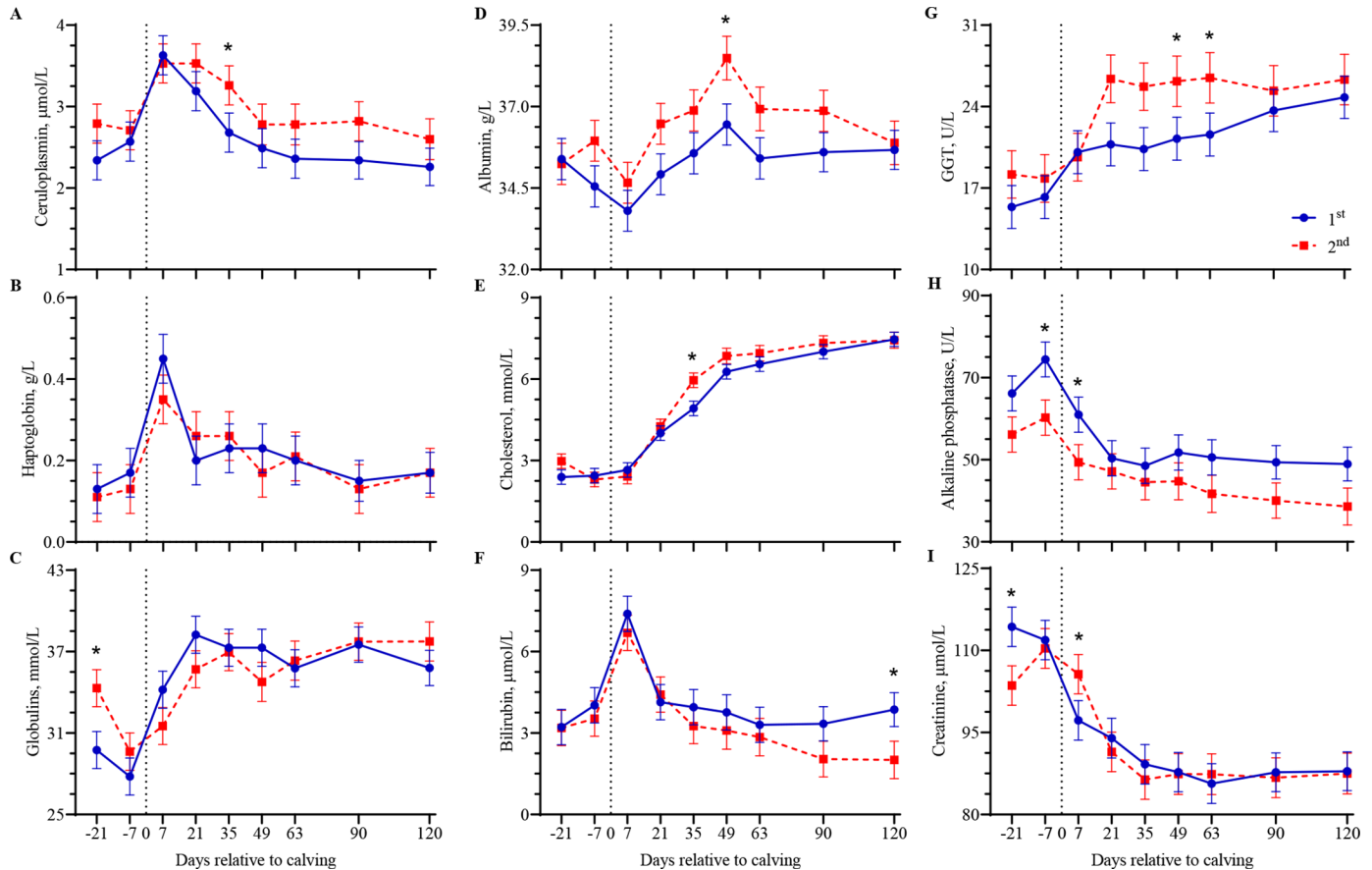


Figure 4. Plasma concentrations of ceruloplasmin, total protein, globulin, albumin, cholesterol, bilirubin, γ -glutamyl transferase (GGT), alkaline phosphatase, and creatinine from -21 to 120 d relative to calving in Holstein dairy cows during their first and second lactations. Vertical dotted line indicates calving day. Significant differences ($P < 0.05$) between first and second lactation at each time point are denoted with an asterisk (*).

