A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis

J. A. A. McArt,* D. V. Nydam,*¹ P. A. Ospina,† and G. R. Oetzel‡

*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

†Department of Animal Science, Cornell University, Ithaca, NY 14853

‡School of Veterinary Medicine, University of Wisconsin, Madison 53706

ABSTRACT

The purpose of this study was to determine the effect of oral propylene glycol (PG) administration on ketosis resolution and milk yield in cows diagnosed with subclinical ketosis (SCK). Cows from 4 freestall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 d in milk on Mondays, Wednesdays, and Fridays. Subclinical ketosis was defined as a β -hydroxybutyrate (BHBA) concentration of 1.2 to 2.9 mM/L, and clinical ketosis was defined as ≥ 3.0 mM/L. Cows with SCK were randomized to the treatment group (oral PG) or control group (no PG); treatment cows were drenched with 300 mL of PG once daily from the day they tested 1.2 to 2.9 mM/L until the day they tested <1.2 mM/L. Outcomes evaluated for all farms included time from SCK until BHBA test <1.2 mM/L or until BHBA test ≥ 3.0 mM/L. Individual milk weights for the first 30 d of lactation were evaluated for the 3 farms monitoring daily milk. Semiparametric proportional hazards models were used to evaluate time to event outcomes; repeated-measures ANOVA was used to assess milk weights. A total of 741 of 1,717 (43.2%) eligible enrolled cows had at least one BHBA test of 1.2 to 2.9 mM/L. Of these, 372 were assigned to the treatment group and 369 to the control group. Based on hazard ratios, PG-treated cows were 1.50 times more likely (95% confidence interval = 1.26 to 1.79) to resolve their SCK and 0.54 times less likely (95% confidence interval = 0.34 to 0.86) to develop clinical ketosis than control cows. Across the 3 herds measuring individual milk weights, treated cows produced 0.23 kg more milk per milking in the first 30 d of lactation than control cows, for a total difference of 0.69 kg/cow per day. After identification of a treatment by herd interaction, stratification by herd showed that treated cows produced more milk per milking on farm A (0.44 kg) and farm B (0.53 kg) in the first 30 d of

lactation than control cows, for a total difference of 1.34 and 1.59 kg/d, respectively; milk production did not differ (0.02 kg per milking) between the 2 groups on farm D. These results show the positive effects of oral PG administration in fresh cows with SCK by helping to resolve SCK and preventing clinical ketosis. In addition, oral PG improves milk yield during early lactation in cows diagnosed with SCK.

Key words: dairy cow, ketosis, propylene glycol, milk yield

INTRODUCTION

Early lactation is a difficult period for dairy cows, which must transition from the demands of late gestation to those of early lactation. Those unable to adapt to this period of negative energy balance are prone to metabolic disorders and decreased milk production (Cameron et al., 1998; Drackley, 1999; Herdt, 2000). Among the sequelae of a poor adaptive response is an excessive elevation of circulating ketone bodies in the blood (Herdt, 2000), which can present clinically as a decrease in appetite, weight loss, and a decrease in milk production. The economic losses due to clinical ketosis are not trivial because of treatment costs, decreased milk yield, increased culling, and decreased reproductive efficiency (Fourichon et al., 1999; Ostergaard and Gröhn, 1999; Gröhn et al., 2003). Cows also suffer from subclinical ketosis (**SCK**), defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988), which places them at an increased risk of other parturient diseases such as displaced abomasum and metritis (Duffield et al., 2009; Ospina et al., 2010a,b). In addition to the effects on disease events, SCK has been found to decrease milk yield in early lactation (Dohoo and Martin, 1984; Ospina et al., 2010b). The lactational incidence of SCK, which can be as high as 80% in some herds, is much greater than the 2 to 15% found with clinical ketosis (Duffield, 2000).

Propylene glycol (**PG**) has long been used to treat clinical ketosis (Johnson, 1954; Maplesden, 1954) and is known to be antiketogenic by increasing plasma glucose concentrations through decreased peripheral tissue glu-

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¹Corresponding author: dvn2@cornell.edu

cose demand (Kristensen and Raun, 2007) and lowering NEFA and liver triglyceride levels, resulting in a decrease of plasma BHBA (Sauer et al., 1973; Grummer et al., 1994; Chung et al., 2009). Although many trials have been conducted with PG using various dosages, lactational stages of administration, routes of delivery, and length of treatment (Studer et al., 1993; Miyoshi et al., 2001; Nielsen and Ingvarsten, 2004), the small scale of these trials may have resulted in a lack of significant findings or have poor external validity in relation to larger herds. In a review of 12 papers concerning the effect of PG on milk production in dairy cows (Nielsen and Ingvarsten, 2004), the general trend of PG administration was to increase milk production over control cows, although these trials used prophylactic dosing of PG rather than a treatment based on results of ketosis testing.

