The objective of this study was to assess the effects of treatment with propylene glycol (PG) and cyanocobalamin (B12) on health, milk production, and reproductive outcomes of cows diagnosed with hyperketonemia (HK), hypoglycemia (HG), or concurrent HKHG. Glucose and β-hydroxybutyric acid (BHBA) concentrations were assessed in whole blood using a handheld device in lactating dairy cows (n = 2,418) between 3 and 9 d postpartum. Cows categorized as HK (n = 232, BHBA ≥1.2 mmol/L), HG (n = 161, glucose ≤2.2 mmol/L), and concurrent HKHG (n = 204, BHBA ≥1.2 mmol/L, and glucose ≤2.2 mmol/L) were randomized to receive treatment or to remain untreated (control). Treatment consisted of a single dose of B12 (10 mg, intramuscularly) and 300 mL of PG orally for 5 d, starting on the day of cow-side testing. Milk production, health, and reproductive outcomes were analyzed according to groups. Statistical analysis was carried out using SAS version 9.4 (SAS/STAT, SAS Institute Inc.). Treatment in HG cows decreased clinical ketosis, increased milk production in the fifth week of lactation for multiparous cows, and tended to increase 305-d mature-equivalent milk yield (305ME) for primiparous cows compared with untreated cows with the same metabolic profile. For cows with HKHG, treatment increased 305ME in multiparous cows and tended to increase 305ME in primiparous cows. No differences were found for treatment among any of the metabolic groups regarding reproductive outcomes, nor were any treatment effects found among HK cows. Glycemic status may help identify metabolically challenged early postpartum dairy cows, which may have differential response to PG and B12 treatment.

Key words: ketosis, metabolic disorders, therapy, vitamin B12

INTRODUCTION

Periparturient dairy cows experience a period of negative energy balance, and the degree and duration of negative energy balance vary across farms, parity, and postpartum disorders such as metritis, hypocalcemia, and hyperketonemia (HK; Taylor et al., 2004; Duffield et al., 2009). Despite major advancements in the past several decades in understanding dairy cow nutrition, management, and metabolic disease prevention and treatment, HK remains an important production and animal-welfare concern in the dairy industry (McArt et al., 2012a; Tatone et al., 2016a). Recently, the introduction of handheld devices expanded the ability of farm personnel to detect subclinical HK by measuring β-hydroxybutyric acid (BHBA) as a cow-side test (Tatone et al., 2015, 2016a, b; Bach et al., 2016; Dubuc and Denis-Robichaude, 2017; Mann et al., 2017). Several handheld devices have been validated and treatment protocols were developed for on-farm use (Iwersen et al., 2009; McArt et al., 2011, 2012b; Gordon et al., 2017). Treatment for ketosis may include administration of 50% dextrose solution, in combination with glucocorticoid, and daily oral administration of a glucose precursor such as propylene glycol (PG) for 3 to 5 d, and a single dose of cyanocobalamin (B12) and butaphosphan (Gordon et al., 2017; Capel et al., 2021). Numerous studies compared the average response to treatment of HK cows based on BHBA cut-points ranging from 1.0 to 1.4 mmol/L (Duffield et al., 2009; Óspina et al., 2010; McArt et al., 2012a). Treatment of HK has dem-
onstrated variable production responses across studies, and one study found that benefit of treatment was increased in cows whose glucose concentrations were ≤2.2 mmol/L (Gordon et al., 2017). The benefits of treatment have been debatable and contingent with the farm conditions. For instance, a study modeling the economic benefit of an HK monitoring and treatment program revealed a net benefit between $7 and $11 per cow using a herd with a 40% incidence of HK, and that farms using thrice-weekly monitoring protocols may incur an economic loss when HK incidence is below 25% (McArt et al., 2014). Labor costs have been estimated to be the second-highest cost for dairy farms (USDA ERS, 2022), and identifying cows that do not respond to treatment may be a means to reduce labor inputs and enhance adoption of HK management. Because of the relatively low return on investment associated with monitoring and treatment, it may be beneficial to identify a subpopulation of HK cows that demonstrate the greatest response to treatment.

