The objective of this prospective cohort study was to characterize the metabolic profile, health, milk production, and reproductive outcomes of cows diagnosed with hyperketonemia (HK; β-hydroxybutyrate ≥1.2 mmol/L), hypoglycemia (HG; glucose ≤2.2 mmol/L), or concurrent HK and HG (HKHG). Glucose and β-hydroxybutyrate concentrations in whole blood were assessed using a handheld device (Precision Xtra, Abbott Laboratories) in lactating dairy cows (n = 2,418) between 3 and 9 d postpartum. Cows were categorized into 4 groups: no HK or HG (healthy; Norm = 1,821), HK only (HK = 232), HG only (HG = 161), and concurrent HK and HG (HKHG = 204). Subsequent milk production, along with health and reproductive outcomes, as recorded by farm personnel, were analyzed according to metabolic category. Serum collected on the day of cow-side diagnosis of hyperketonemia and hypoglycemia was evaluated for total calcium (tCa), magnesium (Mg), nonesterified fatty acids (NEFA), triglycerides (TG), and urea using an automated chemistry analyzer (Randox Daytona; Randox Laboratories Ltd.). Statistical analysis was carried out using SAS version 9.4 (SAS Institute Inc.). Hyperketonemia in multiparous cows was associated with greater incidence of metabolic abnormalities (hypomagnesemia, hypocalcemia, and elevated NEFA and urea). Hyperketonemia in primiparous and multiparous cows led to increased adverse health events (culling rate, retained fetal membranes, puerperal metritis, clinical ketosis, left displaced abomasum) relative to Norm cows. Multiparous cows with HKHG had fewer metabolic disturbances (hypomagnesemia, hypocalcemia, elevated NEFA) than HK cows. Cows with HKHG had an increased incidence of clinical ketosis and left displaced abomasum relative to Norm cows. Cows with HG had similar metabolic profiles to Norm cows and had lower incidence of retained fetal membranes and puerperal metritis than cows with HK. Multiparous cows with HG produced more milk than Norm cows from wk 10 to 20, whereas multiparous cows with HK produced less milk than Norm cows. For primiparous cows, HK did not have a negative effect on milk production compared with Norm cows.

**Key words:** ketosis, metabolic disorders, hypocalcemia, metritis

**INTRODUCTION**

Metabolic disturbances such as hyperketonemia (HK) and hypoglycemia are common in postpartum dairy cows, leading to detrimental effects on health, milk yield, and fertility (Martinez et al., 2012; McArt et al., 2012). Often, HK is associated with decreased milk production (Dohoo and Martin, 1984; Duffield et al., 2009; Raboisson et al., 2014), but a recent study demonstrated an association between concurrent HK and hypoglycemia (HG) in multiparous cows with increased DHI first-test milk yield compared with cows with either HK alone and cows that had neither HK nor HG (Ruoff et al., 2017). Gordon et al. (2017) demonstrated that cows with concurrent HK and HG (HKHG) had a beneficial milk production response to prolonged propylene glycol therapy, whereas cows with HK only tended to have a negative milk production response to lengthening the duration of treatment. Previous studies have also demonstrated that lower plasma glucose during the early postpartum period was associated with decreased first-service conception rate (Green et al., 2012; Garverick et al., 2013). Similarly, Cardoso et al. (2013) demonstrated that decreased blood glucose during wk 3 postpartum was associated with increased days open. Therefore, glycemic status may be used to...
assess production and reproductive performance among HK cows. Several possible mechanisms have been suggested by which increased blood glucose could improve reproduction, including increased insulin and IGF-1 signaling, leading to earlier postpartum follicular development (Lucy et al., 2014). Another proposed mechanism is that increased blood glucose leads to increased glycogen stores in polymorphonuclear cells which, in turn, leads to improved oxidative burst and improved uterine health postpartum (Galvão et al., 2010). Somewhat surprisingly, Bicalho et al. (2017) identified a relationship between increased glucose concentration on d 3 postpartum and increased odds of being diagnosed with metritis or endometritis. Other metabolic disturbances such as hypocalcemia have been associated with impaired uterine health, reproductive performance, and milk production (Martinez et al., 2012; McArt and Neves, 2020); therefore, the potential associations of hypocalcemia, other metabolic disturbances, and HK warrant further investigation.

There is a critical need for further exploration of overall metabolic status, health, milk production, and reproductive performance of cows based on early postpartum HK and HG status. Our aim was to determine the concurrent serum total calcium (tCa), Mg, non-esterified fatty acids (NEFA), urea, and triglyceride (TG) concentrations of cows categorized by cow-side HK and HG status, and to compare the health, milk production, and reproductive performance of cows categorized by cow-side metabolic status using measurements of both BHB and glucose.

**MATERIALS AND METHODS**

**Ethics and Study Population**

All experimental procedures carried out in this study were approved by the Institutional Care and Use Committee of the University of Illinois at Urbana-Champaign (Protocol #19109). This study was conducted on 3 commercial dairy farms: 2 in northern Illinois (farms A and B) and 1 in southern Wisconsin (farm C), which were visited weekly from August 2020 to August 2021. These farms were chosen because they were willing to participate in treatment of a portion of cows detected with metabolic disturbances (Hubner et al., 2022). All 3 herds were enrolled in regular DHIA, and farms A and C also had daily milk weights recorded. Herd size ranged from 400 to 960 lactating cows, and daily milk production ranged from 38 to 45 kg/d (herd A: lactating cows = 887, daily milk = 44 kg/d; herd B: lactating cows = 400, daily milk = 38 kg/d; herd C: lactating cows = 960, daily milk = 45 kg/d). All cows were housed in freestall barns and fed a TMR delivered once daily. Two farms bedded cows with dried manure solids and one farm bedded cows with chopped straw over mattresses.

Data for this study were part of a larger study examining the effects of treatment of HK and HG. For events occurring before or at the time of enrollment into the study (retained fetal membranes and measurement of tCa, Mg, NEFA, TG), all cows were included in the analysis. For events that happened subsequent to enrollment, only those cows that were not treated were included in the analysis (health events except retained fetal membranes, milk production, and reproductive outcomes).

**Data Collection and Study Design**

This was an observational prospective cohort study. Herds were visited once a week for the duration of the study. Individual herds were visited on the same day and at the same time of day throughout the study. Sampling at herd A occurred 30 min after feed delivery, sampling at herd B occurred 3 h after feed delivery, and sampling at herd C occurred 8 h after feed delivery. Within each visit, a list of cows between 3 and 9 DIM was generated using the herd software program, DairyComp 305 (Valley Agricultural Software) or PCDart (DRMS). For sample collection, cows were restrained in headlocks and approximately 6 mL of blood was collected from the coccygeal vessels using an 18-gauge × 2.54-cm needle and 6-mL syringe. Immediately after blood collection, BHB was measured using a handheld Precision Xtra meter (Abbott Laboratories) with a blood ketone test strip. Immediately after BHB testing, blood glucose was tested using the same meter with a blood glucose test strip. The remainder of the blood was evacuated from the syringe into a 6-mL serum blood collection tube and placed on ice within the hour. Blood was centrifuged within 4 h at 2,000 × g and 22°C for 20 min and serum was collected and stored at −80°C for later evaluation. The body condition of all cows was assessed at enrollment by the first author using a scoring system from 1 (emaciated) to 5 (obese) according to Ferguson et al. (1994), as depicted in the Elanco BCS chart (Elanco Animal Health, 2009). For analysis, BCS was categorized into low, moderate, and high (low: ≤2.75; moderate: 3.00-3.50; high: ≥3.75).