Among liver and kidney function biomarkers, GOT and GGT increased after calving (T; $P < 0.01$). γ -Glutamyl transferase was lower during the first lactation (20.5 vs. 23.5 ± 1.6 U/L; LACT; $P < 0.01$; Figure 4G) due to the more marked increase during the second lactation from 21 to 63 DRC, whereas GOT did not differ between lactations (Supplemental Figure S1). Alkaline phosphatase and creatinine decreased sharply after calving to reach steady concentrations during lactation (T; $P < 0.01$; Figure 4H,I). Alkaline phosphatase was higher during the first compared with the second lactation (55.7 vs. 47.0 ± 2.4 U/L; LACT; $P < 0.01$).

Blood Hormones

Least squares means of blood hormones are shown in Supplemental Table S4. The concentrations of all investigated hormones varied with time (T; $P < 0.01$). Insulin and IGF-1 were higher prepartum, reached their nadir at 7 DRC (4.86 ± 0.73 mU/mL and 23.6 ± 8.0

$\mu\text{g/L}$, respectively; Figure 5A,B), and the subsequent increase did not recover in the considered period (until 120 DRC). Both insulin and IGF-1 were lower during the second lactation (7.99 vs. 6.77 ± 0.54 mU/mL and 83.3 vs. 69.6 ± 6.6 $\mu\text{g/L}$ during the first and second lactation, respectively). In contrast, bST peaked at 7 DRC (3.32 ± 0.27 ng/mL; Figure 5C) but was not affected by parity in the transition period. However, bST was higher during the first lactation at 90 DRC (2.62 vs. 1.41 ± 0.35 ng/mL; $P < 0.01$). Glucagon slowly increased after calving, achieving its greatest concentrations from 49 DRC (121.6 ± 14.4 pg/mL; T; $P < 0.01$), and was lower during the first lactation (105.5 vs. 110.8 ± 11.5 pg/mL; LACT; $P < 0.01$; Figure 5D). Triiodothyronine decreased immediately after calving with a nadir at 7 DRC (0.90 ± 0.05 ng/mL) and then progressively increased to values much higher than those recorded prepartum (T; $P < 0.01$). Moreover, T_3 tended to be higher during the first lactation (1.23 vs. 1.18 ± 0.03 ng/mL; LACT; $P = 0.10$; Figure 5E).