One possible means to identify different subpopulations of HK cows would be to use additional metabolite measurements which could potentially differentiate between cows that do and do not respond to treatment. Another metabolite directly related to energy metabolism in dairy cows which can easily be measured as a cow-side test is glucose. The same whole blood handheld meter routinely used to measure BHBA can be used to measure glucose. Previous work has shown that early postpartum whole blood glucose concentrations are associated with future reproductive and milk production performance (Green et al., 2012; Cardoso et al., 2013; Garverick et al., 2013).

There is, therefore, a critical need to explore the effects of treatment of early postpartum dairy cows based not only on HK status, but on a combination of both HK and hypoglycemia (HG). The objective of this study was to assess the effects of treatment with PG and vitamin B12 on health, milk production, and reproductive outcomes of cows diagnosed with HK, HG, or concurrent HKHG.

We hypothesized that improvements due to treatment for health, milk production, and reproductive performance would be greater in cows with concurrent HKHG than HK or HG.

**MATERIALS AND METHODS**

**Ethics and Study Population**

All experimental procedures carried out in this study were approved by the Institutional Care and Use Committees of the University of Illinois at Urbana-Champaign (protocol no. 19109). This study was conducted on 3 commercial dairy farms, 2 in northern Illinois (A, B) and 1 in southern Wisconsin (C), visited weekly from August 2020 to August 2021. Two farms had daily milk weights (farms A and C) and all 3 farms had monthly DHIA testing performed. Farm B milked twice daily, while farms A and C milked thrice daily. 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 were bedded with dried manure solids, and one farm was bedded with chopped straw over mattresses.

**Data Collection and Study Design**

This was a randomized controlled trial conducted on 3 commercial dairy farms comparing cow-level outcomes. Individual herds were visited on the same day and at the same time of day throughout the study. 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, BHBA was measured using a Precision Xtra meter (Abbott Laboratories) with a blood ketone test strip. 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, 2009). For analysis, BCS was categorized into low, moderate, and high (low ≤2.75, moderate = 3.00 to 3.50; high ≥3.75).

Cows were classified into metabolic category based on cow-side testing. Those individuals that tested ≥1.2 mmol/L of whole blood BHBA were classified as hyperketonemic (HK only), whereas those that tested ≤2.2 mmol/L for whole blood glucose were classified as hypoglycemic (HG; Gordon et al., 2017). Cows that were both HK and HG were considered to have concurrent metabolic abnormalities (HKHG). Once the metabolic category was determined, cows were randomly assigned to treatment or control (no treatment) using the random number generator in Microsoft Excel (Microsoft Corp.). Treatment consisted...
of PG (300 mL administered orally for 5 d, starting on the day of cow-side testing) and cyanocobalamin (B12; 10 mg injected intramuscularly once on the day of cow-side testing). Treatment on d 1 was provided by study personnel, whereas the following 4 d of treatment were provided by farm personnel. Cows were sampled 1 wk after enrollment, in a similar fashion as previously described, to determine BHBA and glucose concentration.

**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 input in Excel 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 cows.

Adverse, naturally occurring health outcomes were determined by on-farm personnel. Farm personnel that determined adverse health events were the same as those administering treatment for those cows that qualified, and therefore there was no blinding for determination of adverse health events except for mastitis which was diagnosed by different farm personnel in the milking parlor. Health monitoring and recording was performed as described in Hubner et al. (2022). Briefly, each farm performed daily health monitoring of cows in the first 21 d postpartum. 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 is described in Hubner et al. (2022). Cows that were detected with apparent decreased feed intake, determined to have clinical ketosis, and had previously been enrolled in the study as a treatment or control cow were switched to the farm’s standard treatment for clinical ketosis. Clinical ketosis treatment on all farms included intravenous dextrose (50% dextrose; 500 mL administered once at the time of clinical ketosis detection), vitamin B-complex injection, and oral administration of PG for 3 to 7 d. Cows that were diagnosed with left displaced abomasum (LDA) that were enrolled as treatment or control cows were switched to the farm’s standard treatment postsurgery, which was similar to the treatment for clinical ketosis. Diagnosis and treatment of other diseases did not interfere with enrollment in this study.