Cows were classified into metabolic categories based on cow-side testing. Cows with whole-blood BHB ≥1.2 mmol/L were classified as HK, and those with whole-blood glucose ≤2.2 mmol/L were classified as HG (Gordon et al., 2017). Cows classified as both were considered to have concurrent metabolic abnormalities (HKHG),...
and those with neither were considered healthy (normal; Norm). Farm personnel were informed of the metabolic status of cows that were sampled.

For evaluation of serum metabolites, a subset of cows was created by matching a cow with a metabolic abnormality to a Norm cow. Primiparous cows were matched to primiparous cows from the same week of testing. An attempt was made to match multiparous cows to a cow of identical parity or a cow ±1 parity from the same farm, other than matching parity-2 cows with primiparous cows. If a match could not be found within the weekly cohort of multiparous cows, then one was used from the previous or following week; however, if a match could not be found in those cohorts, the cow remained unmatched.

Reproductive Management

Farms A and C were visited weekly by their herd veterinarian for pregnancy exams; farm B was visited every other week for pregnancy exams. Pregnancy outcomes were entered into herd software and then manually entered into Excel (Microsoft Corp.) to determine pregnancies per AI (P/AI) and days from parturition until pregnancy. Farm A used a Double Ovsynch protocol for all cows for first insemination. Farm B used a Double Ovsynch program for primiparous cows only and a program that included 2 injections of PGF2α, 14 d apart followed by an Ovsynch program 12 d after the second PGF2α, for multiparous cows. Farm C used a strategy for first insemination that included 2 injections of PGF2α, 14 d apart followed by an Ovsynch program 12 d after the second PGF2α, with cows inseminated if detected in estrus after the second PGF2α injection. For all farms, cows that were found open at pregnancy diagnosis were re-enrolled into an Ovsynch program. For all farms, any cow detected in estrus after the first insemination was re-inseminated at the time of estrus detection. The first pregnancy exam took place between 32 and 38 d post-insemination for farms A and C, and between 32 and 45 d post-insemination for farm B. A re-examination for pregnant cows took place between 60 and 66 d post-insemination for farms A and C, and between 60 and 73 d post-insemination for farm B.

Health Monitoring

Farm personnel performed daily health monitoring of cows in the first 21 d postpartum. Farm personnel were trained by their herd veterinarians to detect disease, and disease definitions were similar across all farms for the diseases reported herein. Cows were observed in headlocks after returning from the milking parlor for appetite, rumen fill, and general appearance. Cows that were deemed to have decreased feed intake based on these observations were further examined. Examination on all farms included auscultation and percussion for presence of a displaced abomasum, rectal temperature, whole-blood testing for BHB concentration, and a rectal examination. Health events were then recorded as written records and later transferred to herd software.

Adverse health outcomes reported here were determined subsequent to study sampling except for retained fetal membranes, which was recorded before sampling. Data from the herd software programs was manually extracted and entered into an Excel spreadsheet (Microsoft Corp.). The following health events were recorded (through 100 d postpartum unless otherwise noted): cows sold or died (i.e., left the herd), retained fetal placental membranes by 48 h postcalving (RFM), clinical metritis (defined as a cow with reduced appetite and fetid uterine discharge), clinical ketosis (defined as a cow with reduced appetite and whole-blood BHB ≥1.2 mmol/L), left displaced abomasum (LDA; defined as a cow with reduced appetite and a high-pitched “ping” heard during simultaneous auscultation and percussion within or cranial to the paralumbar fossa), clinical mastitis up to 60 d postpartum (CM60; defined as any visual abnormality of milk observed at pre-stripping during the regular milking routine), clinical mastitis up to 100 d postpartum (CM100; defined similarly as CM60), lameness (defined as a lameness score ≥4 on a scale from 1 to 5; Thomsen et al., 2008), any disease event (morbidity) up to 60 d postpartum (morb60) and up to 100 d postpartum (morb100). All events starting after the day of cow-side testing were recorded. Although all cows between 3 and 9 d were tested, cows that had been previously diagnosed with ketosis or LDA by farm personnel were excluded from the analysis.

Milk Production

Farms A and C had daily milk production recorded via milking parlor flow meters, and data were extracted from DairyComp 305 and transferred to an Excel (Microsoft Corp.) spreadsheet. Daily milk yield was averaged for each week through the first 20 wk of lactation. Farm B had monthly DHIA testing done, and the data were extracted from PCDart. Milk production data for farm B were then entered into the appropriate week of lactation to be analyzed with weekly milk from farms A and C. Milk production was analyzed using repeated measures across the first 20 wk in milk. Predicted milk production, based on mature-equivalent milk yield through 305 d of lactation (305ME), was extracted from herd software and analyzed across metabolic category.
Serum Chemistry

All analytes were measured using an automated clinical chemistry analyzer (RX Daytona, Randox Laboratories Ltd.) using reagents supplied by the manufacturer. Calibrations followed the manufacturer’s recommendations, and quality control checks were performed daily before sample analyses. Intra- and interassay coefficients of variation, respectively, were as follows: 2.0 and 3.2% for tCa (arsenazo III method), 4.2 and 3.9% for Mg (xylinid blue method), 2.5 and 4.9% for NEFA (methodology based on 3 enzymatic reactions: acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase), 1.8 and 2.5% for urea (UV method), and 3.3 and 3.5% for TG (colorimetric method).

Statistical Analysis

Sample Size. A sample size calculation was conducted for the companion paper (Hubner et al., 2022) to detect a difference in milk production between treated and untreated cows. Briefly, power analyses were performed using G Power 3 (Universität Düsseldorf). The sample size was calculated to detect a difference in milk production of 0.68 ± 0.09 kg/d (McArt et al., 2011) between treated and control cows within each metabolic category (Norm, HK, HG, HKHG). The sample size was calculated considering an error probability (α) of 5% and a power of 80% using a 2-tailed test. This resulted in a sample size of 164 cows (treatment = 82; control = 82). We conservatively estimated that 7% of cows would be classified as HKHG (Gordon et al., 2017). Herein, we have compared cows with different metabolic status using only cows in the HK, HG, and HKHG groups that remained untreated, unless otherwise noted.

Descriptive Statistics. For all analyses, metabolic category was treated as the independent variable and milk production, health events, and reproductive outcomes were treated as the dependent variable. Farm descriptive data were analyzed using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc.) and without adjustment for covariates to reflect actual farm outcomes.

