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Cows with feed restriction–induced hyperketonemia early postpartum have a different immunometabolic profile than healthy cows or cows with inflammatory disorders

J. K. Drackley,[1]* H. M. Dann,[1]† G. Bertoni,[2] and E. Trevisi[2,3]
[1]Department of Animal Sciences, University of Illinois, Urbana, IL 61801
[2]Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy
[3]Romeo and Enrica Invernizzi Research Center for Sustainable Dairy Production of the Università Cattolica del Sacro Cuore (CREI), 29122 Piacenza, Italy

ABSTRACT

Immune activation and subsequent inflammation lead to difficult transitions from pregnancy to lactation. Whether postpartum hyperketonemia can occur independently of immune activation remains controversial. Our objective was to compare immunometabolic profiles in blood serum from healthy cows, healthy feed-restricted cows, and cows with naturally occurring inflammatory disorders. Multiparous Holsteins (n = 32) were fed a dry period diet until parturition. After parturition, all cows were fed a lactation diet at ad libitum intake until 5 DIM. At 4 DIM, cows underwent a thorough physical examination by a veterinarian and were classified as healthy (n = 15) or having at least 1 periparturient disorder or disease (n = 17), which were inflammatory conditions. Healthy cows were assigned to a control ad libitum-fed group (CON; n = 6) or to a group subjected to 50% feed restriction beginning at d 5 (FR; n = 9) and continuing until diagnosis of clinical ketosis or d 14, when they were returned to ad libitum DMI. The periparturient inflammatory disorders (PID; n = 17) group consisted of cows with metritis, retained placenta, foot and leg problems, or mastitis. During the dry period, the serum ratio of albumin:globulin tended to be lower and the concentration of β-carotene was lower for cows destined to be sick than for cows that were healthy postpartum. At d −1 prepartum, haptoglobin and globulin tended to be greater for cows that were sick postpartum than for healthy cows. The albumin:globulin ratio and creatinine at d −1 tended to be lower for sick cows than for healthy cows. At d 1 postpartum, cows that were sick tended to have greater BHB and had lower Zn, albumin, and retinol than healthy cows. Aspartate aminotransferase tended to be greater for sick cows than for healthy cows. At d 7 (during feed restriction but before diagnosis of clinical ketosis), glucose and cholesterol were lower, and nonesterified fatty acids and BHB were greater, in FR cows than in PID cows. Concentration of Ca tended to be lower for cows in FR and PID than for CON cows. Albumin concentration and the ratio of albumin:globulin were lower for PID cows than for FR cows, whereas haptoglobin was greater for PID than for FR. Paraoxonase was lower for cows in FR and PID than for CON. Activity of γ-glutamyltransferase was greater for cows in FR and PID than for CON cows. Bilirubin tended to be greater for cows in FR and PID compared with CON. Retinol tended to be lower for cows with disorders and lower for PID than FR. β-Carotene was greater for FR cows than for PID cows. The liver functionality index, a measure of cow resilience in the transition period, showed the lowest value in PID and the highest in CON, with FR intermediate. Overall, healthy cows with feed restriction–induced hyperketonemia showed little evidence for involvement of inflammation.

Key words: periparturient period, inflammation, ketosis

INTRODUCTION

The importance of immune activation and inflammation in determining the success of the transition period has become well documented (Bertoni et al., 2008; Bradford et al., 2015; Horst et al., 2021). Inflammation can lead to decreased DMI and milk yield (Bertoni et al., 2008; Horst et al., 2021), hallmarks of cows with "difficult" transitions. Inflammation is triggered by immune activation in response to a variety of infectious and noninfectious stimuli, which leads to the release of

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*Corresponding author: drackley@illinois.edu
†Current address: William H. Miner Agricultural Research Institution, Chazy, NY 12921.

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Table 1. Serum concentrations of analytes (LSM and 95% confidence limits) during the prepartum period[1] for cows that were either healthy or had inflammatory disorders (sick) after parturition

	Postpartum health at d 4		*P*-value		
Analyte	Healthy	Sick	Health	Time	Time × health
Glucose, mg/dL	59 (54, 63)	60 (56, 64)	0.86	<0.001	0.57
NEFA, μEq/L	226 (142, 310)	232 (148, 316)	0.83	<0.001	0.51
BHB, mg/dL	3.8 (2.2, 5.4)	3.9 (2.3, 5.5)	0.72	0.004	0.64
Cholesterol, m*M*	2.88 (2.65, 3.12)	2.93 (2.71, 3.17)	0.72	<0.001	0.48
Ca, m*M*	2.64 (2.56, 2.75)	2.67 (2.59, 2.75)	0.61	0.12	0.52
P, m*M*	2.17 (2.04, 2.31)	2.07 (1.96, 2.19)	0.25	0.04	0.18
Mg, m*M*	0.88 (0.83, 0.94)	0.88 (0.83, 0.93)	0.90	<0.001	0.36
Zn, μ*M*	14.4 (13.54, 15.31)	14.3 (13.5, 15.2)	0.91	<0.001	0.63
Total protein, g/L	81.6 (79.0, 84.28)	83.5 (81.0, 86.1)	0.31	<0.001	0.68
Albumin, g/L	36.2 (35.2, 37.1)	35.8 (34.9, 36.6)	0.54	0.009	0.39
Globulin, g/L	45.2 (42.6, 47.9)	47.7 (45.1, 50.4)	0.19	<0.001	0.54
Albumin:globulin	0.83 (0.78, 0.87)	0.77 (0.72, 0.82)	0.10	<0.001	0.005
Haptoglobin, g/L	0.21 (0.17, 0.27)	0.22 (0.18, 0.28)	0.77	0.81	0.86
Paraoxonase, U/L	41.3 (37.5, 45.4)	39.4 (36.0, 43.2)	0.48	0.09	0.82
AST, U/L	53.6 (47.8, 60.1)	59.6 (53.4, 66.4)	0.18	0.48	0.20
GGT, U/L	21.1 (19.0, 23.5)	22.7 (20.5, 25.1)	0.32	<0.001	0.76
Bilirubin, μ*M*	0.31 (0.17, 0.56)	0.25 (0.14, 0.44)	0.58	<0.001	0.74
Creatinine, μ*M*	123 (118, 129)	119 (114, 124)	0.31	0.81	0.17
Retinol, μg/100mL	36.9 (34.1, 39.9)	35.2 (32.7, 37.9)	0.39	<0.001	0.09
Tocopherol, μg/100mL	0.83 (0.59, 1.18)	0.65 (0.47, 0.90)	0.30	0.02	0.26
β-carotene, μg/100mL	0.054 (0.046, 0.062)	0.042 (0.35, 0.50)	0.04	0.85	0.86