**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 a Microsoft Excel spreadsheet. Daily milk yield was averaged for each week through the first 20 wk of lactation. Farm B had monthly DHI testing done and that data were extracted from PCDart. Milk production data for farm B was 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 through 305 d of lactation (305ME) was extracted from herd software and analyzed as well.

**Statistical Analysis**

Sample size calculation was 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. The sample size was calculated considering an α error probability of 5% and a power of 80% using a 2-tailed test. This resulted in a sample size of 164 cows (treat = 82; control = 82). We conservatively estimated that 7% of cows would be classified as HKHG (Gordon et al., 2017). Considering this, we estimated that 2,343 cows would need to be tested. We tested an additional 40 cows in the HKHG group to collect adequate cow numbers in the other metabolic categories. Although all cows between 3 and 9 d were tested, cows that had been previously diagnosed with ketosis or a displaced...
Continuous and categorical data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS/STAT; SAS Institute Inc.) using multivariable linear and logistic regression considering a Gaussian and binary distribution, respectively. Descriptive statistics were analyzed with univariate statistical methods using the UNIVARIATE procedure of SAS version 9.4. Data were tested for normality of residuals, and no variables required transformation. The ILINK function in SAS was used to return the data to the original scale for the depiction of least squares means (LSM) and standard error of the mean (SEM). The models include the fixed effects of metabolic status and treatment, 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 treatment and other covariates that did not cause issues with data convergence. The rationale to include covariates was a recognized association of covariates with metabolic abnormalities, reproductive outcomes, and milk production. The categorical variables analyzed include if cows left the herd (sold and dead), P/AI at first service with pregnancy exams occurring 32 to 45 d post-AI and 60 to 73 d post-AI, pregnancy loss between the 2 pregnancy exams, retained fetal membranes, metritis, clinical ketosis, LDA, clinical mastitis through 60 d of lactation, overall mastitis, lameness, any of the previous listed morbidities through 60 d of lactation, and overall morbidity. 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. Time to pregnancy was analyzed by survival analysis with Cox’s proportional hazard model using the PHREG procedure of SAS with parity and season included in the covariation model. For time from calving to pregnancy, those that did not become pregnant were censored when left, or at 300 DIM, whichever occurred first. The adjusted hazard ratio (HR) and respective 95% CI were calculated. 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, LSM ± SEM, median days to the event, and models retained farm, parity, BCS and season. Differences with \( P \leq 0.05 \) were considered statistically significant, and those with \( 0.05 < P \leq 0.10 \) were considered tendencies.

RESULTS

Descriptive Statistics

A total of 2,621 cows were tested with 597 enrolled in the study (HK-control: 115; HK-treat: 117; HG-control: 85; HG-treat: 76; HKHG-control: 100; HKHG-treat: 104). A total of 203 cows were excluded due to a diagnosis with clinical ketosis or displaced abomasum before enrollment (clinical ketosis: 187; LDA: 16). Days in milk at enrollment tended to be greater (\( P = 0.08 \)) for HKHG (DIM = 6.43) than for HK (DIM = 6.04) and HG (DIM = 6.07) cows. A description of milk production, percent of cows tested which were non-Holstein, serum BHBA 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.

Health Outcomes

A total of 23 HK cows received the farm’s standard treatment for clinical ketosis (HK-control: n = 12; HK-treatment: n = 11; P = 0.76) and 10 HK cows were diagnosed with LDA (HK-control: n = 6; HG-treat: n = 4; \( P = 0.51 \)). A total of 12 HG cows received the farm’s standard treatment for clinical ketosis (HG-control: n = 10; HG-treat: n = 2; \( P = 0.04 \)) and 8 HG cows were diagnosed with LDA (HG-control: n = 6; HG-treat: n = 2; \( P = 0.20 \)). A total of 31 HKHG cows received the farm’s standard treatment for clinical ketosis (HKHG-control: n = 18; HKGH-treat: n = 13; \( P = 0.28 \)) and 16 HKHG cows were diagnosed with LDA (HKHG-control: n = 8; HKHG-treat: n = 8; \( P = 0.76 \)). Mean days from parturition to diagnosis of both clinical ketosis and LDA was 15 d. Mean days to diagnosis of LDA tended to be different between HK-control and HKHG-treat cows (HK-control: 20 d; HKHG-treat: 10 d; \( P = 0.08 \)), but no other statistically significant differences existed between groups for time to clinical ketosis or LDA (\( P > 0.19 \)).

