A randomized controlled trial of dexamethasone as an adjunctive therapy to propylene glycol for treatment of hyperketonemia in postpartum dairy cattle

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ABSTRACT

Treatment of hyperketonemia with oral propylene glycol has proven efficacy but the cure rate remains moderate. Dexamethasone has long been suggested as a treatment for hyperketonemia, even though evidence of its efficacy is contradictory. The objective of this randomized controlled trial was to evaluate the effect of adding a single intramuscular injection of 20 mg of dexamethasone to oral propylene glycol therapy for hyperketonemia [blood β -hydroxybutyrate (BHB) ≥ 1.2 mmol/L]. All cows between 3 and 16 d in milk on 4 dairy farms in New York State were tested once weekly for hyperketonemia using a handheld ketone meter. All enrolled animals received 312 g (300 mL) of propylene glycol orally once daily for 4 d and either a single injection of dexamethasone or an equivalent volume of sterile saline. A total of 509 animals were enrolled, with 254 and 255 in the placebo and dexamethasone groups, respectively. Treatment with dexamethasone decreased the odds of being hyperketonemic in the second week posttreatment; however, the odds of hyperketonemia in the first week posttreatment only decreased in those animals that were treated at a BHB blood concentration between 1.2 and 1.5 mmol/L. For the 8% of cows with blood BHB >3.2 mmol/L at enrollment, receiving dexamethasone increased the odds of being hyperketonemic the following week. We detected no difference between treatment groups in the odds of postpartum disease or in milk production. For cows with initial BHB of 1.2 to 1.5 mmol/L, treatment with dexamethasone tended to reduce the odds of pregnancy at first insemination. Based on the small and conditional benefits of dexamethasone and a lack of difference in milk yield

or disease incidence, we do not recommend the use of dexamethasone to treat hyperketonemia.

Key words: hyperketonemia, dexamethasone, glucocorticoid, randomized controlled trial

INTRODUCTION

Hyperketonemia (**HK**), a blood BHB concentration of 1.2 mmol/L or greater, is associated with impaired health and performance and is a common and costly disease (McArt et al., 2015). Despite its prevalence in North America (McArt et al., 2012a; Gordon, 2013), several commonly used treatment protocols remain unproven (Gordon et al., 2013a). Treatment of HK with oral propylene glycol (**PG**) has been recognized to decrease the risk of displaced abomasum, clinical ketosis, and removal from the herd before 30 DIM, as well as increase the risk of conception at first insemination and improve milk yield (McArt et al., 2011, 2012b). In a recent study, the rate of cure from HK was improved with PG treatment; however, even with 5 d of therapy, approximately 40% of cows remained hyperketonemic (McArt et al., 2012a).

Glucocorticoids have been used for the treatment of HK since the 1950s (Hatzitolos and Shaw, 1950); however, the efficacy of glucocorticoid therapy remains controversial. Studies evaluating glucocorticoids in healthy animals (Neff et al., 1960; Hartmann and Kronfeld, 1973) and those that did not include a control group (Jonsgård et al., 1974; Kauppinen, 1984; Shpigel et al., 1996) make the results difficult to interpret. Studies that included control groups and were tested in hyperketonemic, or high-risk populations, have contradictory results. Robertson (1966) found that treatment of clinical ketosis with intramuscular (**i.m.**) dexamethasone or flumethasone decreased clinical signs 40% faster and improved milk yields compared with treatment with a placebo. However, the sample size was small and the difference between groups was not tested statistically