[1]Means of samples from d −28, −21, −14, and −7.

pro-inflammatory cytokines. Because of the irregular release and short half-life of the cytokines, they are less useful for detecting chronic inflammation that occurs in periparturient dairy cows; instead, various biomarkers of inflammation are used as stable indicators (Bertoni and Trevisi, 2013). Inflammation leads to decreased serum concentrations of albumin, retinol, total cholesterol, paraoxonase, Ca, Mg, Zn, tocopherol, β-carotene, and creatinine, whereas concentrations of globulin, haptoglobin, bilirubin, aspartate aminotransferase (**AST**), and γ-glutamyltranspeptidase (**GGT**) increase (Bertoni and Trevisi, 2013).

Cows with health problems in early lactation generally have elevated nonesterified fatty acids (**NEFA**) and BHB and lower Ca and Mg in blood than healthy cows (Bertoni and Trevisi, 2013). Metabolic changes during clinical ketosis include increases of NEFA and BHB, and decreased glucose (Mann and McArt, 2023). Signs of inflammation were detected in the dry period for cows with postpartum hyperketonemia (Zhang et al., 2016) and metritis (Dervishi et al., 2016). Given that inflammation can result in some of the same metabolic changes as in hyperketonemia, Horst et al. (2021) argued that hyperketonemia is not a disease per se but is a consequence either of high milk production in healthy cows or of an activated immune system and the resulting inflammation. Furthermore, they postulated that inflammation might be causative for hypocalcemia (Horst et al., 2021). Inflammatory conditions have variable effects on concentrations of NEFA and BHB, with some studies showing unchanged or decreased concentrations (Bertoni et al., 2008), and others showing increases (Trevisi et al., 2012). The effects seem to depend on stage of lactation, with NEFA decreasing in response to inflammation in mid to late lactation, but increasing in early lactation. The concentration of insulin (Waldron et al., 2003) increases during inflammation. These situations cast doubt on whether inflammation can be the cause of uncomplicated hyperketonemia in all cases. Cows with hyperketonemia often are affected by other disorders and conditions (Dohoo and Martin, 1984; Rico and Barrientos-Blanco, 2024), which complicates understanding of the immunometabolic profile of uncomplicated primary hyperketonemia. Studies to untangle the complex interaction between metabolic and inflammatory conditions during the periparturient period are needed (Trevisi et al., 2025).

Our hypothesis was that otherwise healthy cows with uncomplicated hyperketonemia induced by feed restriction in early lactation would have a different immunometabolic profile than cows with naturally occurring inflammatory conditions. Our primary objectives were (1) to compare the immunometabolic profile in blood serum from healthy cows with those of healthy cows during feed restriction or with inflammatory conditions, and (2) to determine if cows during feed restriction differed in their immunometabolic profile from those with inflammatory conditions. A secondary objective was to determine if prepartum measurements of immunometa-

Table 2. Serum concentrations of analytes (LSM and 95% confidence limits) at d −1 prepartum for cows that were either healthy or had inflammatory disorders (sick) after parturition

	Postpartum health at d 4		*P*-value
Analyte	Healthy	Sick	Health
Glucose, mg/dL	54 (50, 58)	54 (51, 57)	0.80
NEFA, μEq/L	472 (278, 666)	513 (321, 605)	0.48
BHB, mg/dL	5.8 (3.0, 8.6)	5.9 (3.1, 8.7)	0.91
Cholesterol, m*M*	2.28 (2.02, 2.57)	2.33 (2.11, 2.57)	0.80
Ca, m*M*	2.53 (2.43,2.63)	2.48 (2.40, 2.56)	0.43
P, m*M*	1.95 (1.67, 2.29)	1.68 (1.47, 1.92)	0.15
Mg, m*M*	0.81 (0.70, 0.92)	0.78 (0.69, 0.87)	0.64
Zn, μ*M*	10.58 (9.26, 12.08)	9.57 (8.56, 10.69)	0.24
Total protein, g/L	73.4 (70.1, 76.9)	76.9 (74.0, 80.0)	0.13
Albumin, g/L	35.2 (33.9, 36.6)	34.8 (33.7, 35.9)	0.56
Globulin, g/L	38.1 (35.1, 41.3)	41.9 (39.1, 44.9)	0.08
Albumin:globulin	0.93 (0.85, 1.01)	0.84 (0.78, 0.90)	0.07
Haptoglobin, g/L	0.24 (0.14, 0.44)	0.46 (0.28, 0.76)	0.10
Paraoxonase, U/L	38.7 (34.0, 44.2)	37.5 (33.6, 41.8)	0.70
AST, U/L	49.2 (41.9, 57.9)	57.3 (50.0, 65.6)	0.15
GGT, U/L	18.9 (16.5, 21.8)	21.8, 19.4, 24.4)	0.11
Bilirubin, μ*M*	2.24 (1.51, 3.30)	2.51 (1.81, 3.49)	0.63
Creatinine, μ*M*	127.2 (120.6, 134.3)	119.8 (114.6, 125.3)	0.09
Retinol, μg/100mL	28.6 (24.0, 34.1)	23.8 (20.6, 27.6)	0.11
Tocopherol, μg/100mL	1.53 (1.02, 2.29)	1.64 (1.17, 2.29)	0.79
β-carotene, μg/100mL	0.056 (0.042, 0.069)	0.047 (0.035, 0.058)	0.29