Health and Serum Analytes. Continuous and categorical data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc.) using multivariable linear and logistic regression considering a Gaussian and binary distribution, respectively. Data were tested for normality of residuals. The ILINK function in SAS was used to return the data to the original scale for the depiction of least squares means and standard error of the mean. The models include the fixed effects of metabolic status (Norm, HK, HG, HKHG), farm (A, B, C), parity (primiparous and multiparous), season of parturition (warm: April—September; cool: October–March), breed (Holstein, Jersey, crossbred) BCS category (low: ≤2.75; moderate: 3.00–3.50; high: ≥3.75), as well as interactions between metabolic status and other covariates that did not cause issues with data convergence. The rationale for including covariates was a recognized association of covariates with metabolic abnormalities, reproductive outcomes, and milk production. The categorical variables analyzed include whether cows left the herd (sold or dead), RFM, metritis, clinical ketosis, LDA, CM60, CM100, lameness, morb60, and morb100. All analytes (tCa, Mg, NEFA, urea, TG) were examined as continuous data, and tCa and NEFA were also examined as binary data considering tCa ≤2.15 mmol/L (Martinez et al., 2012) as hypocalcemia and NEFA ≥0.7 mmol/L (Ospina et al., 2010a,b) as elevated blood NEFA concentration.

Milk Production. Other continuous variables analyzed included the effects of estimated 305ME and weekly milk production. Weekly milk through the first 20 wk of lactation was analyzed by ANOVA for repeated measures, with cow nested within study as a random variable and using a compound symmetry covariance structure. Adjustment for multiple comparisons was done using Tukey’s post hoc test.

Reproduction. Time to pregnancy was analyzed by survival analysis with Cox’s proportional hazard model using the PHREG procedure of SAS. For time from calving to pregnancy, cows that did not become pregnant were censored when they left the herd or at 300 DIM, whichever occurred first. Proportionality of the hazards was assessed using ASSESS, PH, and RESAMPLE in the PHREG procedure of SAS. The LIFETEST procedure of SAS was used to generate the survival curves, least squares means ± standard error of the mean, and median days to the event.

Binary outcomes for reproduction included P/AI at first service for pregnancy exams occurring both at 32 to 45 d post-AI and 60 to 73 d post-AI, as well as pregnancy loss between reproductive exams. These outcomes were assessed using logistic regression using similar models to those used for health events. For all analyses, differences with P ≤ 0.05 were considered significant and those with 0.05 < P ≤ 0.10 were considered tendencies.

RESULTS

Descriptive Statistics

A total of 2,621 cows were tested, and 203 cows were excluded due to a previous diagnosis of clinical ketosis or LDA. A description of milk production, percent of cows
tested that were non-Holstein, serum BHB and glucose at enrollment, disease incidence, and P/AI at first insemination according to farm enrolled in the study can be found in Table 1. A description of parity, season, BCS, and farm according to metabolic category can be found in Table 2. A total of 1,008 cows had additional serum metabolites evaluated (Norm-primiparous: n = 94; Norm-multiparous: n = 365; HK-primiparous: n = 57; HK-multiparous: n = 149; HG-primiparous: n = 18; HG-multiparous: n = 133; HKHG-primiparous: n = 22; HKHG-multiparous: n = 170). There was a difference in BHB at enrollment between metabolic category with BHB of Norm cows being lower than that of HK (P < 0.001) and HKHG (P < 0.001) cows; BHB of Norm cows tended to be lower than that of HG (P = 0.06). There were also significant differences between HK, HG, and HKHG cows (P < 0.001; Table 3).

### Table 1. Description of milk production, percent of non-Holstein cows tested, serum BHB and glucose concentrations at enrollment, BCS, disease incidence, and pregnancy per AI (P/AI) at first insemination

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm A (n = 926)</th>
<th>Farm B (n = 476)</th>
<th>Farm C (n = 1,016)</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>305ME, kg (n)</td>
<td>13,527.3 ± 121.8 (848)</td>
<td>11,999.5 ± 144.1 (435)</td>
<td>13,831.4 ± 118.6 (995)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Percent non-Holstein, % (n/n)</td>
<td>8.6 ± 0.8 (80/926)</td>
<td>2.3 ± 1.2 (11/476)</td>
<td>0.4 ± 1.0 (4/1,016)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>BHB at enrollment, mmol/L</td>
<td>0.78 ± 0.03</td>
<td>0.69 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Glucose at enrollment, mmol/L</td>
<td>3.1 ± 0.01</td>
<td>3.1 ± 0.01</td>
<td>3.2 ± 0.01</td>
<td>0.24</td>
</tr>
<tr>
<td>Left the herd (sold or died), % (n/n)</td>
<td>8.8 ± 1.3 (82/926)</td>
<td>9.2 ± 1.5 (44/476)</td>
<td>6.7 ± 1.3 (68/1,016)</td>
<td>0.51</td>
</tr>
<tr>
<td>Pregnancy per AI at first insemination</td>
<td>10.4 ± 1.6 (96/926)</td>
<td>15.1 ± 1.9 (72/476)</td>
<td>13.6 ± 1.6 (138/1,016)</td>
<td>0.02</td>
</tr>
<tr>
<td>Clinical ketosis, % (n/n)</td>
<td>4.6 ± 1.0 (43/926)</td>
<td>2.9 ± 1.2 (14/476)</td>
<td>5.0 ± 1.0 (51/1,016)</td>
<td>0.08</td>
</tr>
<tr>
<td>Left displaced abomasum, % (n/n)</td>
<td>1.7 ± 0.7 (16/926)</td>
<td>1.1 ± 0.8 (5/476)</td>
<td>2.6 ± 0.7 (26/1,016)</td>
<td>0.07</td>
</tr>
<tr>
<td>Mastitis up to 60 DIM, % (n/n)</td>
<td>10.5 ± 1.6 (97/926)</td>
<td>14.9 ± 2.3 (71/476)</td>
<td>14.1 ± 1.9 (143/1,016)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis up to 100 DIM, % (n/n)</td>
<td>17.8 ± 1.9 (165/926)</td>
<td>22.3 ± 1.9 (106/476)</td>
<td>24.0 ± 1.6 (244/1,016)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lame, % (n/n)</td>
<td>10.0 ± 1.6 (93/926)</td>
<td>10.7 ± 1.9 (51/476)</td>
<td>20.3 ± 1.6 (206/1,016)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Morbidity up to 60 DIM, % (n/n)</td>
<td>30.9 ± 2.3 (286/926)</td>
<td>40.8 ± 2.7 (194/476)</td>
<td>44.0 ± 2.3 (417/1,016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morbidity up to 100 DIM, % (n/n)</td>
<td>38.0 ± 2.3 (352/926)</td>
<td>46.6 ± 2.7 (194/476)</td>
<td>54.3 ± 2.3 (552/1,016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P/AI at first insemination, % (n/n)</td>
<td>40.5 ± 2.6 (331/817)</td>
<td>47.9 ± 3.0 (205/428)</td>
<td>44.5 ± 2.7 (405/909)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

1Results (± SEM) are for the total number of cows tested at each farm, including those without hyperketonemia or hypoglycemia. Disease incidence is through 100 DIM unless otherwise noted. Data are from all of the cows sampled from the respective farms unless otherwise noted.