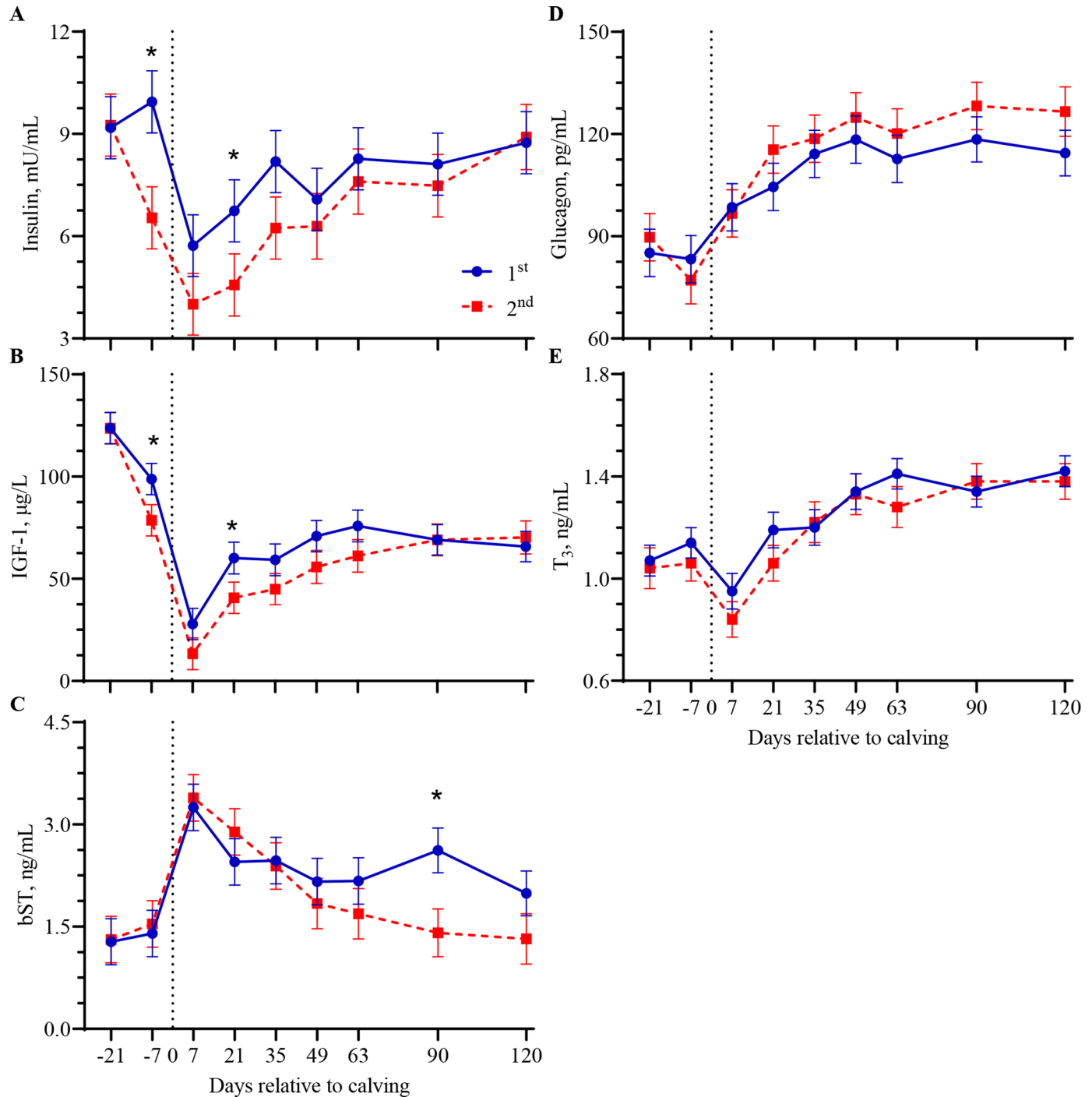


Figure 5. Plasma concentrations of insulin, IGF-1, bST, glucagon, and triiodothyronine (T_3) from -21 to 120 d relative to calving in Holstein dairy cows during their first and second lactations. Vertical dotted line indicates calving day. Significant differences ($P < 0.05$) between first and second lactation at each time point are denoted with an asterisk (*).

DISCUSSION

When heifers calve for the first time, their full body development has yet to be achieved. During the first lactation, mammary tissue increases in volume and

mature BW is gradually reached. These aspects have been extensively studied in the past, but little is known about the differences in the adaptation to the first and second calving in terms of metabolism, inflammation, and endocrine signaling. Previous studies investi-

gated differences in productivity, growth, development, metabolic responses, and fertility (Meikle et al., 2004; Wathes et al., 2007a,b). Nevertheless, to our knowledge, this study is the first to compare the same cows that were managed under the same conditions during their first 2 lactations, thus limiting as much as possible the confounding effects of individual variability due to genetic merit, diet, housing, and environment. Considering that different environmental conditions might affect animal status (milk production, metabolic profile, and hormone secretion), with primiparous and multiparous cows that do not always have the same response to variations in these factors (Dahl et al., 2000; Bernabucci et al., 2014; Menta et al., 2022), the present study was carried out in a controlled setting. Results of our study should be interpreted taking into account the limitation represented by the small sample size.

Metabolism and Endocrine System at the Beginning of Lactation

During the transition period, dramatic changes in bovine physiology related to the calving event and the start of lactation occur. The endocrine system plays a pivotal role in this context. In fact, nutrient partitioning, which is regulated by hormone signaling, represents a key aspect of the transition period, and bST and insulin are among the principal hormones involved. Under homeostatic conditions, insulin stimulates glucose storage after meals and causes a reduction in protein degradation in peripheral tissues, whereas glucagon stimulates hepatic gluconeogenesis and glycogen mobilization in the postabsorptive phase (Baumgard et al., 2017). As the calving event approaches, however, mammary gland glucose uptake greatly increases and, thus, cows implement a series of mechanisms orchestrated mainly by bST (Bauman, 2000) to direct an adequate amount of glucose toward the mammary gland.