Blood concentration of BHBA and glucose 1 wk after enrollment was not affected by treatment within any of the metabolic categories (\( P > 0.62 \)). The covariates farm (\( P < 0.001 \)), parity (\( P < 0.001 \)), season (\( P < 0.001 \)), and BCS (\( P = 0.007 \)) all had statistically significant effects on BHBA concentration 1 wk after enrollment. The covariates parity (\( P < 0.001 \)), season (\( P < 0.001 \)), and BCS (\( P = 0.007 \)) all had statistically significant effects on blood glucose concentration 1 wk after enrollment, whereas farm (\( P = 0.24 \)) did not.

For HG cows, treatment significantly decreased (\( P = 0.04 \)) diagnosis of clinical ketosis (HG-control: 11.8...
Table 1. Description of milk production (305-d mature equivalent, 305ME), percent of cows that were non-Holstein, serum β-hydroxybutyric acid (BHBA) and glucose concentration at enrollment, BCS, disease incidence, and P/AI (pregnancy per artificial insemination) at first insemination according to each farm enrolled in the study.

<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^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>305ME (kg)</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>&lt;0.001</td>
</tr>
<tr>
<td>% Non-Holstein (%)</td>
<td>8.6 ± 0.8 (89/926)</td>
<td>2.3 ± 1.2 (11/476)</td>
<td>0.4 ± 1.0 (4/1,016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BHBA at enrollment (mmol/L)</td>
<td>0.78 ± 0.03</td>
<td>0.69 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose at enrollment (mmol/L)</td>
<td>3.2 ± 0.01</td>
<td>3.1 ± 0.01</td>
<td>3.2 ± 0.01</td>
<td>0.24</td>
</tr>
<tr>
<td>Left (%)</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>Puerperal metritis (%)</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 (%)</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 (%)</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>Mastitis60 (60 DIM, %)</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 (%)</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 (%)</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>&lt;0.001</td>
</tr>
<tr>
<td>Morbidity60 (60 DIM, %)</td>
<td>30.9 ± 2.3 (286/926)</td>
<td>40.8 ± 2.7 (194/476)</td>
<td>44.0 ± 2.3 (447/1,016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morbidity (%)</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 first insemination (%)</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 are for the total number of cows tested at each farm, including those without HK (hyperketonemia) or HG (hypoglycemia). Numbers and proportions in parentheses represent number of cows and number affected/number at risk, respectively. Numbers of cows and number at risk differ for 305ME and P/AI because a small number of cows did not contribute to these items: cows may have been designated “do not breed” and not received an insemination or may not have had sufficient milk production data to generate 305ME. Disease incidence is through 100 DIM unless otherwise noted. Data are from all of the cows sampled from the respective farms unless otherwise noted. All values ± SEM.

^2Mastitis60 = clinical mastitis through 60 d of lactation; morbidity60 = any of the previously listed morbidities through 60 d of lactation.

^3P-values calculated using regression models.

Reproductive Outcomes

No treatment effect was present within any of the metabolic categories for P/AI at first insemination among primiparous (HK-control: 0.61 vs. HK-treat: 0.33; HG-control: 0.14 vs. HG-treat: 0.44; HKHG-control: 0.50 vs. HKHG-treat: 0.46) or multiparous (HK-control: 0.38 vs. HK-treat: 0.33; HG-control: 0.42 vs. HG-treat: 0.42; HKHG-control: 0.36 vs. HKHG-treat: 0.37) cows (P > 0.12; Figure 1; panels A and B, respectively).

Parity and BCS did not have statistically significant effects on P/AI for first insemination for either first
or second pregnancy exam (P > 0.16). Season had a statistically significant effect on P/AI for first insemination at both first (P = 0.009) and second (P = 0.02) pregnancy exam.