bolic status were predictive for inflammation-related disorders in early lactation.

MATERIALS AND METHODS

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. The original experiment was conducted between April 2000 to January 2001. The experimental design, treatments, and management procedures were described in Dann et al. (2005). Briefly, 32 multiparous Holstein cows housed in tiestalls were fed a dry period diet at either ad libitum (n = 15) or restricted intake (n = 17) until parturition. After parturition, all cows were fed the same lactation diet at ad libitum intake until 5 DIM. At 4 DIM, cows underwent a thorough physical examination by a veterinarian and were classified as healthy (n = 15) or having at least one periparturient disorder or disease (n = 17). The physical examination was described in detail in Dann et al. (2005). Healthy cows were assigned randomly to a control ad libitum-fed group (**CON**; n = 6) or to a group in which hyperketonemia was induced by 50% feed restriction beginning at d 5 (**FR**; n = 9). Cows in this group were feed restricted until diagnosis of clinical ketosis or d 14, when they were returned to ad libitum DMI. The restricted amount of TMR was divided into 2 meals fed at ~10- and 14-h intervals. Blood samples used for the feed-restriction period were always before diagnosis of clinical ketosis if induced; no blood samples were used after returning to full feed. Clinical ketosis was diagnosed as cows with strong degree of ketones in urine, inappetence, and lethargy or excitability. Cows with inflammatory conditions (**PID**; n = 17) consisted of cows with metritis, retained placenta, foot and leg problems, or mastitis. Some of the cows had more than one inflammatory condition, and the incidence of disorders is reported in Dann et al. (2005).

Blood was sampled before the a.m. feeding on d −28, −21, −14, −7, −1, 1, 7, 14, 21, and 28 relative to parturition, and serum was frozen at −20°C until analyses. Samples were analyzed for concentrations of glucose, NEFA, and BHB as described in Dann et al. (2005). Samples frozen for ~3 yr before thawing were analyzed for total cholesterol, Ca, P, Mg, Zn, total protein, albumin, haptoglobin, paraoxonase, AST, GGT, total bilirubin, creatinine, retinol, tocopherol, and β-carotene as described in Bionaz et al. (2007). Total globulin was calculated as the difference between total protein and albumin. The liver functionality index (**LFI**) was calculated from concentrations of cholesterol, albumin, and bilirubin at d 7 and d 28 as described by Bertoni and Trevisi (2013).

Statistical analysis of data was performed using PROC GLIMMIX in SAS (version 9.4; SAS Institute). Cow was the experimental unit. Prepartum data (d −28, −21, −14, and −7) were analyzed statistically using a mixed effects model consisting of precalving diet (ad libitum or restricted), d 4 postpartum health status (healthy or sick), the repeated effect of day relative to

Table 3. Serum concentrations of analytes (LSM and 95% confidence limits at d 1 postpartum for cows that were either healthy or had inflammatory disorders (sick) after parturition

	Postpartum health at d 4		*P*-value
Analyte	Healthy	Sick	Health
Glucose, mg/dL	52 (48, 56)	54 (50, 58)	0.45
NEFA, µEq/L	1,097 (692, 1476)	1,112 (752, 1515)	0.92
BHB, mg/dL	7.9 (4.2, 11.5)	10.1 (6.5, 13.7	0.08
Cholesterol, m*M*	2.19 (2.00, 2.39)	2.04 (1.87, 2.21)	0.25
Ca, m*M*	2.05 (1.78, 2.37)	1.98 (1.73, 2.28)	0.73
P, m*M*	1.50 (1.30, 1.72)	1.65 (1.44, 1.89)	0.32
Mg, m*M*	0.81 (0.73, 0.90)	0.84 (0.75, 0.92)	0.69
Zn, µ*M*	8.00 (6.80, 9.39)	6.32 (5.42, 7.37)	0.04
Total protein, g/L	76.4 (73.5, 79.4)	76.7 (74.0, 79.6)	0.87
Albumin, g/L	36.2 (35.2, 37.1)	34.7 (33.8, 35.6)	0.03
Globulin, g/L	40.0 (37.3, 43.0)	41.8 (39.0, 44.7)	0.38
Albumin:globulin	0.91 (0.84, 0.98)	0.84 (0.78, 0.90)	0.12
Haptoglobin, g/L	0.77 (0.52, 1.14)	0.68 (0.47, 0.99)	0.65
Paraoxonase, U/L	35.3 (28.5, 43.7)	29.0 (23.7, 35.5)	0.18
AST, U/L	71.2 (61.0, 83.1)	84.9 (73.3, 98.4)	0.10
GGT, U/L	22.1 (19.2, 25.3)	24.5 (21.5, 27.9)	0.27
Bilirubin, µ*M*	4.66 (3.31, 6.55)	5.18 (3.78, 7.10)	0.64
Creatinine, µ*M*	123.1 (116.7, 129.8)	120.7 (114.7, 127.0)	0.59
Retinol, µg/100mL	24.0 (20.8, 25.1)	18.7 (16.3, 21.4)	0.02
Tocopherol, µg/100mL	1.62 (1.37, 1.91)	1.58 (1.35, 1.86)	0.86
β-carotene, µg/100mL	0.050 (0.040, 0.060)	0.042 (0.032, 0.051)	0.21