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(Robertson, 1966). In one study, postpartum cows with unknown HK status at treatment were treated with isoflupredone within 8 d of calving. Treated animals had increased blood concentrations of BHB, decreased concentrations of glucose, and a higher prevalence of HK 1 wk posttreatment compared with placebo-treated controls (Seifi et al., 2007). Another study found that cows in the early postpartum period with elevated urine ketones treated with oral PG, intravenous dextrose, vitamin B₁₂, and dexamethasone were 40% more likely to be removed from the herd compared with cows that received no treatment (Carrier et al., 2011). Two recent studies have reported more positive results. van der Drift et al. (2015) compared the short-term effect of treating clinically ketotic cows with PG alone or with a single injection of long-acting dexamethasone (dexamethasone-21-isonicotinate). The group mean serum BHB was lower in the group that received dexamethasone (van der Drift et al., 2015). Sami et al. (2015) treated postpartum animals, regardless of energy status, with dexamethasone with or without insulin. The group mean BHB was lower 2 d after treatment for all groups compared with those that did not receive treatment; however, there was no difference in serum BHB at later time points up to 14 d posttreatment (Sami et al., 2015).

Glucocorticoids produce hyperglycemia by shunting glucose away from cellular usage and into circulation (Herdt and Emery, 1992). However, glucocorticoids do not reduce and may exacerbate lipolysis, which could be detrimental in some animals (Herdt and Emery, 1992). The impact of glucocorticoids may depend on the extent of fatty acid mobilization of the individual.

The objective of this randomized controlled trial was to evaluate the effect of a single dose of 20 mg of dexamethasone, in conjunction with oral PG therapy, on the reduction of HK persistence, the incidence of subsequent disease, milk production and pregnancy at first insemination.

MATERIALS AND METHODS

Enrollment

Four herds in New York State milking between 900 and 3,100 predominantly Holstein cows were visited weekly from May until August 2014. Herds were a convenience sample based on willingness to participate and proximity to the participating veterinary clinic. To participate, herds were required to have Dairy Comp 305 (DC305; Valley Agricultural Software, Tulare CA), be enrolled in a DHIA service, and to routinely monitor for, and record, disease events in fresh cows.

All cows between 3 and 16 DIM were tested once weekly for HK with the Precision Xtra handheld ketone meter (Abbott Diabetes Care Canada, Mississauga, ON, Canada). Each herd was visited at the same time and day each week. Cows with a blood BHB concentration of ≥ 1.2 mmol/L were determined to be hyperketonemic and enrolled in the study. All cows not enrolled at the first sample between 3 and 9 DIM were tested again the following week and enrolled if blood BHB was ≥ 1.2 mmol/L. Cows with recorded disease events before the day of testing, and those with visible signs of disease were excluded.

Randomization, Random Allocation, and Sample Size

Enrolled cows were randomly allocated to either the treatment or the placebo group. Randomization was blocked by farm and balanced in sets of 6, using a random number generator in Excel (Excel for Mac, version 15.5.4, Microsoft Corp., Redmond, WA). Allocation was concealed until the time of treatment. Those responsible for treatment allocation and outcome assessment, research assistants, and farm employees, were blinded to treatment group.

The sample size was calculated to detect an increase in cure rate from 50 to 65% with dexamethasone treatment, assuming 80% power and a confidence of 95%. The sample size was increased to allow for a 10% loss to follow-up. At the resulting sample size of 250 cows per treatment group with a standard deviation of 1.8 kg per test, a 1-kg difference in test-day milk production could be detected.

Treatments

The treatment group received a single 20-mg i.m. injection of dexamethasone solution (Dexaject 2 mg/mL, Henry Schein Animal Health, Dublin, OH). The control group was given a placebo of an equivalent volume (10 mL) i.m. injection of sterile 0.9% NaCl solution (Henry Schein Animal Health). A research assistant blinded to treatments gave the i.m. injections at the time of enrollment with syringes labeled "A" and "B". Farm employees administered 312 g (300 mL) of oral PG once daily for 4 d to all enrolled cows starting on the day of enrollment. All treatments were recorded.