For time to pregnancy treatment had no effect among HK cows (HK-control: 137 d; HK-treat: 155 d; HR: 1.07, CI: 0.88–1.39). For time to pregnancy treatment had no effect among HG cows (HG-control: 141 d; HG-treat: 137 d; HR: 0.91, CI: 0.63–1.32). For time to pregnancy treatment had no effect among HKHG cows (HKHG-control: 140 d; HKHG-treat: 134 d; HR: 0.95, CI: 0.68–1.33). We observed no effect of farm (P = 0.11) or BCS (P = 0.14) on time to pregnancy (Figure 2). We observed an effect of parity (P = 0.01) and a tendency for an effect of season (P = 0.09) on time to pregnancy.

Milk Production Outcomes

Treatment tended to increase 305ME among primiparous HG (P = 0.07) and HKHG (P = 0.09) cows (HG-control: 10,079.1 ± 969.8 kg vs. HG-treat: 12,266.4 ± 860.0 kg; HKHG-control: 10,055.0 ± 947.0 kg vs. HKHG-treat: 12,157.3 ± 862.0 kg), however, treatment had no effect among HK primiparous cows for 305ME (Figure 3, panel A). Treatment increased 305ME among multiparous HKHG (P = 0.02) cows (HKHG-control: 9873.6 ± 575.9 kg vs. HKHG-treat: 11,437.3 ± 575.8 kg); however, treatment had no effect among HK or HG multiparous cows for 305ME (Figure 3, panel B).

Trend in milk production among multiparous cows to hormone therapy and disease incidence according to control versus treatment within metabolic category

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (n = 115)</th>
<th>Treat (n = 117)</th>
<th>Control (n = 85)</th>
<th>Treat (n = 76)</th>
<th>Control (n = 100)</th>
<th>Treat (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHBA 7 d after enrollment (mmol/L)</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Glucose 7 d after enrollment (mmol/L)</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Left (%)</td>
<td>19.1 ± 5.0</td>
<td>18.2 ± 4.7</td>
<td>10.6 ± 4.5</td>
<td>9.2 ± 4.3</td>
<td>11.0 ± 3.9</td>
<td>11.5 ± 4.0</td>
</tr>
<tr>
<td>Puerpatal ketosis (%)</td>
<td>29.6 ± 6.2</td>
<td>25.6 ± 6.0</td>
<td>4.7 ± 3.0</td>
<td>6.6 ± 3.6</td>
<td>17.0 ± 5.9</td>
<td>18.3 ± 6.2</td>
</tr>
<tr>
<td>Clinical ketosis (%)</td>
<td>10.4 ± 2.7</td>
<td>9.4 ± 2.5</td>
<td>11.8 ± 2.9</td>
<td>2.6 ± 1.0*</td>
<td>18.0 ± 4.6</td>
<td>12.5 ± 3.4</td>
</tr>
<tr>
<td>LDA (%)</td>
<td>5.2 ± 2.2</td>
<td>3.4 ± 1.8</td>
<td>7.1 ± 3.3</td>
<td>2.6 ± 2.0</td>
<td>8.0 ± 4.0</td>
<td>7.7 ± 3.5</td>
</tr>
<tr>
<td>Mastitis60 (60 DIM, %)</td>
<td>10.4 ± 2.5</td>
<td>17.1 ± 3.8</td>
<td>17.7 ± 3.4</td>
<td>15.8 ± 3.2</td>
<td>9.0 ± 2.0</td>
<td>8.7 ± 2.0</td>
</tr>
<tr>
<td>Mastitis (100 DIM, %)</td>
<td>17.4 ± 3.7</td>
<td>25.6 ± 4.7</td>
<td>25.9 ± 4.9</td>
<td>27.6 ± 5.2</td>
<td>17.0 ± 3.6</td>
<td>17.3 ± 3.5</td>
</tr>
<tr>
<td>Lame (%)</td>
<td>16.5 ± 4.0</td>
<td>13.7 ± 3.5</td>
<td>16.5 ± 4.5</td>
<td>15.8 ± 4.3</td>
<td>13.0 ± 4.1</td>
<td>20.2 ± 5.0</td>
</tr>
<tr>
<td>Morbidity60 (60 DIM, %)</td>
<td>53.9 ± 5.9</td>
<td>51.3 ± 6.0</td>
<td>42.4 ± 6.8</td>
<td>35.5 ± 6.4</td>
<td>50.0 ± 6.7</td>
<td>50.0 ± 6.5</td>
</tr>
<tr>
<td>Morbidity (100 DIM, %)</td>
<td>58.3 ± 5.8</td>
<td>57.3 ± 5.9</td>
<td>52.9 ± 7.1</td>
<td>48.7 ± 7.1</td>
<td>55.0 ± 6.6</td>
<td>57.7 ± 6.3</td>
</tr>
</tbody>
</table>

*Tendency for an effect of season (P < 0.05) on time to pregnancy.