2P-values calculated using regression models.

3305-d mature-equivalent milk yield.

### Table 2. Descriptive statistics (%; n/n in parentheses) for 2,418 cows enrolled in the study according to metabolic status category diagnosed between 3 and 9 DIM

<table>
<thead>
<tr>
<th>Variable and level</th>
<th>Metabolic status</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Primiparous, % (n = 1,041)</td>
<td>51.6 (940/1,821)</td>
<td>25.9 (60/232)</td>
</tr>
<tr>
<td>Multiparous, % (n = 1,377)</td>
<td>48.4 (881/1,821)</td>
<td>74.1 (172/232)</td>
</tr>
<tr>
<td>Season&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Cool, % (n = 1,103)</td>
<td>46.3 (843/1,821)</td>
<td>36.2 (84/232)</td>
</tr>
<tr>
<td>Warm, % (n = 1,315)</td>
<td>53.7 (978/1,821)</td>
<td>63.8 (148/232)</td>
</tr>
<tr>
<td>BCS&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Low, % (n = 118)</td>
<td>4.7 (86/1,821)</td>
<td>6.9 (16/232)</td>
</tr>
<tr>
<td>Moderate, % (n = 2,099)</td>
<td>87.8 (1,598/1,821)</td>
<td>83.6 (194/232)</td>
</tr>
<tr>
<td>High, % (n = 201)</td>
<td>7.5 (137/1,821)</td>
<td>9.5 (22/232)</td>
</tr>
<tr>
<td>Farm&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>A, % (n = 926)</td>
<td>37.2 (372/1,821)</td>
<td>44.8 (104/232)</td>
</tr>
<tr>
<td>B, % (n = 476)</td>
<td>18.8 (342/1,821)</td>
<td>20.3 (47/232)</td>
</tr>
<tr>
<td>C, % (n = 1,016)</td>
<td>44.0 (802/1,821)</td>
<td>34.9 (81/232)</td>
</tr>
</tbody>
</table>

1Cows were diagnosed with hyperketonemia (HK, BHB ≥1.2 mmol/L) and hypoglycemia (HG, glucose ≤2.2 mmol/L) and categorized into 4 groups: no HK or HG (Norm); HK only, HG only, or concurrent HK and HG (HKHG).

2P-values calculated using logistic regression.

3Season of parturition (warm: April–September; cool: October–March).

4BCS categorized as low (≤2.75), moderate (3.00 to 3.50), or high (≥3.75).

Serum tCa was first considered a continuous variable and examined by parity. Among primiparous cows, HK cows had lower serum tCa than Norm cows (P = 0.008)
but were similar to HG and HKHG cows ($P = 0.15$ and $P = 0.95$, respectively). Cows in HKHG tended to have lower $tCa$ than Norm cows ($P = 0.06$; Figure 1A). Among multiparous cows, HK cows had lower serum $tCa$ than cows in Norm, HG, and HKHG ($P < 0.001, P = 0.03, P = 0.03$; respectively; Figure 1A). Farm ($P < 0.001$), parity ($P = 0.02$), season ($P < 0.001$), BCS ($P < 0.001$), and the interaction between metabolic category and farm ($P = 0.005$) all had significant effects on $tCa$, whereas breed and other covariate interactions with metabolic category were not significant ($P > 0.39$).

Serum $tCa$ was categorized as those cows with serum $tCa \leq 2.15$ mmol/L being considered hypocalcemic and those with serum $tCa > 2.15$ mmol/L being considered normocalcemic. Among primiparous cows, HK cows tended to have a higher prevalence of hypocalcemia than Norm cows ($P = 0.06$); no other differences in prevalence of hypocalcemia existed between primiparous cows of different metabolic status (Figure 2A). Among multiparous cows, HK cows had an increased prevalence of hypocalcemia compared with Norm, HG, and HKHG cows ($P < 0.001, P < 0.001, P = 0.005$, respectively), whereas HKHG cows tended to have a greater prevalence of hypocalcemia than HG cows ($P = 0.09$; Figure 2A). Farm ($P < 0.001$) and season ($P < 0.001$) had significant effects on hypocalcemia, whereas BCS ($P = 0.06$) tended to have an effect. The interaction between metabolic category and parity ($P = 0.02$) had a significant effect on hypocalcemia, whereas parity, breed, and other covariate interactions with metabolic category did not ($P > 0.11$).

**Serum Concentration of Mg**

Serum Mg was considered a continuous variable and examined by parity. Among primiparous cows, HK cows had lower serum Mg than Norm cows ($P = 0.02$; Figure 1B). There were no other significant differences among metabolic status category for primiparous cows. Among multiparous cows, HK cows had lower serum Mg than Norm cows ($P = 0.001$) and tended to have lower Mg than HG and HKHG cows ($P = 0.06, P = 0.06$; respectively; Figure 1B). Farm ($P = 0.006$) and breed ($P = 0.008$) had significant effects on serum Mg, whereas parity, season, BCS, and interactions between covariates and metabolic category did not ($P > 0.10$).

**Serum Concentration of NEFA and Prevalence of Elevated NEFA**

Serum NEFA was first considered a continuous variable and examined by parity. Among primiparous cows, those in HK had greater serum NEFA concentrations than cows in Norm and HG ($P < 0.001, P = 0.001$; respectively), whereas HKHG cows had higher serum NEFA concentrations than Norm cows ($P = 0.02$) and tended to have higher serum NEFA concentrations than HG cows ($P = 0.09$). (Figure 1C). Among multiparous cows, HK cows had greater serum NEFA than Norm, HG, and HKHG cows ($P < 0.01$), whereas HKHG cows had greater serum NEFA than Norm and HG cows ($P < 0.01, P = 0.02$; respectively; Figure 1C). Farm ($P < 0.001$), parity ($P = 0.005$), season ($P < 0.001$), and BCS ($P = 0.06$) all had significant effects on serum NEFA. Among multiparous cows, HK cows had greater serum NEFA than Norm, HG, and HKHG cows ($P < 0.01$) and tended to have greater serum NEFA concentrations than HG cows ($P = 0.09$). (Figure 1C). Among multiparous cows, HK cows had greater serum NEFA than Norm, HG, and HKHG cows ($P < 0.01$), whereas HKHG cows had greater serum NEFA than Norm and HG cows ($P < 0.01, P = 0.02$; respectively; Figure 1C). Farm ($P < 0.001$), parity ($P = 0.005$), season ($P < 0.001$), and BCS ($P = 0.06$) all had significant effects on serum NEFA. Among multiparous cows, HK cows had greater serum NEFA than Norm, HG, and HKHG cows ($P < 0.01$), whereas HKHG cows had greater serum NEFA than Norm and HG cows ($P < 0.01, P = 0.02$; respectively; Figure 1C). Farm ($P < 0.001$), parity ($P = 0.005$), season ($P < 0.001$), and BCS ($P = 0.06$) all had significant effects on serum NEFA.