Interest exists in developing drenching programs on farms with intensive monitoring protocols to evaluate the effect of PG on milk production in early lactation and to determine whether PG administration decreases the incidence of metabolic diseases (Pickett et al., 2003). The recent identification and validation of the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL; Iwersen et al., 2009; Konkol et al., 2009), a rapid, accurate, and relatively inexpensive cow-side test for SCK, eases many of the previous difficulties associated with intensive monitoring programs.

The objective of this study was to determine the effect of oral PG administration on SCK resolution, clinical ketosis prevention, and milk yield in cows diagnosed with SCK in early lactation with an intensive monitoring program.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York State from May 18 until September 8, 2010, and from 2 dairy farms (farms C and D) in Wisconsin, from June 11 until August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing and treatment protocol. Detailed information concerning farm management structure and nutrition can be found in the Appendix.

Data Collection and Study Design

Enrollment into the study occurred at calving (Figure 1). Cows were tested from 3 to 16 DIM on Mondays,

Wednesdays, and Fridays for ketosis using a Precision Xtra meter. The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 96 to 100% and 98 to 100%, respectively, when using a cut-off value of ≥ 1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mM/L; clinical ketosis was defined as ≥ 3.0 mM/L (Oetzel, 2004).

On farms A and B, 10 mL of blood was collected from the coccygeal vessels of each cow using a tube without anticoagulant and a 20-gauge, 2.54-cm blood collection needle. On farms C and D, approximately 0.5 mL of blood was collected from the coccygeal vessels using a 22-gauge, 2.54-cm needle and a 1-mL syringe. β -Hydroxybutyrate testing was completed according to Precision Xtra meter instructions and performed immediately after blood collection. A ketone strip was attached to the Precision Xtra meter until the "add blood" symbol appeared on the meter display. The lot number of the inserted ketone strip was then checked to ensure matching with the lot number displayed on the meter. For each cow test, a drop of blood was applied to the ketone test strip test chamber; the meter indicated when the chamber was full. After 10 s, the BHBA concentration was displayed on the meter and the value recorded.

All testing and treatment of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Cows with BHBA concentrations of 1.2 to 2.9 mM/L were sequentially randomized to treatment group (oral PG drench) or control group (no PG) after their first SCK positive test. Randomization to treatment group for the first cow on each farm was completed in Excel (Microsoft, Redmond, WA) using the random number function. Cows assigned to treatment were drenched with 300 mL of PG (E.H. Wolf & Sons, Green Bay, WI) once daily from the day they tested ≥ 1.2 mM/L until the day they tested < 1.2 mM/L or reached 17 DIM. Administration of a 300-mL volume was chosen because it is a common dose used on farms and delivers approximately 310 g of PG. Drenching on farms A and B was completed by the research team; drenching on farms C and D was completed by on-farm personnel. Cows with BHBA concentrations of ≥ 3.0 mM/L were treated by on-farm personnel per farm protocol for cows diagnosed with ketosis.

Cows were excluded from the study if their previous days carried calf was less than 260 d, if they died or were sold before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before

Figure 1. Flowchart showing total enrollment, reasons for exclusion before testing, reasons for nonrandomization, reasons for removal from analysis, and final analysis by treatment group for 2,115 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM.

their first BHBA test, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included lactation number and individual milking weights for the first 30 d of lactation (farms A, B, and D only). Individual milking weights were exported throughout the study period from each farm's Dairy Comp 305 program. All milking values recorded as "0" pounds of milk were reentered as missing data points.

The study aimed to enroll 2,400 cows. Based on previous research by Ospina et al. (2010c), with approximately 22% of cows testing positive for SCK, enrollment of 2,400 cows would render approximately 530 cows available for randomization, with 265 cows randomized to each treatment group. This sample size, assuming a desired type I error rate of 5%, a power of 80%, and a standard deviation of 1.8 kg of milk per milking, would allow detection of a 0.5-kg difference in individual milk yield. A proposal was reviewed

and approved by the Cornell University Institutional Animal Care and Use Committee (#2008-0099) and the University of Wisconsin Institutional Animal Care and Use Committee (#V01479-0-05-10). All farms were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions including clinical milk fever, retained placenta, metritis, displaced abomasum, and clinical ketosis.