DISCUSSION

This study was carried out to assess treatment benefits of cows classified as HK, HG, and HKHG. This approach was performed to determine whether cows could be selectively treated under these 3 metabolic categories. Up to this point, limited work has been done to identify subpopulations of cows with greater potential response to treatment. Some farms may struggle to screen and treat ketonemic cows due to the high cost of labor associated with procedures. Potentially reducing the number of treated cows could be beneficial, as some...
farms may have greater than 50% of cows classified above the typical cut-point for treatment (Gordon et al., 2017).

Lactation represents a substantial draw of nutrients, and the mammary gland’s demand for glucose and production of lactose is the primary determinant of milk production (Bell, 1995). It is not surprising then that high-producing dairy cows may be hypoglycemic during the early postpartum period as milk production is increasing. Ruoff et al. (2017) showed that hyperketonemic multiparous cows decline in blood glucose through the third week of lactation before glucose concentrations stabilize, whereas nonhyperketonic cows had an mild drop in blood glucose during lactation which appears to stabilize during the second week of lactation. It would be expected that low blood glucose would favor lipolysis and that hypoglycemic cows would also have increased nonesterified fatty acids (NEFA) concentrations in blood (Herdt, 2000; Boden, 2011). However, that was not the case for the cows in our concurrent study where hypoglycemic cows had lower serum NEFA concentration (Hubner et al., 2022). It is also recognized that NEFA inhibit nonhepatic tissues from using glucose primarily by inhibiting insulin-dependent glucose transport, thereby increasing blood glucose (Cadórniga-Valiño et al., 1997; Boden, 2011). The fact that hypoglycemic cows in our concurrent study also had lower concentrations of circulating NEFA may indicate that low serum NEFA concentration in these cows leads to decreased insulin resistance and therefore greater glucose utilization and subsequent HG.

It is generally thought that insulin resistance in the early postpartum is a mechanism to spare glucose for the insulin independent mammary gland (De Koster and Opsomer, 2013). It could be hypothesized then that cows with HG would produce less milk due to the efficient uptake of glucose by nonmammary cells. Surprisingly we found that was not the case in our concurrent study (Hubner et al., 2022) nor in the previous study by Ruoff et al. (2017) where hypoglycemic cows produced the greatest quantity of milk. Even though the mammary gland does contain glucose transporters that operate independently of insulin, it is also true that mammary tissue has insulin-dependent glucose transporters (Zhao, 2014). Therefore, some level of insulin sensitivity could help the mammary gland capture circulating glucose. Although whole-body insulin resistance may be a mechanism for increased milk production, relative levels of insulin resistance between dairy cows may be insufficient to explain modest but important differences in milk production. Moreover, there has been limited work to identify differing levels of insulin sensitivity among different organs within the early postpartum cow and so it is also possible that the mammary gland may increase in glucose sensitivity even though other tissues elsewhere in the body become more insulin resistant (Zachut et al., 2013).

The idea that cows with both low NEFA and low glucose concentrations may more efficiently use glucose may help to explain why only hypoglycemic cows with or without HK responded to treatment with a glucose precursor. Both PG and B12 are expected to raise blood glucose concentration (Kennedy et al., 1990; Studer et
Figure 2. Time to pregnancy comparing treatment vs. control within metabolic categories. Hyperketonemia defined as 3-hydroxybutyric acid $\geq 1.2$ mmol/L, hypoglycemia defined as glucose $\leq 2.2$ mmol/L whole blood concentration. HK = hyperketonemic only; HG = hypoglycemic only; HKHG: hyperketonemic and hypoglycemic. (A) HKHG, (B) HK, (C) HG. Blue line = control, red line = treatment. (HKHG-control: n = 85; HKHG-treat: n = 89; HK-control: n = 91; HK-treat: n = 99; HG-control: n = 73; HG-treat: n = 66). Proportion remaining not pregnant shown on the y-axis.
al., 1993; Fürll et al., 2010), but perhaps only cows with decreased insulin resistance are able to use this glucose effectively at a variety of tissues including the mammary gland.