**Table 3.** β-Hydroxybutyrate concentration at enrollment and disease incidence according to metabolic category for diseases diagnosed by farm personnel

<table>
<thead>
<tr>
<th>Metabolic status</th>
<th>Norm (n = 1,821)</th>
<th>HK (n = 115)</th>
<th>HG (n = 85)</th>
<th>HKHG (n = 100)</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHB at enrollment, $^a$ mmol/L ± SEM</td>
<td>0.71 ± 0.02$^a$</td>
<td>1.61 ± 0.04$^b$</td>
<td>0.80 ± 0.04$^a$</td>
<td>2.04 ± 0.04$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left the herd (sold or died), %</td>
<td>6.2 ± 1.2$^a$</td>
<td>19.0 ± 6.8$^b$</td>
<td>10.1 ± 10.5$^b$</td>
<td>10.8 ± 11.1$^b$</td>
<td>0.12</td>
</tr>
<tr>
<td>Retained fetal membranes, $^3,4$ %</td>
<td>3.1 ± 0.7$^a$</td>
<td>9.0 ± 2.7$^b$</td>
<td>0.6 ± 0.5$^a$</td>
<td>3.9 ± 1.7$^b$</td>
<td>0.0004</td>
</tr>
<tr>
<td>Puerperal metritis, %</td>
<td>10.9 ± 1.5$^a$</td>
<td>29.3 ± 5.4$^b$</td>
<td>4.5 ± 2.5$^a$</td>
<td>17.7 ± 5.3$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical ketosis, %</td>
<td>2.3 ± 0.5$^a$</td>
<td>10.3 ± 3.4$^b$</td>
<td>11.2 ± 5.7$^b$</td>
<td>17.7 ± 9.1$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left displaced abomasum, %</td>
<td>0.7 ± 0.2$^a$</td>
<td>5.2 ± 2.3$^b$</td>
<td>6.7 ± 4.1$^b$</td>
<td>7.8 ± 7.5$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mastitis up to 60 DIM, %</td>
<td>12.9 ± 1.5$^a$</td>
<td>10.3 ± 3.0$^a$</td>
<td>18.0 ± 4.3$^b$</td>
<td>8.8 ± 2.9$^a$</td>
<td>0.23</td>
</tr>
<tr>
<td>Mastitis up to 100 DIM, %</td>
<td>21.4 ± 1.8$^a$</td>
<td>17.2 ± 3.4$^a$</td>
<td>27.0 ± 4.6$^b$</td>
<td>16.7 ± 3.4$^a$</td>
<td>0.14</td>
</tr>
<tr>
<td>Lame, %</td>
<td>13.2 ± 1.4$^a$</td>
<td>16.4 ± 3.7$^b$</td>
<td>15.7 ± 3.6$^a$</td>
<td>13.7 ± 4.2$^b$</td>
<td>0.65</td>
</tr>
<tr>
<td>Morbidity up to 60 DIM, %</td>
<td>35.3 ± 2.5$^a$</td>
<td>53.5 ± 9.9$^b$</td>
<td>41.6 ± 15.8$^b$</td>
<td>50.0 ± 14.2$^b$</td>
<td>0.27</td>
</tr>
<tr>
<td>Morbidity up to 100 DIM, %</td>
<td>43.9 ± 2.6$^a$</td>
<td>57.8 ± 9.6$^b$</td>
<td>52.8 ± 15.3$^b$</td>
<td>54.9 ± 14.4$^b$</td>
<td>0.62</td>
</tr>
</tbody>
</table>

$^a$Values within a row with different superscripts are significantly different ($P < 0.05$).

$^b$Cows were diagnosed with hyperketonemia (HK, BHB ≥1.2 mmol/L) or hypoglycemia (HG, glucose ≤2.2 mmol/L) and categorized into 4 groups: no HK or HG (Norm); HK only, HG only, or concurrent HK and HG (HKHG).

$^c$P-values calculated using logistic regression.

$^d$All cows, including those that received treatment: Norm = 1,821, HK = 232, HG = 161, HKHG = 204.

$^e$Disease event before metabolic sampling.
Figure 1. Serum metabolite concentrations (mmol/L) according to parity and metabolic status: (A) total Ca, (B) Mg, (C) nonesterified fatty acids (NEFA), (D) urea, and (E) triglycerides. Norm = not hyperketonemic or hyperglycemic; HK = hyperketonemic only; HG = hypoglycemic only; HKHG = hyperketonemic and hypoglycemic. Hyperketonemia was defined as BHB ≥1.2 mmol/L; hypoglycemia was defined as glucose ≤2.2 mmol/L (whole-blood concentration). Numbers at the bottom of bars indicate the serum analyte value, and numbers at the top of bars indicate the number of cows in each category. Different letters (a–c) above bars represent significant differences between metabolic categories (P ≤ 0.05). Error bars represent standard error of the mean.
and the interactions between metabolic category and farm \((P = 0.002)\), metabolic category and BCS \((P = 0.04)\), and metabolic category and season \((P = 0.05)\) all had significant effects on serum NEFA, whereas breed, BCS, and other covariate interactions with metabolic category did not \((P > 0.31)\). Cows with serum NEFA \(\geq 0.7\) mmol/L were considered to have elevated NEFA and those with serum NEFA \(< 0.7\) mmol/L were considered to have normal serum NEFA. Among primiparous cows, HK cows had a greater prevalence of elevated NEFA than cows in Norm \((P < 0.001)\) and HG \((P = 0.02)\). Among primiparous cows, HKHG cows tended to have a higher prevalence of elevated NEFA than Norm cows \((P = 0.06)\) but no other differences existed between primiparous cows of different metabolic statuses (Figure 2B). Among multiparous cows, HK cows had a greater prevalence of elevated NEFA than Norm cows \((P < 0.001)\) and HG cows \((P < 0.001)\). Among multiparous cows, HKHG cows had a greater prevalence of elevated NEFA than in HG and HK \((P < 0.003)\) or Norm \((P = 0.009)\) cows; no other differences existed between multiparous cows of different metabolic statuses (Figure 2B). Farm \((P < 0.001)\) and season \((P < 0.001)\) had significant effects on prevalence of elevated NEFA, whereas BCS \((P = 0.08)\) and the interaction between metabolic category and BCS \((P = 0.10)\) tended to have significant effects on prevalence of elevated NEFA. Parity, breed, and other covariate interactions with metabolic category did not have significant effects \((P > 0.10)\).

**Serum Concentration of Urea**

Serum urea was considered a continuous variable and examined by parity. Among primiparous cows, cows with HK tended to have greater serum urea than Norm cows \((P = 0.09)\); no other significant differences were found among metabolic status category for primiparous cows (Figure 1D). Among multiparous cows, HK cows had greater serum urea than Norm cows \((P = 0.02)\); Figure 1D). Farm \((P < 0.001)\), season \((P < 0.001)\), and BCS \((P = 0.007)\) all had effects on serum urea, whereas parity, breed, and other covariate interactions with metabolic category did not \((P > 0.11)\).