Data Collection and Follow-Up

Enrolled cows were followed for 2 wk after treatment. At each weekly visit, blood BHB and glucose concentrations were measured using the handheld meter and

recorded. The sensitivity of the meter for the determination of HK at a threshold of 1.2 mmol/L BHB has been reported to be between 85 and 95%, with a specificity that ranged from 94 to 97% (Iwersen et al., 2009; Voyvoda et al., 2010; Panousis et al., 2011; Gordon et al., 2013b). The repeatability of the meter was good, with a coefficient of variation of 10% when using a slightly higher threshold of 1.4 mmol/L, resulting in little change to the classification of ketotic animals (Oetzel, 2010). The meter has a correlation of 95% compared with a gold standard for the measurement of blood glucose in cattle (Wittrock et al., 2013). Body condition at enrollment was measured on a scale of 1 to 5, with increments of 0.25 (Elanco Animal Health, 1996). A single research assistant measured BCS at each herd. Test-day milk yield and milk components were collected for the first 90 DIM. Milk production data, number of inseminations, date of pregnancy, and disease events up to 90 d after enrollment were retrieved from DC305.

An animal use protocol for the study was submitted and approved by the University of Guelph Animal Care Committee (AUP# 2918).

Statistical Analysis

Data input and management were completed in Excel (Microsoft Corp.) and all statistical analyses were completed in SAS software (SAS 9.3, SAS Institute Inc., Cary, NC). For the outcomes of HK at 1 or 2 wk posttreatment, disease events (metritis, displaced abomasum, clinical ketosis) within the first month of lactation (but subsequent to enrollment), pregnancy at first insemination, and pregnancy by 120 DIM, multi-level logistic regression models were constructed using the PROC GLIMMIX function, controlling for herd as a random effect. A Cox proportional frailty hazard model was constructed for the outcome of time to pregnancy up to 120 DIM, with herd controlled for as a frailty. The effect of treatment on milk production was explored using the outcomes of projected mature-equivalent 305-d (**305ME**) yield at third DHIA test, as well as milk yield at the first 3 DHIA test days; 305ME was modeled using PROC MIXED of SAS with herd as a random effect. A repeated-measures model (PROC MIXED) was constructed for milk yield at the first 3 DHIA test days using a heterogeneous Toeplitz correlation structure, chosen based on data structure and the lowest Akaike information criterion. The outcome was modeled by cow, and nested by herd and treatment group. Herd 1 was missing milk data for one-third of enrolled cows. The cows without milk data were missing transponders but no systematic reason existed for not having a transponder tag. Missing data were not

related to lactation, disease, or HK status. Because information was presumed to be missing at random, production data from herd 1 were included in the analysis.

The variables offered to all models included parity group, BCS, BHB and blood glucose concentrations at the time of initial treatment, and week at enrollment postpartum (1 or 2). The model of pregnancy at first insemination also included DIM at first insemination as a covariate. Production models included a variable for test number or DIM at test. Body condition was categorized into 3 groups based on previously reported HK risk (Duffield, 2000; Gillund et al., 2001): ≤ 3.0 , 3.25 to 3.50, and ≥ 3.75 (scale of 1 to 5). Parity was categorized into first, second, and third or greater lactation. The covariate for blood BHB concentration at enrollment was transformed with the natural logarithm to satisfy the assumption of linearity. The quadratic of blood glucose concentration was tested in all models and retained in the milk production models due to significance. Univariable associations of predictors and the outcomes were explored. Variables deemed significant with a $P \leq 0.2$ were then tested in the multivariable model. Treatment group was forced into all models regardless of significance. Multivariable models were constructed in a backward stepwise manner, removing those with a $P > 0.05$. Before removal, all nonsignificant variables were assessed for confounding, determined by a change in the model estimates for treatment of 20% or more. Interactions of significant covariates with treatment group were tested. The best linear unbiased predictors were evaluated for outliers or data patterns. Outliers were examined for data recording errors and their effect on model outputs was assessed. All observations were retained in the models presented.