calving, and all interactions. Postpartum recovery data (d 21 and 28) were analyzed statistically using a mixed effects model consisting of precalving diet (ad libitum or restricted), postpartum health group (CON, FR, or PID), the repeated effect of day relative to calving, and all interactions. Cow was considered a random effect. Several covariance structures for the repeated effect were tested; the unstructured option was chosen based on the lowest Akaike information criterion value. Interactions of prepartum diet with health status were not significant and are not presented or discussed herein. Experimental data on discrete days (d −1, d 1, and d 7) were analyzed in a similar model without repeated measures. The latter approach was taken to account for distinct metabolic milestones (immediate prepartum, immediate postpartum before feed restriction, during feed restriction) where treatments or groups differed. All models except for the albumin:globulin ratio and β-carotene used a lognormal distribution because of improved model fit; LSM and 95% confidence limits were back-transformed for presentation. Model residuals were examined for homogeneity of variance and homoscedasticity. Data points whose model studentized residuals were >4 were deleted and the models were re-run. Two orthogonal contrast statements were constructed using the ESTIMATE statement for data from d 7 and d 21 to 28: healthy cows versus cows with disorders (CON vs. FR and PID), and feed restriction versus inflammatory disorder (FR vs. PID). The *P*-values for the estimates were adjusted for multiple comparisons using the Sidak option. Significance was declared when $P \leq 0.05$, and tendencies are discussed when $P \leq 0.10$ but >0.05.

RESULTS

During the dry period (means of d −28, −21, −14, and −7), mean DMI across diets did not differ ($P = 0.51$) between cows that were healthy postpartum (10.6 ± 0.42 kg/d) and those that were sick postpartum (11.0 ± 0.42 kg/d). Most variables did not differ between cows that were healthy postpartum and those that were sick postpartum (Table 1). The albumin:globulin ratio tended ($P = 0.10$) to be lower for cows destined to be sick and concentration of β-carotene was lower ($P = 0.04$) than for cows that were healthy postpartum. The significant interaction of health and day relative to expected calving for the albumin:globulin ratio showed that cows that were healthy after calving had a faster increase of the ratio and reached a higher value by d −7 than cows that were sick after calving (data not shown).

At d −1 prepartum, the DMI did not differ ($P = 0.36$) between cows that were healthy postpartum (7.9 ± 0.67 kg/d) and those that were sick postpartum (6.7 ± 0.67 kg/d). The concentration of globulin tended to be greater ($P = 0.08$) for cows that were sick postpartum than for healthy cows (Table 2). As a result, the albumin:globulin ratio tended to be lower ($P = 0.07$)

Table 4. Serum concentrations of analytes (least squares means and 95% confidence limits) at d 7 postpartum for cows that were healthy (CON), were undergoing feed restriction (FR), or had inflammatory disorders (PID) after parturition

	Postpartum health group			*P*-value	
Analyte	CON	FR	PID	CON vs. FR and PID	FR vs. PID
Glucose, mg/dL	53 (41, 65)	41 (29, 53)	52 (42, 62)	0.23	0.003
NEFA, μEq/L	552 (342, 894)	1,573 (1,060, 2335)	749 (561, 1002)	0.03	0.008
BHB, mg/dL	7.2 (2.7, 11.4)	16.7 (12.3, 21.0)	9.5 (5.2, 13.8)	0.05	<0.001
Cholesterol, m*M*	2.26 (1.90, 2.69)	2.26 (1.96, 2.60)	1.88 (1.69, 2.09)	0.55	0.08
Ca, m*M*	2.53 (2.33, 2.75)	2.37 (2.22, 2.54)	2.25 (2.14, 2.36)	0.09	0.34
P, m*M*	2.09 (1.74, 2.51)	2.33 (2.00, 2.72)	2.28 (2.03, 2.56)	0.56	0.96
Mg, m*M*	0.85 (0.74, 0.96)	0.78 (0.69, 0.87)	0.72 (0.65, 0.78)	0.16	0.46
Zn, μ*M*	11.9 (8.6, 16.4)	10.5 (8.0, 13.6)	10.3 (8.4, 12.5)	0.68	0.99
Total protein, g/L	79.7 (74.4, 85.4)	75.8 (71.7, 80.3)	75.6 (72.4, 78.8)	0.33	0.99
Albumin, g/L	33.9 (31.7, 36.3)	34.2 (32.4, 36.2)	30.5 (29.3, 31.8)	0.37	0.004
Globulin, g/L	45.4 (40.3, 51.1)	41.5 (37.6, 45.7)	44.7 (41.6, 48.1)	0.67	0.38
Albumin:globulin	0.76 (0.65, 0.87)	0.83 (0.74, 0.92)	0.69 (0.63, 0.76)	0.99	0.04
Haptoglobin, g/L	0.70 (0.36, 1.37)	0.53 0.30, 0.92)	1.25 (0.82, 1.89)	0.90	0.03
Paraoxonase, U/L	35.2 (24.3, 50.9)	19.1 (14.1, 25.8)	25.3 (20.1, 31.8)	0.05	0.26
AST, U/L	74.4 (55.2, 101.7)	79.6 (62.3, 101.7)	106.8 (88.8, 128.4)	0.36	0.10
GGT, U/L	18.3 (13.8, 24.3)	26.2 (20.8, 33.1)	24.7 (20.7, 29.4)	0.08	0.89
Bilirubin, μ*M*	1.84 (0.89, 3.80)	3.74 (2.06, 6.78)	3.68 (2.36, 5.76)	0.10	0.99
Creatinine, μ*M*	113.5 (105.4, 122.1)	107.7 (101.4, 114.4)	103.7 (99.1, 108.5)	0.10	0.52
Retinol, μg/100mL	25.4 (20.8, 31.0)	23.8 (20.2, 28.0)	17.3 (15.3, 19.6)	0.09	0.007
Tocopherol, μg/100mL	0.58 (0.31, 1.08)	0.97 (0.69, 1.37)	0.67 (0.49, 0.92)	0.51	0.22
β-carotene, μg/100mL	0.022 (0.013, 0.046)	0.039 (0.032, 0.046)	0.025 (0.020, 0.030)	0.05	0.004