In the present study, we compared the same cows at their first and second calvings. Cows were in good overall health status with only mild diseases that did not result in intake drops or relevant positive acute-phase protein increases, suggesting a lack of acute immune activation phenomena (Bertoni and Trevisi, 2013). Regardless of parity, after the first week of lactation, milk yield was >30 kg/d, which is roughly equivalent to an increase in glucose requirements of 2,300 g/d in a few days (Kronfeld, 1982). Concurrently, DMI increased but because its increase was slower than that of milk yield (as can be seen in the different shapes of the curve of DMI and milk yield shown in Figures 1 and 2, respectively), dietary energy intake could not cope with the large requirements of the first 2 mo of lactation. Thus, net energy balance was negative until the beginning of

the third month of lactation, as reflected in the lower plasma glucose and the body reserve mobilization, as confirmed by the rapid decrease in BCS and the peaks in circulating BHB and NEFA immediately after calving. The hormonal framework supports this process. Starting in late gestation, insulin pancreatic secretion diminished in both lactations, as previously reported (Rhoads et al., 2004), and tissue sensitivity and responsiveness to insulin action are reduced, basically pushing the cow into an insulin-resistant state (De Koster and Opsomer, 2013). Moreover, the mammary gland is insulin independent. In fact, most glucose transport to the gland can be attributed to an insulin-independent glucose transporter (GLUT1), whereas the insulin-dependent GLUT4 is absent (Komatsu et al., 2005). This finding implies that most of the energy available (in the form of glucose) is captured by the mammary gland for milk synthesis. Moreover, the capability of insulin to inhibit lipolysis is reduced in this phase, and NEFA are known to further inhibit pancreatic insulin secretion (Bossaert et al., 2008). Furthermore, the thyroid hormone T_3 , which is decreased in blood immediately after calving, was found to be related to the energy state and lactation initiation. This decrease can be explained by the decrease in hormone secretion rate due to the negative energy balance and the increase in demand by the mammary gland (Ronge et al., 1988; Fiore et al., 2015). In fact, an increase in the number of T_3 receptors in the mammary gland secretory cells during lactation has been reported (Wilson and Gorewit, 1980). Moreover, during the transition period, the transcription of iodothyronine deiodinases (the enzymes that convert the precursor thyroxine, T_4 , into the active form, T_3) changes. The conversion of T_4 to T_3 decreases in liver and increases in the mammary gland (Capuco et al., 2008). Such changes may lead to an increase in the sensitivity of mammary tissue to thyroid hormones to establish the metabolic priority for the mammary gland and boost the galactopoietic effects of these hormones.

Moreover, bST, which stimulates gluconeogenesis and lipolysis—thus leading to an increase in the concentrations of plasma NEFA and promoting BCS loss (Lucy et al., 2001, 2009)—gradually increases after calving. The higher bST concentration observed postpartum was paired with the decrease in circulating IGF-1. In fact, these hormones are strictly related and IGF-1 elicits negative feedback control on bST (Le Roith et al., 2001). In addition, IGF-1 is the main mediator of bST action on milk production and regulates milk synthesis by the mammary gland (Etherthon and Bauman, 1998). Together, the greater bST and lesser amounts of IGF-1 in blood are part of the homeorhetic state that supports milk synthesis by promoting a catabolic state (Burton et al., 1994).

Glucagon's gradual increase during lactation supports the recovery of homeostatic glycemia. Glucagon is a hyperglycemic hormone that, in response to low glucose, promotes hepatic glucose output by causing an increase in glycogenolysis and gluconeogenesis from AA, thereby leading to increases in glycemia (Flakoll et al., 1994; Jiang and Zhang, 2003). Infusion of glucagon in periparturient cows resulted in an increase in plasma glucose and insulin and a decrease in plasma NEFA and BHB and hepatic triglycerides (Bobe et al., 2003; Nafikov et al., 2006), similar to what we observed in the present study after the first month of lactation when glucagon increased and blood metabolites returned to physiological concentrations (Premi et al., 2021).

Metabolic and endocrine variations observed in the present study are those typical of the period in clinically healthy subjects (Cozzi et al., 2011; Premi et al., 2021), with an upper middle production level and fed with a program that allows maximization of DMI with a gradual increase in energy and protein content. These results confirm that the turning point in terms of negative energy balance lies in the first 2 wk of lactation, even though some hormones (insulin and IGF-1) and blood markers (alkaline phosphatase and globulin) demonstrated relevant alterations even 7 d before calving. Therefore, the hypothesis of the prominent role of the weeks preceding the calving event in establishing the success of the transition period (Grummer, 1995; Drackley, 1999; Mezzetti et al., 2021) is emphasized.