RESULTS

Descriptive Statistics

In total, 2002 animals were screened for hyperketonemia, with 509 (25.4%) enrolled in the trial; 254 were allocated to the placebo group and 255 to the dexamethasone-treated group (Figure 1). Herd sizes ranged from 900 to 3,100 milking cows (mean = 1,855; SD = 1,073). The mean 305ME was 12,344 kg (SD = 780). The mean weekly HK prevalence across herds was 28% (SD = 14%); however, herds varied from a mean weekly prevalence of 15% in herd 4 to 47% in herd 3. The proportion of animals with HK with blood BHB < 2.0 mmol/L at enrollment did not differ between treatment groups ($P = 0.92$). Treatments were evenly distributed in each herd and there was no difference in parity ($P = 0.63$) or BCS ($P = 0.92$) between treatment groups (Table 1). The group mean of blood glucose

Table 1. Description and outcomes of cows enrolled in a randomized controlled trial using a single dose of dexamethasone for treatment of hyperketonemia (HK)¹

Variable	Treatment group [no./total (%)]		P-value
	Placebo	Dexamethasone	
Parity group			0.63
1	36 (14.2)	44 (17.3)	
2	81 (31.9)	80 (31.4)	
≥3	137 (53.9)	131 (51.4)	
BCS			0.92
≤3.0	17 (6.7)	18 (7.1)	
3.25 to 3.5	71 (28.1)	75 (29.4)	
≥3.75	165 (65.2)	162 (63.5)	
Time of enrollment			0.43
3 to 9 DIM	211 (83.1)	205 (80.4)	
10 to 16 DIM	43 (16.9)	50 (19.6)	
HK 1 wk after treatment	132/248 (53.2)	120/251 (47.8)	0.23
HK 2 wk after treatment	87/246 (35.4)	58/244 (23.8)	0.005
HK both weeks after treatment	71/241 (29.5)	45/242 (18.6)	0.005
Displaced abomasum	8/254 (3.1)	8/253 (3.2)	0.99
Metritis	16/254 (6.3)	16/253 (6.3)	0.99
Clinical ketosis	4/254 (1.6)	10/253 (4.0)	0.11
Removal from herd	7/254 (2.8)	5/253 (2.0)	0.55

¹All cows received 4 d of oral propylene glycol. All postpartum diseases and removal from the herd were measured within 30 d from calving.

at enrollment decreased with increasing blood BHB at enrollment (Figure 2); however, blood BHB and blood glucose at enrollment were only 41% correlated in individual animals.

HK in Wk 1 Posttreatment

One week after treatment, 52% of the placebo group and 47% (*P* = 0.23) of the dexamethasone group were hyperketonemic (Table 1). When controlling for herd, BCS, and blood glucose at enrollment, we detected an interaction of blood BHB concentration at enrollment

with treatment (Table 2). At blood BHB = 1.2 mmol/L (19% of cows with HK), the dexamethasone group had an odds of HK at 1 wk posttreatment of 0.48 compared with the placebo group (*P* = 0.004; Figure 3). However, at higher blood BHB concentrations at the time of treatment, the dexamethasone-treated cows had higher odds of HK than the placebo group. At a blood BHB of ≥3.2 mmol/L (8% of cows), the odds of HK at 1 wk posttreatment was 1.95 times greater in cows treated with dexamethasone than in those that received the placebo (*P* = 0.05), with the difference between the 2 groups increasing with increasing BHB at treatment.

Figure 1. Flowchart of total number animals screened for hyperketonemia (HK) and enrolled in the study, the number of observations included in each analysis and reason for missing data for 2002 dairy cattle in 4 New York State dairy herds tested for HK between 3 and 16 DIM. 305ME = 305-d mature-equivalent milk yield.

Figure 2. Group mean blood glucose concentration (mmol/L; $\pm$ SE) separated by blood BHB concentration at enrollment (mmol/L) for prepartum cows diagnosed with hyperketonemia (HK; BHB $\geq$ 1.2 mmol/L) between 3 and 16 DIM in 4 herds in New York State.

There was no difference between treatments in HK at 1 wk posttreatment for BHB >1.5 and <3.2 mmol/L at treatment. Cows with HK with BCS ≤ 3.0 at diagnosis (7% of cows) were less likely to have HK in the first week posttreatment than hyperketonemic cows with a BCS of 3.25 to 3.50 (odds ratio: 0.33; 95% CI: 0.13 to 0.85) and we detected no difference between odds of HK for cows with an ideal BCS (3.25 to 3.50) and overconditioned cows (≥ 3.75).