Increased milk production as a response to treatment for hyperketonemic cows has been variable across studies (McArt et al., 2011; Østergaard et al., 2020; Capel et al., 2021). Our study may help to explain some of this variation. Although mean glucose at enrollment was consistent across the 3 farms in this study, farm had an effect for the proportion of HKHG cows enrolled into our concurrent study (Hubner et al., 2022). Even though we observed a milk production response to treatment among HKHG cows in our study detected by 305ME, this response was not present among HK cows. Based on this information it is possible that in studies where glucose precursor treatment did not improve milk production that the farms enrolled in those studies had more cows with HK. In most previous studies regarding treatment of HK, glycemic status is unknown. Future studies are warranted to critically evaluated this hypothesis.

Treated HG cows had a reduction in on-farm clinical ketosis diagnosis compared with nontreated HG cows. Although HG cows had similar concentrations of NEFA to healthy cows, it is reasonable to surmise that NEFA concentrations increased in nontreated HG cows over time (Hubner et al., 2022). Considering that we did not have more frequent testing, we were unable to assess blood glucose and NEFA concentrations during treatment. However, a possible explanation in the difference between clinical ketosis incidence for treatment among HG cows is that treatment helped to reduce the duration of time for which HG cows experienced hypoglycemia and that longer-term hypoglycemia in untreated HG cows led to mobilization of adipose tissue eventually leading to clinical ketosis. Treated HG multiparous cows also had greater milk production at wk 5 of lactation than untreated HG cows, although it seems unlikely that this was a direct consequence of glucose precursor administration during the first and second week of lactation, a case can be made that PG administration affected feed intake during the postpartum period and helped with overall milk production long-term. Clinical ketosis has been associated with decreased feed intake and so the reduction in clinical ketosis among treated HG cows may have meant that feed intake was greater for treated HG cows around the time of treatment. Indeed, apparent appetite was the primary criterion used by the farm personnel in the study to identify cows to be examined more closely for disease including clinical ketosis. If, in fact, treated HG cows had greater feed intake during the early postpartum period then this would explain the increased milk production among multiparous HG treated cows early in lactation and the reduction in clinical ketosis. The mechanism by which PG would increase feed intake cannot be elucidated from this study, and it would seem somewhat counterintuitive that PG would have this effect. Hypoglycemia has been associated with increased appetite in several species (Cai et al., 2001; Schultes et al., 2003), and therefore raising