Statistical Analysis

Descriptive statistics were generated with the FREQ and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC). The effect of PG on time to resolution of SCK and time to diagnosis of clinical ketosis were analyzed by semiparametric proportional hazards (Cox, 1972) models using the PHREG procedure of SAS. The time-series variables for the models were first BHBA test of 1.2 to 2.9 mM/L until BHBA test <1.2 mM/L and first BHBA test of 1.2 to 2.9 mM/L until BHBA test ≥ 3.0 mM/L, respectively. Censoring variables were used to identify cows that had the event of interest from cows that either died (or were culled) or did not have the event by 16 DIM. In addition to the PG treatment variable, the potential confounding variables lactation group (lactation 1, lactation 2, and lactation ≥ 3) and herd were offered to the model as independent variables. Independent variables and their respective interaction terms were manually removed by backward stepwise elimination if considered statistically nonsignificant ($P > 0.15$) or biologically not important. Proportional hazards assumptions were verified by evaluating the time-dependent covariates (Allison, 1995); noninformative censoring was evaluated using sensitivity analysis. Difference between treatment groups in milk yield for individual milk weights until 30 DIM was analyzed using repeated-measures ANOVA with first-order autoregressive covariance using the MIXED procedure of SAS (Littell et al., 1998, 2000). Results were analyzed using different covariance structures; the 3 covariance structures producing outcomes with the smallest Akaike information criterion were retained for further discussion. A first-order autoregressive covariance structure was chosen over compound symmetry and Toeplitz covariance structures for logical and model simplicity reasons. Compound symmetry covariance does not take into account the fact that individual milk weights are more likely to be correlated with weight measurements taken in close time proximity and less correlated with weight measurements taken farther away in time. The first-order covariance model was retained over the model using a Toeplitz covariance structure because a simpler model (fewer parameters) is more desirable.

Variables offered to the model included PG treatment, lactation group (lactation 1, 2, ≥ 3), DIM, and herd as a random effect. A second model was developed using the variables lactation group, DIM, herd, and a treatment by herd interaction. If the interaction between treatment and herd was found to be significant, then the outcome would be stratified by herd. Independent variables and their respective interaction terms were considered statistically significant if $P \leq 0.05$.

RESULTS

Descriptive Statistics

Of the 2,115 cows enrolled in the trial, 741 were diagnosed with SCK and randomized, with 372 cows in the PG treatment group and 369 control cows (Figure 1). The control group was composed of 106, 97, and 166 cows in lactations 1, 2, and ≥ 3 , respectively (median = 2); the treatment group contained 109 cows in lactation 1, 92 in lactation 2, and 171 in lactation ≥ 3 (median = 2). A Chi-squared test showed no difference in parity between the 2 groups ($P = 0.89$). The incidence of SCK was 40.4% on farm A, 26.4% on farm B, 40.9% on farm C, and 55.7% on farm D.

Time to Resolution of SCK and Time to Clinical Ketosis

Figure 2 shows the Cox proportional hazards curve for the effect of PG on time to resolution of SCK; the final model included only PG treatment as an independent variable. Based on hazard ratios, PG-treated cows were 1.50 times more likely (95% CI = 1.26 to 1.79; $P < 0.001$) to resolve their SCK than control cows. Figure 3 shows the Cox proportional hazards curve for the effect of PG on time to development of clinical ketosis; the final model included only PG treatment as an independent variable. Cows treated with PG were 0.54 times less likely (95% CI = 0.34 to 0.86; $P = 0.009$) to develop clinical ketosis than control cows. The final Cox proportional models can be seen in Table 1.

Milk Yield

In total, 622 cows from farms A, B, and D were used in the analysis to determine the effect of PG treatment on early lactation cows diagnosed with SCK. The variables PG treatment, lactation group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights. The first model using the variable herd as a random effect found that treated cows produced 0.23 kg more milk per milking in the first 30 d of lactation than control cows ($P < 0.001$), for

Figure 2. Cox proportional hazards analysis illustrating the time from diagnosis of subclinical ketosis (BHBA concentration of 1.2 to 2.9 mM/L) until resolution of subclinical ketosis (BHBA concentration <1.2 mM/L) for 741 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM. Cows treated with propylene glycol (PG; $n = 372$) were 1.50 (95% CI = 1.26 to 1.79) times more likely ($P < 0.0001$) to resolve their subclinical ketosis than control cows ($n = 369$).

a total difference of 0.68 kg/d. The second model, using herd as a fixed effect and including a treatment by herd interaction, was stratified by herd after a significant treatment by herd interaction was found. Treated cows produced more milk per milking on farm A (0.44 kg; $P < 0.001$) and farm B (0.53 kg; $P < 0.001$) in the first 30 d of lactation compared with control cows, for a total difference of 1.34 and 1.59 kg/d, respectively, based on 3 times a day milking. Milk production per milking did not differ (0.02 kg; $P = 0.70$) between the 2 groups on farm D, with treated cows making 0.074 kg more milk per day than control cows. Table 2 shows PG treatment results by herd.