HK at Wk 2 Posttreatment

At 2 wk after treatment, 35% of the control group and 24% of the dexamethasone group had HK. In contrast to HK at 1 wk posttreatment, dexamethasone treatment was associated with lower odds of HK at 2 wk

Figure 3. Predicted natural logarithm odds and 95% CI of having hyperketonemia (HK) in wk 1 after treatment with 4 d of oral propylene glycol and a single intramuscular injection of 20 mg of dexamethasone (black circles) or an equivalent volume of sterile saline (placebo, gray diamonds); 95% CI are presented. The treatment effect was modified by BHB blood concentration at the time of diagnosis. Least squares means account for BCS and blood glucose concentration at diagnosis.

after treatment regardless of BHB blood concentration at the time of diagnosis. The odds of HK 2 wk after diagnosis (10 d after the end of PG treatment) in the dexamethasone-treated group were 0.54 (95% CI: 0.35 to 0.84) compared with the placebo-treated group ($P = 0.007$; Table 3). Overconditioned cows (BCS ≥ 3.75) and cows in parity ≥ 3 had higher odds of persistent HK at wk 2 posttreatment.

HK at Both Weeks Posttreatment

Similar to HK at 1 wk posttreatment, the effect of treatment on having HK at both wk 1 and wk 2 after

Table 2. Multivariable model estimates for having hyperketonemia (HK) 1 wk after treatment with 4 d of oral propylene glycol and a single intramuscular dose of 20 mg of dexamethasone or an equivalent volume of sterile saline (placebo)¹

Variable	Estimate	SE	Odds ratio (OR)	95% CI of OR	P-value
Treatment					
Placebo	Referent				
Dexamethasone	-1.15	0.38	0.82	0.55 to 1.21	0.003
Treatment $\times$ BHB at diagnosis					
Placebo $\times$ BHB	Referent		1.08	0.99 to 1.17	
Dexamethasone $\times$ BHB	0.18	0.06	1.28	1.16 to 1.42	0.003
BCS					
≤ 3.0	-1.10		0.33	0.13 to 0.85	0.02
3.25 to 3.5	Referent				
≥ 3.75	0.33	0.22	1.38	0.89 to 2.15	0.15
Natural log BHB at diagnosis (0.1 unit)	0.74	0.41			0.08
Blood glucose (0.1 mmol/L)	-0.53	0.22	0.59	0.39 to 0.90	0.02

¹Herd was controlled for as a random effect.

Table 3. Multivariable model estimates for having hyperketonemia (HK) 2 wk after treatment with 4 d of oral propylene glycol and a single intramuscular dose of 20 mg of dexamethasone or an equivalent volume of sterile saline (placebo)¹

Variable	Estimate	SE	Odds ratio (OR)	95% CI of OR	P-value
Treatment					
Placebo	Referent				
Dexamethasone	-0.61	0.23	0.54	0.35 to 0.84	0.007
BCS					
≤3.0	0.095	0.52	1.10	0.39 to 3.08	0.86
3.25 to 3.5	Referent				
≥3.75	0.71	0.27	2.03	1.20 to 3.44	0.008
Parity group					
1	Referent				
2	0.11	0.38	1.11	0.53 to 2.33	0.78
≥3	0.68	0.34	1.97	1.01 to 3.85	0.05
Time of enrollment					
3 to 9 DIM	0.24	0.30	1.27	0.70 to 2.31	0.44
10 to 16 DIM	Referent				
Natural log BHB (0.1 unit)	0.06	0.04	1.06	0.99 to 1.14	0.08
Blood glucose (0.1 mmol/L)	-0.07	0.03	0.93	0.88 to 0.98	0.007

¹Herd was controlled for as a random effect.

diagnosis and dexamethasone treatment was modified by blood BHB concentration at the time of diagnosis (Table 4). The proportion of the animals with HK at both follow-ups was 30% for the placebo group and 19% for the dexamethasone group. A difference between treatment groups could only be detected at lower blood BHB concentrations (Figure 4).