**Serum Concentration of TG**

Serum TG was considered a continuous variable and examined by parity. No differences were found among metabolic categories for TG in either primiparous or multiparous cows (Figure 1E). Farm tended \((P = 0.08)\) to have effects on serum TG, whereas parity, season, breed, BCS, and other covariate interactions with metabolic category did not \((P > 0.14)\).

**Health Outcomes**

A greater proportion of HK cows left the herd by 100 d postpartum compared with Norm cows, whereas HG and HKHG cows were similar to both Norm and HK cows (Norm, 6.2 ± 1.2%; HK, 19.0 ± 6.8%; HG, 10.1 ± 10.5%; HKHG 11.8 ± 11.1%; Table 3). The covariates farm, parity, season, and breed did not have an effect on cows leaving the farm \((P > 0.34)\), whereas BCS \((P = 0.07)\) and the interaction between metabolic category and farm \((P = 0.05)\) tended to have effects on cows leaving the farm. A greater proportion of HK cows were diagnosed with RFM compared with Norm \((P = 0.0002)\) and HG \((P = 0.005)\) cows, but the proportion was similar to that HKHG cows (Norm, 3.1 ± 0.7%; HK, 9.0 ± 2.7%; HG, 0.6 ± 0.5%; HKHG 3.9 ± 1.7%). Farm \((P < 0.001)\) and BCS \((P < 0.001)\) affected the incidence of RFM. A greater proportion of HK cows were diagnosed with clinical metritis than Norm cows \((P < 0.01)\), but this number was similar to that in HG and HKHG cows (Norm, 10.9 ± 1.5%; HK, 29.3 ± 5.4%; HG, 4.5 ± 2.5%; HKHG 17.7 ± 5.3%). Farm \((P = 0.009)\) and BCS \((P < 0.001)\) affected the incidence of clinical metritis. A greater proportion of HKHG cows were diagnosed with clinical ketosis compared with Norm \((P < 0.001)\) or HK \((P = 0.02)\) cows, but not HG \((P = 0.11)\) cows. Furthermore, a greater proportion of HK and HG cows were diagnosed with clinical ketosis compared with Norm cows \((P = 0.001\) and \(P = 0.002\), respectively), whereas HK and HG cows had similar incidence of ketosis (Norm, 2.3 ± 0.5%; HK, 10.3 ± 3.4%; HG, 11.2 ± 5.7%; HKHG 17.7 ± 9.1%). Farm \((P = 0.004)\) influenced the incidence of clinical ketosis, and the interaction between metabolic category and parity \((P = 0.05)\) tended to affect the incidence of clinical ketosis. A lesser proportion of Norm cows were diagnosed with LDA compared with HK \((P = 0.03)\), HG \((P < 0.001)\), and HKHG \((P < 0.001)\) cows, and no differences were found between HK, HG, and HKHG cows (Norm, 0.7 ± 0.2%; HK, 5.2 ± 2.3%; HG, 6.7 ± 4.1%; HKHG 7.8 ± 5.7%). Farm \((P = 0.009)\) affected the incidence of LDA. There were no differences among groups for CM60 or CM100. Farm \((P = 0.02)\) had significant effects on both CM60 and CM100. There were no differences among groups regarding diagnosis of lameness. Farm \((P < 0.001)\) and BCS \((P = 0.01)\) affected the incidence of lameness. There were no differences between groups for morb60 and morb100 postpartum \((P > 0.12)\). Farm \((P = 0.10)\) and parity \((P = 0.07)\) tended to have effects on morb60, and the interaction between metabolic category and farm \((P = 0.02)\) had significant effects on morb60. Farm \((P = 0.03)\) and parity \((P = 0.01)\) affected morb60 and morb100 (Table 3).
Reproductive Outcomes

Pregnancy per AI for first insemination was examined at the first pregnancy examination (32–45 d postinsemination) and second pregnancy examination (60–73 d postinsemination), and pregnancy loss was examined between the first and second pregnancy exams. No differences \((P > 0.78)\) for proportion pregnant at first pregnancy exam were found among metabolic categories for primiparous cows (Norm, 51.3 ± 3.0%; HK, 64.3 ± 9.8%; HG, 42.9 ± 16.6%; HKHG 50.0 ± 14.4%) or multiparous cows (Norm, 46.4 ± 2.8%; HK, 43.1 ± 6.9%; HG, 43.9 ± 7.2%; HKHG 38.2 ± 6.0%; Figure 3A). No differences \((P > 0.17)\) for proportion pregnant at second pregnancy exam were found among metabolic categories for primiparous cows (Norm, 49.2 ± 3.0%; HK, 60.7 ± 9.5%; HG, 14.3 ± 13.5%; HKHG 50.0 ± 14.4%) or multiparous cows (Norm, 42.4 ± 2.8%; HK, 38.1 ± 6.6%; HG, 42.2 ± 7.2%; HKHG 36.0 ± 6.0%; Figure 3B). Season affected the proportion pregnant at first \((P = 0.02)\) and second \((P = 0.02)\) pregnancy exam.

Pregnancy loss was increased among primiparous HG cows compared with primiparous Norm, HK, and HKHG cows (Norm, 3.6 ± 0.9%; HK, 5.6 ± 3.8%; HG, 66.7 ± 7.3%; HKHG, 0 ± 6.5%; \(P < 0.01\)). Pregnancy loss did not differ among multiparous cows (Norm, 7.6 ± 1.4%; HK, 7.7 ± 3.7%; HG, 33.3 ± 3.5%; HKHG, 3.6 ± 3.5%; Figure 3C). Metabolic status \((P = 0.002)\) and the interaction of metabolic status and parity \((P = 0.002)\) affected pregnancy loss, whereas parity \((P = 0.06)\) tended to affect pregnancy loss.

Milk Production Outcomes

Predicted milk production, measured by 305ME, among primiparous cows did not differ \((P > 0.13)\) between metabolic categories (Norm, 12,923.2 ± 141.8 kg; HK, 13,393.2 ± 529.1 kg; HG, 13,090 ± 834.4 kg; HKHG 11,888 ± 838.4 kg). Predicted milk production among multiparous cows was greater for HG than for HK \((P = 0.005)\) and HKHG \((P = 0.02)\) cows. Moreover, 305ME was greater \((P = 0.005)\) for Norm than for HK cows, and tended \((P = 0.05)\) to be greater for Norm than for HKHG cows (Norm, 13,192.7 ± 131.1 kg; HK, 11,910 ± 431.8 kg; HG, 13,985 ± 604.1 kg; HKHG 12,218.2 ± 483.8 kg; Figure 5). Farm \((P < 0.001)\), breed \((P < 0.001)\), and the interaction between metabolic category and parity \((P < 0.001)\) all affected time to pregnancy.