for sick cows than for healthy cows. The concentration of haptoglobin also tended to be greater ($P = 0.10$) for cows that were sick postpartum than for healthy cows. Creatinine tended to be lower ($P = 0.09$) for cows that were sick postpartum. Other analytes were not affected by postpartum health status.

During d 1 to 4 prepartum, DMI was greater ($P = 0.002$) for healthy cows (13.7 ± 0.74 kg/d) than for cows that were sick (10.3 ± 0.74 kg/d). Milk production was 21.4 kg/d and 16.1 kg/d (SE = 1.41 kg/d; $P = 0.01$) for healthy and sick cows, respectively. Calculated energy balance was 89% and 80% of requirements (SE = 7%; $P = 0.38$; Dann et al., 2005). At d 1 postpartum (Table 3), cows that were sick tended to have greater BHB ($P = 0.08$) and had lower Zn ($P = 0.04$) in serum than healthy cows. The concentration of albumin ($P = 0.03$) was lower for sick cows than for healthy cows. The activity of AST tended to be greater ($P = 0.10$) for sick cows than for healthy cows. Retinol was lower ($P = 0.02$) for sick cows than for healthy cows. Other analytes did not differ significantly between health statuses.

During the feed-restriction period from d 5 to 14 postpartum, DMI was 18.9, 9.6, and 14.1 kg/d for CON, FR, and PID groups, respectively (SE = 1.6 kg/d; Dann et al., 2005). Milk production was 34.2, 26.8, and 24.0 kg/d (SE = 2.6 kg/d) and energy balance was 93, 53, and 88% of requirements (SE = 11%; Dann et al., 2005). Four of 9 cows subjected to feed restriction developed signs of clinical ketosis before d 14, but after d 7 when blood was sampled (Dann et al., 2005). At d 7, the concentration of glucose was lower ($P = 0.003$) in FR cows than in PID cows (Table 4). The concentrations of NEFA ($P = 0.03$) and BHB were greater for FR and PID than for CON cows ($P = 0.05$), primarily because NEFA ($P = 0.008$) and BHB were greater for FR cows than for PID cows ($P < 0.001$). Cholesterol concentration tended to be lower for PID cows than FR cows ($P = 0.08$). The concentration of Ca tended to be lower ($P = 0.09$) for FR and PID cows than for CON cows. Albumin concentration ($P = 0.004$) and the ratio of albumin:globulin ($P = 0.04$) were lower for PID cows than for FR cows. Paraoxonase was lower ($P = 0.05$) for FR and PID cows than for CON cows. Activities of AST ($P = 0.10$) and GGT tended to be greater ($P = 0.08$) for FR and PID cows than for CON cows. The concentration of bilirubin tended ($P = 0.10$) to be greater and the concentration of creatinine tended to be lower ($P = 0.10$) for FR and PID than for CON. The concentration of retinol tended to be lower ($P = 0.09$) for FR and PID cows than for CON cows primarily because it was lower for PID cows than for FR cows ($P = 0.007$). The concentration of β-carotene was greater ($P = 0.05$) in FR and PID cows than CON cows primarily because concentrations were greater for FR cows than for PID cows ($P = 0.004$).

During d 15 to d 42, DMI was 21.3, 18.5, and 19.6 (SE = 1.5) kg/d for CON, FR, and PID groups. Milk production was 38.6, 37.1, and 34.1 (SE = 2.5) kg/d, whereas energy balance was 103%, 102%, and 108% (SE = 6%)

Table 5. Serum concentrations of analytes (LSM and 95% confidence limits) during d 21 and d 28 postpartum for cows that were healthy (CON), had undergone feed restriction (FR), or had inflammatory disorders (PID) after parturition