DISCUSSION

The reported study was conducted to determine the effects of oral PG on resolution of SCK, development of clinical ketosis, and milk yield in cows diagnosed with SCK. Results showed that cows treated with oral PG were more likely to resolve their ketosis, less likely to develop clinical ketosis, and, in some herds, produce more milk per milking in the first 30 d of lactation than control cows.

The wide range of SCK incidence found among the research farms (26.4 to 55.7%) illustrates the varying severity of common periparturient diseases between herds. These values are similar to those reviewed by Duffield (2000), who suggested a lactational incidence of SCK of approximately 54% with herds ranging from 8 to 80%. Other studies examining the prevalence of SCK have reported values at 2 wk postpartum of 33% (Duffield et al., 1998), within 2 wk postpartum of 22% (Ospina et al., 2010a), and within the first 2 mo of lactation of 0 to 33.9% (Dohoo and Martin, 1984). Whereas the variability of herd SCK found in the current study did not have an effect on the time to resolution of ketosis or time to diagnosis of clinical ketosis, it may have had an effect on subsequent milk production, as discussed below.

To preserve data quality, individual milking weights were used in the analysis rather than daily milk yields. All farms had cows with missing weights for a variety of reasons, including inability of the parlor system to read electronic identification numbers, temporary malfunctioning of the parlor recording system, or inclusion of sick cows in pens that were not milked. In addition, cows on farm A and farm B that were milked in the

Table 1. Cox proportional models showing hazard ratios (HR) for 741 Holstein cows with at least one positive test for subclinical ketosis from 3 to 16 DIM randomly assigned to propylene glycol treatment (n = 372) or control (n = 369)

Event ¹	Estimate	SE ²	P-value ³	HR	95% CI ⁴
BHBA <1.2 mM/L	0.41	0.091	<0.001	1.50	1.26 to 1.79
BHBA ≥3.0 mM/L	0.62	0.24	0.009	0.54	0.34 to 0.86

¹HR events include time from positive test for subclinical ketosis (BHBA concentration of 1.2 to 2.9 mM/L) to either ketosis resolution (BHBA concentration <1.2 mM/L) or clinical ketosis (BHBA concentration ≥3.0 mM/L)

²SE = standard error for estimate.

³P-value reported for estimate.

⁴CI for hazard ratio.

parlor reserved for colostrum collection and milk withholding did not have weights recorded for these milkings. Rather than determine daily milk yield for cows with missing weights by averaging surrounding values or by data imputation, the analysis was completed using the available individual milking weight data (Gornbein et al., 1992).

Once an interaction between herd and treatment group was identified, milk weight analysis was stratified by herd. On farm A and farm B, SCK cows treated with PG made significantly more milk than control

cows, amounting to 1.34 and 1.59 kg/d, respectively, for the first 30 DIM. To the authors' knowledge, this is the first study that examined the effect of PG on milk yield of cows diagnosed with SCK. Although multiple studies have found a positive effect on milk yield after prophylactic administration of PG during early lactation (Formigoni et al., 1996; Miyoshi et al., 2001; Lien et al., 2010), only the trial completed by Lien et al. (2010) showed a significant difference between groups, with PG-treated cows producing 0.61 kg more milk per day in the first 90 d than control cows. A few studies found

Figure 3. Cox proportional hazards analysis illustrating the time from diagnosis of subclinical ketosis (BHBA concentration ≥1.2 to 2.9 mM/L) until diagnosis of clinical ketosis (BHBA concentration ≥3.0 mM/L) for 741 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM. Cows treated with propylene glycol (n = 372) were 0.54 (95% CI = 0.34 to 0.86) times less likely ($P = 0.009$) to develop clinical ketosis than control cows (n = 369).

Table 2. Individual milking yield stratified by herd for 622 Holstein cows randomly assigned to propylene glycol (PG) or control group with at least one positive blood test for subclinical ketosis (BHBA of 1.2 to 2.9 mM/L) from 3 to 16 DIM

Herd ¹	Ketosis incidence (%)	Yield/milking per cow (kg)	SE ²	Milk difference per day (kg)	P-value ³
Farm A					
Control (n = 70)	40.4	12.41	0.089	1.32	<0.001
PG (n = 73)		12.85	0.087		
Farm B					
Control (n = 52)	26.4	12.26	0.096	1.59	<0.001
PG (n = 54)		12.79	0.091		
Farm D					
Control (n = 189)	55.7	10.97	0.047	0.06	0.70
PG (n = 184)		10.99	0.047		

¹Of the 4 farms in the study, 3 recorded individual milk weights and were included in the analysis.