Differences Between the First and Second Lactation

As expected, milk yield during early lactation increased more than 25% during the second lactation due to completion of growth. The cow's full development is not reached until third lactation. Indeed, BW gradually increases in both lactations, keeping a constant difference between them. Mammary secretory ability greatly increases in the second lactation, likely as a result of a combination of a greater number of secretory cells (Capuco et al., 2001) and their greater metabolic activity in multiparous compared with primiparous cows (Miller et al., 2006). In the mammary gland of multiparous cows, greater expression of genes related to metabolic activity and greater content of DNA and fatty acid synthase protein can be found (Miller et al., 2006). Moreover, as expected, cows showed a faster increase in milk yield during the second lactation, reaching peak milk yield earlier; peak milk yield was also higher than during the first lactation (Wood, 1980). Consequently, besides the similar milk composition, this difference led to higher fat and protein output in milk. At the same time, DMI was around 3 kg/d higher and BW around 70 kg higher in second- versus first-lactation cows,

suggesting greater development of the gastrointestinal tract and a higher flux of nutrients from nutrient digestion in second-lactation cows. However, the increase in DMI and thus in energy intake could not compensate for the requirements of the greater milk yield, resulting in a more marked negative energy balance, in particular 7 d after calving, which is the most critical phase in the postpartum period. Plasma glycemia fully confirmed the latter. In this context, circulating insulin declined even more during the days around calving to support the large glucose uptake by the mammary gland and the basal metabolism in the peripheral tissues (Bauman, 2000; Piccioli-Cappelli et al., 2014). Surprisingly, these variations did not result in differences in circulating glucagon and bST. As a consequence of the more severe negative energy balance and lower insulin, more body reserves were mobilized during second lactation to support milk production. The latter was not shown by variations in BCS itself in this period but, considering the higher BW in the second lactation and the similar decrease in BCS, it can be speculated that during the second lactation, cows mobilized a greater amount of fat reserves. Mobilization from visceral adipose tissue, which cannot be evaluated using any BCS method, might have been involved (Akter et al., 2011). Proceeding through lactation, milk yield curves gradually came closer between the 2 lactations, and BCS corresponded about 4 mo after calving. The latter was the result of the lower persistency and higher DMI of second-lactation cows, 2 aspects that combined led to a greater body reserve accumulation.

The lower IGF-1 observed in the transition period during second lactation was consistent with the more severe negative energy balance. However, its action in prioritizing milk production in nutrient partitioning is usually associated with increased bST concentrations to promote the effects of bST on lipolysis and gluconeogenesis (Lucy et al., 2001). Interestingly, the impaired IGF-1:bST ratio before calving contributes to driving the use of lipid reserves with other possible consequences at the metabolic level, such as during lipid mobilization, when adipokines that act on many tissues and on the immune response are released (Häussler et al., 2022). Moreover, the higher bST that occurred later during the lactation was noteworthy and likely related to the still-growing state of primiparous cows.

The changes observed in metabolism confirmed the above hypothesis, in particular the higher BHB concentrations in the immediate postpartum period during the second lactation. Nevertheless, postpartum BHB concentrations were below the subclinical ketosis threshold (Duffield, 2000). The higher blood urea observed during the second lactation in the postpartum period could also suggest that, alongside greater dietary protein intake

due to higher DMI (Wang et al., 2007), increased reliance on AA as a source for gluconeogenesis in the liver occurred. This speculation appears to be supported by the higher levels of glucagon. In this phase, the glucose supply relies more on gluconeogenesis from endogenous sources such as increased peripheral mobilization and hepatic uptake from muscle AA and glycerol from fat mobilization. The higher creatinine concentration 7 d after calving during the second lactation (as creatinine is also an indicator of protein mobilization) is consistent with these observations (Kokkonen et al., 2005; Osorio et al., 2014). This finding, in turn, could support either higher milk protein output and consequent AA demand or altered N efficiency.