Postpartum Disease

The odds of having one or more of metritis, displaced abomasum, or clinical ketosis subsequent to enrollment

was not associated with treatment ($P = 0.98$). The odds of disease were higher for cows diagnosed with HK in the first week of lactation compared with the second week (odds ratio: 3.80; 95% CI: 1.13 to 12.71) and with higher blood BHB concentrations at diagnosis ($P = 0.001$; Table 4).

Pregnancy at First Insemination and at 120 DIM, and Time to Pregnancy

The average DIM at first breeding was 65 (SE = 0.39). One hundred forty-one (28%) of the 509 cows

Table 4. Multivariable model estimates for having hyperketonemia (HK) for 1 and 2 wk after treatment with 4 d of oral propylene glycol and a single intramuscular dose of 20 mg of dexamethasone or an equivalent volume of sterile saline (placebo)¹

Variable	Estimate	SE	Odds ratio (OR)	95% CI of OR	P-value
Treatment					
Placebo	Referent				
Dexamethasone	-1.55	0.50	0.45	0.27 to 0.74	0.002
Treatment × BHB at diagnosis					
Placebo × BHB	Referent				
Dexamethasone × BHB	0.14	0.07	1.20	1.07 to 1.35	0.04
BCS					
≤3.0	-0.18	0.61	0.84	0.25 to 2.76	0.77
3.25 to 3.5	Referent				
≥3.75	0.72	0.29	2.06	1.16 to 3.68	0.01
Parity group					
1	Referent				
2	-0.01	0.42	0.99	0.44 to 2.26	0.99
≥3	0.78	0.37	2.18	1.04 to 4.54	0.04
Time of enrollment					
3 to 9 DIM	0.51	0.36	1.67	0.82 to 3.38	0.16
10 to 16 DIM	Referent				
Natural log BHB (0.1 unit)	0.04	0.04			0.33
Blood glucose (0.1 mmol/L)	-0.07	0.03	0.93	0.88 to 0.99	0.01

¹Herd was controlled for as a random effect.

were diagnosed pregnant to the first AI. Twenty-seven percent of the dexamethasone-treated cows were diagnosed pregnant to first AI compared with 32% of the cows receiving the placebo ($P = 0.17$). There was an interaction of treatment with blood BHB at diagnosis ($P = 0.06$). At blood BHB of 1.2 to 1.5 mmol/L at diagnosis, cows treated with dexamethasone had lower odds of pregnancy at first AI (Figure 5). At blood BHB = 1.2 mmol/L, the odds of pregnancy were 0.50 (95% CI: 0.29 to 0.89) times lower in the dexamethasone-treated group than in the placebo group. Treatment with dexamethasone tended to decrease odds of pregnancy by 120 DIM by 0.68 times ($P = 0.07$). Treatment did not affect time to pregnancy up to 120 DIM ($P = 0.13$).

Milk Yield

There was no overall difference between treatment groups in milk yield across the first 3 test days. There was an interaction of treatment and test day such that cows in the dexamethasone group produced 3.4% more milk at the third DHIA test day; however, it was not significant ($P = 0.38$; Figure 6). Treatment group did not affect projected 305ME based on the first 3 milk tests ($P = 0.23$).

All models, except for the outcome for pregnancy at first insemination, had an association between blood glucose concentration at the time of diagnosis and the outcome of interest. Having lower blood glucose

Figure 4. Predicted natural logarithm odds and 95% CI of having hyperketonemia (HK) in wk 1 and wk 2 after treatment with 4 d of oral propylene glycol and a single intramuscular injection of 20 mg of dexamethasone (black circles) or an equivalent volume of sterile saline (placebo, gray diamonds). The treatment effect was modified by BHB blood concentration at the time of diagnosis. Least squares means account for BCS and blood glucose concentration at diagnosis.