	Postpartum health group			*P*-value			
Analyte	CON	FR	PID	CON vs. FR and PID	FR vs. PID	Time	Time × Health
Glucose, mg/dL	57 (50, 64)	59 (52, 63)	57 (51, 63)	0.90	0.56	0.66	0.52
NEFA, μEq/L	321 (115, 525)	258 (26, 462)	280 (70, 483)	0.95	0.57	<0.001	0.24
BHB, mg/dL	4.2 (2.4, 6.3)	4.6 (2.9, 6.5)	4.5 (2.6, 6.4)	0.93	0.63	<0.001	0.26
Cholesterol, m*M*	3.81 (3.09, 4.71)	3.64 (2.88, 4.62)	3.00 (2.64, 3.40)	0.44	0.27	<0.001	0.36
Ca, m*M*	2.61 (2.50, 2.72)	2.59 (2.47, 2.71)	2.52 (2.46, 2.59)	0.65	0.53	0.39	0.32
P, m*M*	2.16 (1.95, 2.39)	2.12 (1.89, 2.38)	2.03 (1.91, 2.16)	0.76	0.74	0.74	0.60
Mg, m*M*	0.95 (0.86, 1.04)	0.90 (0.81, 1.01)	0.90 (0.85, 0.96)	0.61	0.99	0.37	0.77
Zn, μ*M*	12.9 (10.7, 15.5)	11.5 (9.3, 14.1)	11.3 (10.1, 12.7)	0.44	0.99	0.17	0.67
Total protein, g/L	84.9 (80.3, 89.8)	82.6 (77.6, 88.0)	83.8 (81.0, 86.6)	0.78	0.91	0.02	0.88
Albumin, g/L	35.5 (33.1, 38.0)	35.4 (32.8, 38.3)	32.1 (30.8, 33.5)	0.40	0.06	0.009	0.96
Globulin, g/L	49.0 (44.7, 53.8)	47.0 (42.4, 52.2)	51.5 (48.6, 54.4)	0.99	0.24	0.04	0.76
Albumin:globulin	0.74 (0.65, 0.83)	0.76 (0.66, 0.86)	0.63 (0.57, 0.68)	0.65	0.05	0.53	0.53
Haptoglobin, g/L	0.15 (0.09, 0.25)	0.17 (0.10, 0.31)	0.30 (0.22, 0.41)	0.32	0.17	0.13	0.23
Paraoxonase, U/L	39.3 (29.3, 52.7)	34.9 (25.1, 48.5)	32.3 (27.1, 38.6)	0.60	0.89	0.001	0.12
AST, U/L	66.7 (54.4, 81.6)	66.2 (52.8, 83.0)	73.5 (65.0, 83.1)	0.91	0.65	0.33	0.39
GGT, U/L	24.3 (19.8, 29.9)	27.2 (21.6, 34.3)	27.5 (24.2, 31.1)	0.55	0.99	0.32	0.20
Bilirubin, μ*M*	0.99 (0.65, 1.49)	1.45 (0.91, 2.33)	1.11 (0.86. 1.44)	0.50	0.52	0.001	0.007
Creatinine, μ*M*	104.2 (97.2, 111.7)	101.6 (93.9, 109.8)	99.3 (95.2, 103.6)	0.60	0.84	0.67	0.08
Retinol, μg/100mL	41.1 (33.4, 50.5)	30.0 (23.5, 38.2)	36.6 (32.2, 41.6)	0.16	0.27	0.05	0.38
Tocopherol, μg/100mL	1.01 (0.42, 2.43)	0.61 (0.23, 1.62)	0.62 (0.37, 1.05)	0.55	0.99	0.15	0.11
β-carotene, μg/100mL	0.033 (0.024, 0.043)	0.031 (0.021, 0.042)	0.029 (0.023, 0.034)	0.78	0.88	0.35	0.67
LFI[1]	1.31 (−1.07, 3.69)	−0.15 (−2.81, 2.50)	−2.73 (−4.24, −1.22)	0.06	0.09	—	—

[1]Liver functionality index.

of requirements for CON, FR, and PID groups (Dann et al., 2005). Measurements of cows during recovery at d 21 and 28 are reported in Table 5. Albumin tended to be lower ($P = 0.06$) for PID cows than for FR cows. The albumin:globulin ratio was lower ($P = 0.05$) for PID cows than FR cows. An interaction of health status and time for bilirubin (Figure 1A) indicated that it increased slightly from d 21 to d 28 for CON cows, but decreased for both FR and PID cows. The tendency for an interaction of health status and time ($P = 0.08$) for creatinine showed that creatinine increased for CON cows from d 21 to d 28, remained essentially constant for FR cows, but decreased for PID cows (Figure 1B). The LFI tended to be greater for CON than for FR and PID ($P = 0.06$) and tended to be greater for FR than for PID ($P = 0.09$).

DISCUSSION

Our experiment was successful in creating 3 distinct physiological phenotypes of cows during the periparturient period: healthy cows, those during FR, and those with PID. The latter 2 groups displayed the primary characteristics for "difficult" transitions of decreased DMI and milk production in early lactation compared with the healthy controls, feed restriction decreasing milk yield by about 22%, and the inflammatory conditions decreasing milk yield by 30% during d 5 to 14 of lactation (Dann et al., 2005). During the same time, DMI was decreased (by design) by 49% in FR and by 25% in PID compared with CON. During feed restriction on d 7 postpartum, cows in FR and PID had greater concentrations of NEFA, BHB, GGT, bilirubin, and β-carotene, and decreased concentrations of Ca, paraoxonase, creatinine, and retinol than CON cows. The LFI also was positive for CON, near zero for FR, and negative for PID.

Higher AST and GGT result partly from hepatocellular injury, possibly from increased lipopolysaccharide uptake or oxidative damage (Giannini et al., 2005; Woreta and Alqahtani, 2014). Bilirubin increases in both hepatocellular and cholestatic injury (Woreta and Alqahtani, 2014). Paraoxonase is a negative acute phase protein that decreases with liver injury (Ceron et al., 2014) and increased inflammation in liver (Bionaz et al., 2007). The decreased creatinine concentration in FR and PID relative to CON indicates that cows with peripartal disorders might have lost more lean body mass during early lactation than CON cows (De Rosa et al., 2023). Retinol decreases because synthesis of its main carrier protein, retinol-binding protein, decreases during an acute phase response (Bertoni et al., 2008; Rubin et al., 2017). Changes in these analytes demonstrate that feed-restricted cows and nonhealthy periparturient cows had a different immunometabolic profile than healthy cows, as expected.