**Figure 3.** Predicted mature-equivalent milk through 305 d of lactation (305ME) according to metabolic category and treatment. Hyperketonemia defined as β-hydroxybutyric acid ≥1.2 mmol/L, hypoglycemia defined as glucose ≤2.2 mmol/L whole blood concentration. HK = hyperketonemic only; HG = hypoglycemic only; HKHG: hyperketonemic and hypoglycemic. Panel A = primiparous; panel B = multiparous. Numbers at the base of each bar are the percentage, and numbers at the top of each bar are the number of cows within the group. Error bars represent SEM.
Figure 4. Weekly milk production for primiparous cows according to metabolic category and treatment. Hyperketonemia defined as \( \beta \)-hydroxybutyric acid \( \geq 1.2 \) mmol/L, hypoglycemia defined as glucose \( \leq 2.2 \) mmol/L whole blood concentration. HK = hyperketonemic only; HG = hypoglycemic only; HKHG = hyperketonemic and hypoglycemic. Letters above each week represent pairwise differences between treatment and control. Panel A = HK; panel B = HG; panel C = HKHG. (HK-control: n = 31; HK-treat: n = 29; HG-control: n = 10; HG-treat: n = 9; HKHG-control: n = 10; HKHG-treat: n = 12). Error bars represent SEM.
Figure 5. Weekly milk production for multiparous cows according to metabolic category and treatment. Hyperketonemia defined as \( \beta \)-hydroxybutyric acid \( \geq 1.2 \) mmol/L, hypoglycemia defined as glucose \( \leq 2.2 \) mmol/L whole blood concentration. HK = hyperketonemic only; HG = hypoglycemic only; HKHG = hyperketonemic and hypoglycemic. Letters above each week represent pairwise differences between treatment and control. Panel A = HK; panel B = HG; panel C = HKHG. (HK-control: n = 84; HK-treat: n = 88; HG-control: n = 75; HG-treat: n = 67; HKHG-control: n = 90; HKHG-treat: n = 92). Error bars represent SEM.
blood glucose with PG may be expected to decrease feed intake. However, most investigations of glycemic status with appetite have analyzed relatively moderate levels of hypoglycemia (Chaput and Tremblay, 2009). Hypoglycemia in our study was whole blood glucose \( \leq 2.2 \text{ mmol/L} \), and it is conceivable that this should not be considered moderate hypoglycemia even for a ruminant animal in which normoglycemia is lower compared with monogastric animals. It is unclear from published literature the effects of moderation of severe hypoglycemia on appetite. Future studies may help to elucidate whether PG may increase appetite in postpartum cows based on metabolic status.

It should also be noted that several control cows received later treatment with PG and dextrose if they were diagnosed with clinical ketosis or had an LDA. It is difficult to determine the precise effects of these treatments or to compare them to the initial treatment for cows enrolled into the treatment group. Previous work has shown that treatment of subclinical hyperketonemia reduces subsequent occurrence of LDA and an association of hyperketonemia with LDA is recognized. Coupled with the fact that farms are likely to be using early diagnosis and treatment of subclinical hyperketonemia to prevent clinical ketosis and LDA, it was important to have these cows remain in the study. Nonetheless, these cows may increase the difficulty in interpreting our results.

Blood glucose concentration is detected by hypothalamic cells which signal GnRH neurons and low blood glucose is recognized to effect ovarian cyclicity (Roland and Moenter, 2011). Therefore, it is possible that treatment of hypoglycemic cows could affect reproductive outcomes especially for reproductive measurements early in lactation. We saw no statistically significant difference in first insemination P/AI for treatment in any of the metabolic groups. However, it is possible that we lacked enough power to demonstrate a statistically significant difference. Our results appear to indicate that differences may exist among HG primiparous cows for treatment with PG and B12. It did appear that HG more negatively affected primiparous cows compared with multiparous cows regarding first insemination P/AI (Hubner et al., 2022). However, it should be noted that the HG status among primiparous cows was very low with only 19 out of 1,041 primiparous cows tested qualifying for HG status. Because of the small numbers of primiparous cows qualifying for HG status and the lack of significance between treatment and control in this metabolic category, these results should be viewed with caution.

Our data regarding milk production varied between the 305ME and weekly milk production among HKHG cows. Weekly milk did not demonstrate an increase in milk production for treated primiparous or multiparous HKHG cows. However, among primiparous HKHG cows, treatment resulted in a tendency for an increase in 305ME and among multiparous HKHG cows, treatment resulted in a statistically significant increase in 305ME. Data for weekly milk production primarily came from daily milk weights produced by on-farm flow meters on the 2 largest farms, whereas 305ME was produced from monthly DHIA milk weights. Because DHIA testing is less frequent and because 305ME is both a predicted number for cows that do not reach 305 DIM and a projected number for young cows, this may be the less accurate measurement. Nonetheless, considering either milk production measurement (weekly milk or 305ME) no treatment effect could be found among HK cows, nor were any treatment effects present for HK cows among any of the other outcomes measured.

**CONCLUSIONS**

Hyperketonemic cows that did not exhibit concurrent HG demonstrated no positive effects of treatment with PG and B12. Treatment in cows that were hypoglycemic with or without concurrent hyperketonemia had improvements in health and milk production. We observed no benefits of treatment to reproductive performance in any of the metabolic groups. Glycemic status may help identify metabolically challenged, early postpartum dairy cows that may have differential responses to PG and B12 treatment.

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