Figure 5. Predicted natural logarithm odds and 95% CI of pregnancy at first insemination after treatment with 4 d of oral propylene glycol and a single intramuscular injection of 20 mg of dexamethasone (black circles) or an equivalent volume of sterile saline (placebo, gray diamonds). We detected a tendency ($P = 0.06$) for a treatment by BHB at diagnosis interaction.

concentration was associated with poorer outcomes, increased odds of HK at all follow-ups, and increased odds of postpartum disease (Tables 2, 3 and 4). Mean blood glucose decreased with increasing blood BHB concentrations; however, there was no interaction between blood glucose and treatment group for any outcome. Blood glucose concentrations had a quadratic association with milk production outcomes, meaning that animals with low blood glucose and those with

Figure 6. Predicted estimates and 95% CI for milk yield (kg/d) on the first 3 DHIA test-days. Treatment for hyperketonemia (HK) with 4 d of oral propylene glycol and a single 20-mg dexamethasone intramuscular injection (dark gray) or an equivalent volume of sterile saline (placebo, light gray) had no effect on milk yield.

high blood glucose produced less milk over the first 3 test days.

Examination of the BLUP identified an obvious outlier animal with a blood glucose concentration at enrollment of 6.8 mmol/L, which was more than 3.0 mmol/L higher than the next highest recorded concentration. There was no change in model estimates for any outcome when the outlying animal was removed from the analysis, and therefore the animal was retained in all models.

DISCUSSION

Treatment of HK with 4 d of PG and a single 20-mg i.m. injection of dexamethasone was conditionally associated with decreased persistence of HK at 1 and 2 wk after treatment. For cows with a blood BHB of 1.2 to 1.5 mmol/L at initial diagnosis, the odds of persistent HK were decreased in the first week after treatment for all cows that received dexamethasone, whereas the odds of HK 2 wk after diagnosis were lowered for all cows that received dexamethasone. The association between treatment and HK in wk 2 after diagnosis was different from that in wk 1 or in both weeks. The other significant covariates explaining increased odds of HK in wk 1 differed from HK in wk 2 and HK in both weeks. The odds of having HK in wk 2 after diagnosis, and both weeks after diagnosis, were higher for overconditioned (≥ 3.75) cows and cows in parity ≥ 3 . Parity was not associated with odds of having HK in wk 1 after diagnosis and there was no difference between cows with an ideal BCS (3.0 to 3.5) and those with a BCS ≥ 3.75 . The difference in treatment effect and population characteristics may represent a difference in underlying pathophysiology. Future work should examine the interplay of ketones, inflammatory mediators, and liver function to better describe the underlying pathophysiology of cows that are diagnosed with HK using blood alone. Although it is a convenient method for identifying the at-risk population for subsequent treatment with PG, better understanding of the pathophysiology will inform our research into other potential diagnostic criteria and treatment options.

Notwithstanding the effects of treatment with dexamethasone in reducing the prevalence of HK 1 and 2 wk after treatment, we detected no effect of treatment on production, the odds of postpartum disease, or pregnancy at first AI. The incidence of disease within 30 DIM was low in both treatment groups (dexamethasone: 10%, placebo: 9.3%). The incidence of metritis was lower than expected (6.3% in both groups; Kelton et al., 1998; Ospina et al., 2010); however, that was likely due to excluding cows with retained placentas and evidence of metritis before enrollment. With a

sample size of 250 cows per treatment group, a 9% difference in disease incidence would be detected with 80% power and 95% confidence. The difference in disease incidence between treatment groups was 1.4%. Although the incidence of postpartum disease was low, the lack of numerical difference between the treatment groups suggests that treatment truly had no effect.

The mean weekly HK prevalence in this study (28%) was similar to that reported by McArt et al. (2012b), where the peak prevalence at 5 DIM was 29%. McArt et al. (2011, 2012b) treated with oral PG from initial HK diagnosis (BHB ≥ 1.2 mmol/L) until BHB < 1.2 mmol/L. After 4 d of PG therapy, 50% still had HK. In the current study, although sampling occurred less frequently (1×/wk compared with 3×/wk), a similar proportion of both groups remained hyperketonemic 7 d after the start of therapy (placebo: 52%; dexamethasone: 47%).