Nevertheless, the 2 groups with disorders differed from one another in that the FR group had increased se-

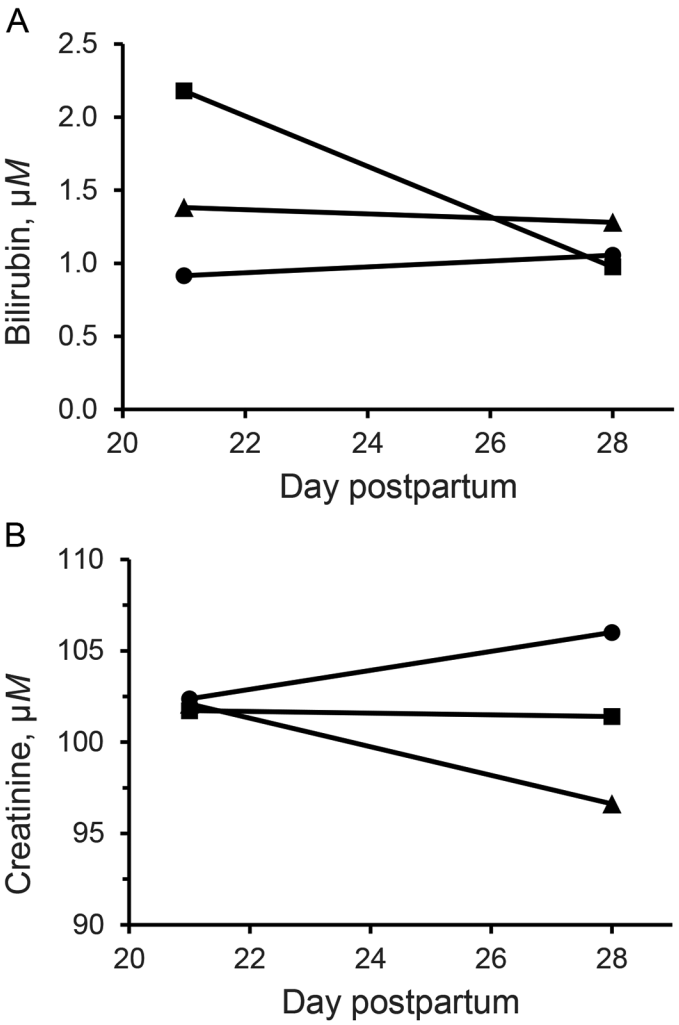

Figure 1. Concentrations on d 21 and 28 of (A) bilirubin, and (B) creatinine in serum of dairy cows that were healthy (CON; circles), undergoing feed restriction (FR; squares), or with inflammatory disorders (PID; triangles). Panel A: Effects in the model: health group, $P = 0.43$, time, $P = 0.001$, and health by time, $P = 0.007$. Panel B: Effects in the model: health group, $P = 0.48$; time, $P = 0.67$, and health by time, $P = 0.08$.

rum NEFA and BHB and decreased glucose, whereas the PID group did not. Thus, a situation where feed intake was drastically decreased (by 49% of d 4 values) in the absence of concurrent disease that affects the immune system (FR) caused a marked shift in metabolism to adapt to the nutrient deficit, whereas the PID group maintained these metabolites at values not greatly different from the CON despite 25% lower DMI. However, milk production decreased by about 30% in the PID group relative to CON, due to lower DMI but also likely from negative effects of elevated cytokines. Lower milk yield could explain the similar energy-related biomarkers in PID and CON. Part of the ability for PID to maintain NEFA and BHB at levels comparable to healthy controls might be attributable to the higher insulin in PID than in FR (3.02 vs. 2.07 μIU/mL), which was not different than the CON cows (3.68 μIU/mL; Dann et al., 2005). Immune activation increases insulin concentrations (Waldron et al., 2003), presumably to ensure glucose uptake by activated immune cells (Calder et al., 2007). The nonsignificantly lower insulin in PID than in CON likely was due to the reduction in DMI for PID.

Conversely, PID cows had a greater number of inflammatory biomarkers that were altered than the FR group, with elevations in haptoglobin and AST and decreases in cholesterol, albumin, the albumin:globulin ratio, creatinine, retinol, and β-carotene. The greater haptoglobin and lower concentrations of cholesterol, albumin, and retinol are clear indicators of inflammation in PID cows (Bertoni and Trevisi, 2013). Cholesterol decreases because of the decreased synthesis of its main apoprotein ApoB100 (Bertoni and Trevisi, 2013) despite the increase in lipomobilization; both of these conditions increase the risk of occurrence of liver lipidosis (Katoh, 2002). As a negative acute phase protein, albumin decreases in response to inflammation, which is believed to be due to competition for EAA being used for positive acute phase protein synthesis but also may be a result of increased vascular permeability that leads to efflux to the extravascular space (Hülshoff et al., 2013). As mentioned earlier, retinol decreases because of decreased synthesis of its main carrier protein. Although some of the biomarkers were also altered in the FR group relative to CON (decreased Ca, paraoxonase, creatinine, and retinol; increased GGT and bilirubin), there was no overall indication that the FR group had significant involvement of an inflammatory response. This conclusion is further supported by the LFI being intermediate to the CON and PID groups (1.31, −0.15, and −2.73 for the CON, FR, and PID groups).

Of note is the fact that feed restriction in rats (Hernandez-Baixauli et al., 2022) and steers (Gao et al., 2022) resulted in systemic immune activation, presumably caused by stress-induced hyperpermeability of the gastrointestinal tract tissues (Mayorga et al., 2020). Feed restriction in pigs increased gut permeability and endotoxin translocation across the gut (Pearce et al., 2013). Feed restriction (to 40% of ad libitum DMI) also resulted in systemic inflammation in mid-lactation cows (Horst et al., 2020). Similar to our data, moderate feed restriction prepartum (to 80% of calculated energy requirements) did not result in increases of inflammatory biomarkers (Efil et al., 2022). Reasons for the discrepancies among studies are not clear but could be related to the length of time feed was not available or the stage of lactation during which the feed restriction was applied, with the initiation of new inflammatory events being more difficult to detect during the transition period.