Across the first 20 wk of lactation, mean daily milk weight for primiparous cows was as follows: Norm: 37.3 kg, HK: 39.3 kg, HG: 38.5 kg, and HKHG: 37.4 kg, whereas mean daily milk weight for multiparous cows was as follows: Norm: 49.6 kg, HK: 48.6 kg, HG: 52.4 kg, and HKHG: 50.8 kg. Among primiparous cows, weekly milk production tended to be greater \((P < 0.10)\) for HK than for Norm cows in wk 3, 9, 15, and 16 (Figure 6A). Among multiparous cows, milk production was greater...
(\(P < 0.05\)) for HG cows than for Norm cows from wk 10 to 20. Among multiparous cows, milk production was greater (\(P < 0.05\)) or tended to be greater (\(P < 0.10\)) for HKHG cows than for Norm cows for wk 9 to 14 and wk 18 to 19. Multiparous HK cows produced (\(P < 0.05\)) or tended to produce (\(P < 0.10\)) less milk than multiparous Norm cows through the first 4 wk of lactation (Figure 6B).

**DISCUSSION**

Our objective was to classify cows by hyperketonemia and hypoglycemia and evaluate differences among these cows, in terms of other metabolites, health events, milk production, and reproductive outcomes. Cows with HKHG did not have greater metabolic disturbances than HK cows. In some instances, HKHG multiparous cows had decreased metabolic disturbances such as higher serum tCa, higher serum Mg, and lower serum NEFA. Recently, McArt and Neves (2020) demonstrated an association between tCa concentration and BHB and NEFA. In their study, they analyzed BHB and NEFA concentrations based on timing of hypocalcemia status. Multiparous cows that had delayed hypocalcemia (i.e., not hypocalcemic at 1 DIM but hypocalcemic at 4 DIM) had greater BHB at 5 DIM than cows that were normocalcemic throughout the first 4 d postpartum. Although not statistically significant at other time points in that study, normocalcemic cows had numerically lower BHB than cows with tCa aberrations, which is consistent...
with our results and suggests an inverse relationship between tCa and BHB. Induced hypocalcemia has been shown to decrease DMI (Martinez et al., 2014), likely due to decreases in smooth muscle contraction of the gastrointestinal tract (Hansen et al., 2003; Martinez et al., 2014). Conversely, in a study by Zarrin et al. (2013), intravenous infusion of Na-dl-β-OH-butyrate increased blood BHB concentration but did not decrease feed intake in mid-lactation cows; however, these cows might not have the same metabolic challenges as an early postpartum dairy cow. The results of the studies cited above may suggest that hypocalcemia is more likely to cause hyperketonemia or that both conditions are caused by a common preceding factor; more research is needed to explore this relationship.

Multiparous HK cows had lower serum Mg than cows in the other metabolic groups. Although the relationship between Mg and metabolic group was not repeated among primiparous cows, primiparous HK cows had lower Mg than primiparous Norm cows. Hypomagnesemia has been associated with hypocalcemia, likely because of the effects of hypomagnesemia on parathyroid hormone (PTH) secretion and sensitivity (Littledike et al., 1983; Rude, 1998). However, the association between hyperketonemia and hypomagnesemia has not been critically evaluated. Gröhn et al. (1989) showed a statistically significant association between ketosis and hypomagnesemia; however, it is unclear whether this effect is solely mediated via calcium. Aberrations in energy metabolism have been reported in clinical hypomagnesemia (grass tetany), with alterations in serum Mg being directly linked to carbohydrate metabolism (Lentz et al., 1976). Several authors have noted the lack of published information regarding subclinical hypomagnesemia in confined dairy cows (Leno et al., 2017; Oetzel, 2017); nonetheless, it is plausible that decreases in serum tCa and Mg and increases in whole-blood BHB are all caused by the decline in feed intake that begins prepartum in lactating dairy cows (Seifi et al., 2011).

Despite the increased aberrations of tCa and Mg among multiparous HK cows, HK and HKHG cows both had increased incidences of clinical ketosis and LDA. Cows with HG also showed an increased incidence of clinical ketosis relative to Norm cows, despite having similar tCa concentrations. This result is consistent with previous work showing BHB to be a better predictor of LDA than serum tCa (LeBlanc et al., 2005). However, the same study showed that postpartum NEFA was also a useful predictor of subsequent LDA. Although HK cows had higher NEFA than HG and HKHG cows, the incidence of LDA was similar among the metabolic groups.

**Figure 4.** Days to pregnancy according to metabolic category. Norm (yellow line) = not hyperketonemic (HK) or hyperglycemic (HG); HK (red line) = hyperketonemic only; HG (green line) = hypoglycemic only; HKHG (blue line) = hyperketonemic and hypoglycemic. Hyperketonemia was defined as BHB $\geq 1.2$ mmol/L; hypoglycemia was defined as glucose $\leq 2.2$ mmol/L (whole-blood concentration). Proportion remaining not pregnant is shown on the y-axis.
groups, in contrast to previous findings (LeBlanc et al., 2005). The results of the current study suggest that BHB may have a stronger relationship with LDA than NEFA and would likely be a more promising target for prediction of LDA.

Postpartum blood NEFA concentration has been used as a predictor of culling and reproductive outcomes. The relative usefulness of NEFA compared with BHB concentration postpartum as a predictor of subsequent culling and reproductive outcomes has been inconsistent across studies (Ospina et al., 2010b; Seifi et al., 2011; Chapinal et al., 2012; Bicalho et al., 2017). In general, blood NEFA concentrations reflect body fat mobilization better than BHB (Herdt, 1988, 2000; Kessel et al., 2008). Surprisingly, multiparous HKHG cows, despite having higher BHB than HK cows, had lower NEFA values. The fact that HKHG cows had lower blood glucose than HK cows may indicate that HKHG cows better utilized glucose in lieu of mobilizing body fat. It should be noted that several of the analytes in this study are likely to fluctuate with time relative to feeding. Glucose, NEFA, and BHB may have varied by farm because our time of sampling, although consistent on each individual farm, was not the same between farms relative to when feed was delivered. This may introduce error so further research confirming our results would be beneficial.

Serum urea was elevated in multiparous HK cows compared with Norm cows, which may reflect the muscle catabolism that occurs during negative energy balance (Haines et al., 2019). Serum urea of HG and HKHG cows was between that of HK and Norm cows and not different from either. Conversely, uterine disease (Paiano et al., 2019) has been associated with lower serum urea and, in our study, puerperal metritis had the highest incidence among HK cows. In Paiano et al. (2019), uterine discharge was scored at 25 DIM, which...
may reflect the long-term effects of uterine disease on serum urea and explain the seemingly contradictory results between that study and ours.

A greater proportion of cows with farm-reported clinical ketosis was found in HKHG cows than in HK cows. Cows in the HKHG group had higher BHB at enrollment than HK cows, so inclusion in the HKHG category based on hypoglycemia also meant that these cows had more severe hyperketonemia. Previous work has shown an inverse relationship between early postpartum whole-blood BHB and glucose (Tatone et al., 2016). Because BHB concentration differed between HKHG and HK cows, this difference in severity of hyperketonemia may explain the increased incidence of clinical ketosis. Conversely to what might be expected, multiparous HKHG cows had higher serum tCa and Mg than multiparous HK cows. In the case of HKHG cows, hypoglycemia may predict more severe hyperketonemia, despite having a positive association with Ca and Mg metabolism.