Consistent with previous research, being diagnosed with HK in the first 3 to 9 DIM, being overconditioned, and having a higher blood BHB concentration increased the odds of having postpartum disease within the first 30 DIM (Duffield et al., 2009; McArt et al., 2012b).

Treatment was not associated with pregnancy at first insemination but there was a tendency for BHB concentration at diagnosis to modify the treatment effect. Contrary to the other models with a treatment by initial BHB concentration interaction, for cows with lower initial BHB, being treated with dexamethasone was associated with lower odds of pregnancy at first insemination. The reason for this relationship is unclear and may warrant further research.

Treatment with dexamethasone was not associated with milk production outcomes, similar to the findings of Sami et al. (2015). Previous studies evaluating glucocorticoids suggested that they had a negative effect on milk production due to a reduction in glucose uptake by the udder (Baird and Heitzman, 1971; Hartmann and Kronfeld, 1973). However, decreases in milk production with glucocorticoid treatment in previous studies occurred only in healthy animals and not in those with HK (Neff et al., 1960; Wierda et al., 1987; Seifi et al., 2007). The lack of difference in milk production between treatment groups in this study confirms that glucocorticoids do not affect milk production in HK animals.

The main limitation of this work was the sampling frequency. Due to labor and financial constraints, sampling could only be completed once a week. By sampling less frequently, HK animals may have been missed, both at enrollment and in follow-up weeks. Missing HK animals contributes to a nondirectional misclassification bias. Due to the equal probability of misclassification between groups, the bias would be toward the null and

therefore the associations identified are likely to be true. However, there could be existing associations that were not discovered with this design. All animals had HK at enrollment and treatment groups did not differ in time at enrollment, parity, or BCS distribution; therefore, the effect on disease incidence and milk production between treatment groups should be comparable. The effect of treatment on HK 1 and 2 wk after treatment is more difficult to interpret based on sampling frequency, as it is unknown whether the decrease in HK prevalence is due to an increase in overall cure rate or a decrease in relapses.

Another limitation includes the lack of precision in glucose testing using the handheld meter. Although glucose, as measured by the meter, was previously reported to be 95% correlated with laboratory glucose, 95% of the results were within 1 mmol/L of the laboratory value (Wittrock et al., 2013). Postpartum blood glucose concentrations are tightly clustered and a difference of 1 mmol/L may be significant. Treatment did not depend on blood glucose concentration as measured by the meter; however, if treatment decisions are to be made based on blood glucose, laboratory confirmation may be necessary.

The contradictory findings in previous research into the use of glucocorticoids to treat HK may be due to the varying effect of glucocorticoids depending on cow-level factors (e.g., BHB concentration at diagnosis as shown here, but not accounted for in many earlier studies) and the dose and frequency of glucocorticoid used. van der Drift et al. (2015) and Sami et al. (2015) both reported improved group-level means of serum BHB in the short term; however, the proportion of animals that were below the ketosis threshold and the long-term consequences were not clear, which may be of more practical importance. It is difficult to compare the current study to van der Drift et al. (2015) and Sami et al. (2015), as the criteria for enrollment, the dose and frequency of PG, the formulation of dexamethasone, and the outcome of interest differed.

Of particular interest are the increased odds of HK in the first week posttreatment in the 8% of animals with high (>3.2 mmol/L) blood BHB concentrations at the time of treatment. Future research should evaluate the use of dexamethasone in cows with high BHB, especially as a treatment for clinical ketosis, as this may represent a pathophysiology different from that of cows with less-elevated BHB and may be detrimental.

CONCLUSIONS

The use of dexamethasone as an adjunctive therapy to oral PG had short-term conditional effects to reduce the prevalence of HK, depending on the blood BHB

concentration at the time of diagnosis and the herd. However, with no effects on postpartum disease or milk production and with a tendency toward a negative association with pregnancy at first AI, we do not recommend one 20-mg i.m. injection of dexamethasone at the time of HK diagnosis as an adjunctive therapy to PG for treatment of HK.

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