Haptoglobin is a positive acute phase protein and its concentration above 0.2 g/L is used as an indicator of inflammation (Bertoni and Trevisi, 2013). Thus, at d 7 postpartum, all groups experienced some degree of inflammation, including CON despite the absence of any clinical signs of disease. One of the limitations of our study is that our cohort selection was based only on the absence of clinical evidence of disease or disorders, and subclinical issues would not be detected. The elevation of haptoglobin in CON was uniform, with all cows having concentrations > 0.2 g/L. It is possible that this increase in haptoglobin resulted from some unidentified environmental- or management-induced stressor. The concentration of haptoglobin was significantly increased in PID cows compared with FR cows. This tendency directly corroborates the other markers for inflammatory response in PID cows. Concentrations of haptoglobin were in the range of other studies reporting haptoglobin in periparturient cows (Huzzey et al., 2011; Janovick et al., 2023; Seminara et al., 2025). The highest values during d 21 to 28 for the PID group compared with FR indicated that the degree and duration of inflammation were maintained longer in the PID cows. This is substantiated by the greater LFI for FR than for PID.

An interesting finding was that β-carotene increased in serum of FR cows but not in PID cows during feed restriction; values for PID were similar to those of CON. β-Carotene normally decreases during inflammation (Erlinger et al., 2001). A possible explanation could be an increase in release of stored β-carotene from adipose tissue during the intense lipomobilization in the FR cows.

Day 1 postpartum was before the start of the feed restriction, and all cows were retrospectively classified as healthy or not based on their d 4 health examination. At this time, sick cows showed slightly higher BHB and AST, and lower Zn, albumin, and retinol. The greater BHB likely resulted from the lower DMI for the sick cows. Serum Zn decreases during inflammation as it is redistributed into cells throughout the body, particularly into the liver (Lubna and Ahmad, 2023). This process is mediated by pro-inflammatory cytokines, which increase Zn transporter activity so that Zn can be used for protein synthesis, neutralization of free radicals, and to prevent microbial invasion (Gammoh and Rink, 2017). Together these changes indicate the presence of inflammation in the sick group, which were affected by inflammatory disorders.

Days 21 and 28 represented the recovery from feed restriction in FR cows, and nearly all biomarkers (with the exception of retinol) had returned to CON values. However, cows in PID had not fully returned to CON values during this time, as shown by data for albumin and the albumin:globulin ratio. In addition, the negative LFI indicates that PDI cows had persistent inflammation. These data might help to explain longer-term effects of peripartal disorders and diseases on production in cows.

A secondary objective was to determine if prepartum measurements were predictive of disease or disorders at parturition and early lactation. During the dry period, only a small decrease in the albumin:globulin ratio and β-carotene occurred in cows that were classified as sick after parturition. In previous studies, the ratio of albumin:globulin was found to be lower in the dry period for cows with a worse postpartum status (Cattaneo et al., 2021; Janovick et al., 2023). By d −1 prepartum the cows that were sick postpartum had elevated globulin, a decreased albumin:globulin ratio, decreased creatinine, and a greater haptoglobin concentration. Although these data do not indicate a large degree of inflammation, it is possible that there was a subclinical inflammation present before calving in cows that had inflammatory disorders postpartum. Our data do not confirm the observations by Dervishi et al. (2016), who found signs of altered innate immunity and inflammation during the dry period in cows that developed metritis postpartum.

Overall, otherwise healthy cows that developed hyperketonemia in response to feed restriction early postpartum (FR) showed a lowered involvement of an activated immune system compared with cows with naturally occurring inflammatory disorders (PID). Our results demonstrate that clinically and subclinically healthy cows at calving time can develop hyperketonemia without clear clinical signs of immune activation, but whether this could occur naturally is unknown. Most conditions that would decrease DMI would involve infection or some relevant stressor that likely would also stimulate an immune response, such as overcrowding or poor feed management. In agreement, Zhang et al. (2016) and Mezzetti et al. (2019) found that cows that developed hyperketonemia during the early postpartum period had alterations in innate immunity and inflammation during the dry period.

CONCLUSIONS

Cows during feed restriction (FR) and those with inflammatory disorders (PID) had immunometabolic profiles that differed from each other and from healthy cows. Overall, there was little evidence of clear inflammatory involvement during feed restriction in early lactating cows as measured by positive acute phase proteins (i.e., haptoglobin) in cows free of other clinical diseases and disorders. Nevertheless, in these cows the liver functionality was affected as demonstrated by the reduction of the main negative acute phase proteins such as paraoxonase and a lower LFI than CON. A few prepartum changes signifying inflammation were detected before parturition in cows that were not healthy postpartum.

NOTES

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Nonstandard abbreviations used: AST = aspartate aminotransferase; CON = control treatment; FR = cows subjected to 50% feed restriction postpartum; GGT = γ-glutamyltransferase; LFI = liver functionality index; NEFA = nonesterified fatty acids; PID = periparturient inflammatory disease group.

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ORCIDS

J. K. Drackley, https://orcid.org/0000-0002-4560-5594
H. M. Dann, https://orcid.org/0000-0002-7372-7011
G. Bertoni, https://orcid.org/0000-0003-1247-0344
E. Trevisi https://orcid.org/0000-0003-1644-1911