There was no difference in first-service P/AI between cows of different metabolic categories, nor did time to pregnancy differ. Previous work has shown that hyperketonemic cows took longer to become pregnant than cows without hyperketonemia (Walsh et al., 2007; Barletta et al., 2017). The fact that we did not find a difference may have been because our HK cows were divided into treated and not treated in the current study (Hubner et al., 2022) and only nontreated cows were used for this analysis, reducing the power to find a difference. If treated and nontreated cows were analyzed together, our results would be consistent with previous findings. Primiparous HG cows experienced greater pregnancy loss after conception to first insemination than did primiparous cows in the other metabolic categories. Glucose signaling to the hypothalamus may have played a role in this phenomenon as glucose-sensing hypothalamic neurons are known to signal to GnRH neurons and may subsequently affect the LH surge and corpus luteum formation. Because we did not measure progesterone in our study, we cannot confirm this hypothesis. Although not significantly different, primiparous HG cows also had increased serum urea concentrations relative to primiparous cows in the other metabolic categories. Increased urea nitrogen in blood and milk has been associated with reproductive failure in several studies (Ferguson et al., 1988; Butler et al., 1996; Melendez et al., 2000); however, it is important to note that the current study included only 10 untreated primiparous HG cows and may not accurately reflect reproductive outcomes.

Metabolic category appeared to affect primiparous cows differently than multiparous cows in terms of milk production. In primiparous cows, HK cows did not show decreased milk production relative to Norm cows, whereas multiparous HK cows did have decreased milk production. In multiparous cows, HG cows had increased milk production relative to Norm cows, whereas primiparous HG cows did not show a milk production increase. In addition, multiparous HK cows produced less milk than Norm cows at the beginning of lactation, but cows in these groups had similar milk production after wk 4 of lactation. In a German study by Ruoff et al. (2017), in which cows were categorized by metabolic category as in this study, hypoglycemic multiparous cows produced the most milk through 30 DIM. Although HG cows did not produce more milk early in the current study, their milk production increased relative to that of Norm cows after wk 10 of lactation. In Ruoff et al. (2017), hyperketonemic and concurrent hyperketonemic-hypoglycemic multiparous cows also produced more milk through 30 DIM than cows without metabolic aberrations, whereas in the current study, HK cows produced less milk in the first 28 DIM than Norm cows. Differences between this study and Ruoff et al. (2017) in sampling strategy and farm management may explain some of the differences in outcomes. Our results agree with previous work that demonstrated an association between HK and decreased milk production, at least for multiparous cows. Nonetheless, our data suggest that HK appears to be more harmful in terms of milk production for multiparous cows than primiparous cows. However, because our sample size was calculated based on treatment effects for the companion paper (Hubner et al., 2022) and not specifically for this observational study, caution is warranted when drawing conclusions, especially for results regarding reproductive outcomes and for differences between milk production of primiparous and multiparous cows.

In a previous study, decreased blood glucose appeared to be associated with increased uterine disease among multiparous cows (Galvão et al., 2010). It is somewhat surprising that HKHG cows did not have a greater incidence of puerperal metritis than HK cows and that HG cows did not have a greater incidence of clinical metritis than Norm cows. Perhaps this difference can be explained in part by the increased serum levels of tCa among HG and HKHG cows, as a previous study showed a strong relationship between early postpartum blood calcium levels and metritis (Martinez et al., 2012). An association between low PMN glycogen levels, impairment of PMN oxidative burst, and increased uterine disease has also been reported (Galvão et al., 2010). In contrast, another study described an association between high blood glucose and uterine disease (Bicalho et al., 2017). Cows in the HG group had the lowest numerical incidence of puerperal metri-
itis and had less metritis than HK cows; therefore, the current findings more closely align with that of Bicalho et al. (2017). It has been hypothesized that hypoglycemic cows do not have adequate circulating glucose to supply PMN cells with glycogen (Galvão et al., 2010). An alternative proposition is that hypoglycemia in the early postpartum cow is a sign of reduced insulin resistance and better glucose uptake by insulin-dependent tissue such as PMN cells.

Insulin resistance in the early postpartum cow is thought to be a mechanism by which glucose is spared for mammary tissue uptake (Bauman and Currie, 1980), so we might expect that cows that better utilize glucose for requirements other than milk production, such as the immune system, may have lower milk production. However, in this study, multiparous cows with low blood glucose did not have reduced milk production. It is possible that measures of whole-body insulin resistance lack the granularity to discern levels of insulin resistance for different body systems. Considering this idea, one possible explanation for improved uterine health without decreased milk production in HG cows is that these cows have a unique phenotype that shunts glucose to both the mammary gland and immune cells. These cows may have a similar degree of whole-body insulin resistance as other high-producing cows but may specifically target glucose to leukocytes by increasing glucose transporters to these cells and decreasing glucose transporters on non-immune cells. Further work is needed to test this hypothesis and may include a simultaneous glucose tolerance test and harvesting of PMN cells to quantify glucose transporters on these cells in cows with low blood glucose relative to normoglycemic cows. Cows that have a physiologic phenotype capable of sparing glucose for both immune function and milk production may represent the ideal cow for modern dairy production. The association of low blood glucose with a low incidence of metritis requires more work to elucidate the causative relationships.

The difficulties of transitioning the modern dairy cow from late gestation to early lactation have been attributed to metabolic and immune dysfunction, with different researchers arguing in favor of one or the other category of dysfunction. The idea that immune dysregulation is the primary culprit suggests that hypoglycemia in the early postpartum period is primarily due to immune dysfunction, and ample research shows that excessive immune stimulation does, in fact, lead to hypoglycemia (Lang et al., 1993; Kvidera et al., 2017). However, it is difficult to view HG cows in this study as developing hypoglycemia due to aberrant immune stimulation, considering the low level of metritis among HG cows. It is also difficult to simply consider hypoglycemia a normal state because these cows had an increased incidence of both clinical ketosis and LDA compared with Norm cows. This is not to say that immune dysregulation does not contribute to the difficulties of transitioning modern dairy cows into lactation. It is clear from work in lactating dairy cows and other species that there is abundant cross-talk between the immune system and metabolic regulation (Spurlock, 1997; Lackey and Olefsky, 2016; Newby et al., 2017). Despite considerable work, understanding the interaction of immune function and metabolic disease in the periparturient dairy cow has been challenging and requires more work to unravel the intricacies of this interaction.

**CONCLUSIONS**

Concurrent HKHG detected between 3 and 9 d postpartum was associated with an increased incidence of clinical ketosis compared with HK only; however, increases in other diseases were not detected among cows with HKHG. Hyperketonemia alone and concurrent HKHG were associated with increased incidence of clinical metritis. Concurrent HKHG was associated with fewer disturbances of Ca, Mg, and NEFA compared with cows with HK only. Multiparous cows with HG had increased milk production compared with multiparous Norm cows after 10 wk of lactation, and HG cows had similar metabolic profiles to Norm cows in terms of serum tCa, Mg, NEFA, urea, and triglycerides.

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