The concentration of NEFA was similar between lactations, despite a greater apparent amount of adipose tissue mobilization during the second lactation. This lack of difference was consistent with the similar blood bST observed and might be related to a greater oxidative capacity of the liver or greater incorporation into milk fat (Lucy et al., 2001). The latter hypothesis is in agreement with the greater fat output during the second lactation despite the similar fat content of milk. Instead, the first might be supported by slightly better liver condition during the second lactation, even though this apparently better status when energy deficit was more severe should be elucidated, and the increase in hepatic synthesis—as revealed by most of the liver functionality biomarkers—should be analyzed. In a recent study, Giannuzzi et al. (2021) reported a greater liver size in multiparous cows compared with primiparous cows despite having a higher fat infiltration, suggesting the capability to support a higher metabolic load. Cholesterol, as an indicator of lipoprotein synthesis, and albumin, which is a blood protein of liver origin (Eckersall, 2008; Cattaneo et al., 2021) was higher during the second lactation, whereas bilirubin, which results from the degradation of red blood cells and thus reflects the efficiency of liver enzymes in its clearance (Bertoni et al., 2008; Bertoni and Trevisi, 2013), was lower. The higher alkaline phosphatase, which is a marker of liver function but can have several different origins (such as bones, placenta, and uterus) and exerts a role in dephosphorylating compounds, in primiparous cows was difficult to interpret. It could be speculated that as a result of greater mobilization from bone tissue as determined by the low blood concentration of calcium during the first lactation, phosphorus accumulated in the blood (Sato et al., 2013; Shipman et al., 2013). Transaminases GOT and GGT are involved in AA metabolism and usually serve as markers of liver damage (Bertoni et al., 2008) when values are above the reference values. Considering the condition described earlier and the lack of difference in GOT,

the higher GGT observed during the second lactation fell within reference limits (Premi et al., 2021) and might be interpreted as a consequence of the increase in metabolic activity due to the higher demand of the mammary gland and the increased liver activity required (Giannuzzi et al., 2021) or of the higher AA metabolism (Calamari et al., 2015).

The calculation of dairy and N efficiency highlighted the imbalance between feed input and milk output at the beginning of the second lactation, both from a mass and a protein standpoint. The N efficiency, moreover, can indicate differences in protein metabolism. The higher efficiency at the onset of the second lactation might be the result of a pronounced reliance on muscle AA as a source of gluconeogenic precursors allowed by the lower circulating insulin. Moreover, the aforementioned higher GGT values during second lactation support this hypothesis and indicate greater protein metabolism activity.

Inflammatory conditions were similar in the 2 lactations in our study, even though ceruloplasmin (after the typical postpartum increase) returned to physiological values later during the second lactation. Ceruloplasmin is a positive acute-phase protein with a longer half-life than haptoglobin and is synthesized mainly by the liver (Bertoni and Trevisi, 2013). However, it can be produced by other organs, including kidneys and mammary gland (Linder, 2016). Although interpreting these variations is challenging, we speculate that the higher concentrations of ceruloplasmin during the second lactation might be related to greater development of such organs; likewise, the greater liver activity mentioned above might contribute.

Milk from cows during their first lactation had higher titratable acidity and better coagulation properties with faster coagulation time (lower RCT and k_{20}) and better curd firmness (higher a_{30}). Better coagulation properties in primiparous than multiparous cows were previously reported (Bittante et al., 2015). These differences might be related to the different milk yields but are mainly due to the lower negative energy balance observed in cows during the first lactation, as often the diet imbalance to requirements results in a worsening of clotting features (Calamari et al., 2010) and to mild chronic inflammatory status (such as high ceruloplasmin; Bertoni et al., 2001; Pegolo et al., 2022). Moreover, as proposed earlier, the higher titratable acidity in primiparous cows could be due to the slightly higher levels of stress hormones (Bertoni et al., 1985) likely caused by adaptation to new experiences (including calving, milking, and diet) and environment (such as group and daily routine), whereas the better coagulation properties were related to the lower degree of negative energy balance.

CONCLUSIONS

Holstein cows managed identically for 3 years were considered healthy during the period evaluated herein, yet their metabolic and endocrine profiles differed significantly during their first and second lactations. Aside from potential genetic and environmental variations, first-lactation cows had a less pronounced negative energy balance compared with the second lactation, as a consequence of lower milk yield, as supported also by metabolic biomarkers and hormones. Moreover, liver activity and metabolic load were greater in the second lactation. The inflammatory profile around parturition was similar between the 2 lactations. These results suggest different metabolic and hormonal milieu between the same cows during their first and second lactation, which should be taken into consideration in the assessment of metabolic profile and the cows' nutrition and management in dairy farms. Potential effects of diurnal and seasonal variation, as well as variations in nutrient utilization, should be taken into account to better understand metabolism in this important phase.

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