²SE = standard error for individual milk yield.

³P-value reported for difference between control and PG treatment individual milk yield.

a numerical decrease in milk yield after prophylactic administration of PG in early lactation (Emery et al., 1964; Fisher et al., 1973; Pickett et al., 2003), in which none of the results were statistically significant. One study evaluating the effect of PG on cows positive for clinical ketosis based on the milk ketone test failed to find a difference in milk production between the treated and control groups (Rueggsegger and Schultz, 1986). In the studies mentioned above that failed to detect a difference or that did not detect a significant difference, the small number of animals assigned to each treatment group (ranging from 7 to 22 cows per group) may have failed to provide enough power to determine if a true difference existed in milk yield between groups.

Considering the milk production differences between SCK cows treated with PG and control cows on farm A and farm B, the lack of difference found on farm D was unexpected. One explanation for this disparity in results is the higher incidence of ketosis on farm D. Although PG administration in SCK cows on farm D may have helped them resolve their ketosis faster and prevent clinical ketosis, the underlying metabolic challenges these cows faced to produce more milk may have been too large to improve with PG treatment alone. Changes in dry cow management and nutrition may decrease the metabolic stresses of parturition and early lactation to a level at which PG treatment is effective in elevating milk production in this herd. Another possible explanation for the differences seen between study herds is the average milk yield of the herds; the average daily milk yield for all lactating cows during the study period for farm A, farm B, and farm D was 41.8, 41.8, and 35.3 kg, respectively. Similarly, the average daily milk produced by study cows in the first 30 DIM on farm A and farm B was higher during the study period than that by cows on farm D, at 37.5, 37.0, and 32.9 kg, respectively. There may be a limiting management or nutrient factor on farm D that did not

allow SCK cows treated with PG to improve as seen in the other herds. Additionally, the on-farm protocol for treating cows with clinical ketosis differed slightly between the farms. On farm A and farm B, cows were tested daily either by the research group (on Mondays, Wednesdays, and Fridays) or by farm personnel (the remaining days of the week) and treated according to the ketosis diagnosis of the given test day. On farm D, cows diagnosed with clinical ketosis were given 4 d of oral PG regardless of their test results for the last 3 of the 4 treatment days. Given this on-farm treatment for clinical ketosis, of the SCK positive cows on farm D that subsequently developed clinical ketosis (6%, n = 48), 22 of 31 cows in the control group and 3 of 17 cows in the treatment group received repeated doses of PG that were unnecessary. Because more cows developed clinical ketosis in the control group than the treatment group, it is possible that the additional PG received by the control group cows may have increased milk production enough to inappropriately bias the finding toward the conclusion that milk yield did not differ between the 2 groups. Thus, the true difference in milk yield found between the 2 groups on farm D is most likely greater than 0.06 kg/d. Cows that developed clinical ketosis were included in the analysis. Because cows in the control group were more likely to develop clinical ketosis than cows in the PG treatment group, excluding cows with clinical ketosis from the milk yield analysis would have removed more control cows with poor milk production than treatment cows, thus falsely inflating the milk yield of the control cows and further biasing the findings toward the conclusion that milk yield did not differ between the 2 groups.

The treatment effect of PG on resolution of SCK was the same for all herds, with PG-treated cows 1.50 times more likely to resolve their ketosis than control cows. Additionally, the PG treatment effect on prevention of clinical ketosis diagnosis was the same for all

herds, with PG-treated cows 0.54 times less likely to be diagnosed with clinical ketosis than control cows. Only a few published studies have looked at the effect of PG on cows with clinical ketosis. In a study by Rueggsegger and Schultz (1986) involving PG treatment in cows testing positive for milk ketones, none of the treated or control cows developed clinical ketosis. In cows treated with prophylactic PG during the dry period or early lactation, 2 studies found a significant decrease in blood BHBA concentration (Grummer et al., 1994; Formigoni et al., 1996), and 1 found no difference between groups (Pickett et al., 2003). Miettinen (1995) showed that treatment with PG and a nicotinamide solution did not prevent clinical ketosis compared with control treatment; however, PG treatment started at 14 DIM. The discrepancies in these results may be due to the differences in PG dose, route of administration, or in the time, method, and frequency of BHBA or ketosis testing. In addition, all studies used a relatively small number of cows per group; thus, the studies without a significant finding may have lacked the necessary cow numbers for adequate statistical power. The randomization of 372 cows to PG treatment and 369 cows to the control group in the current study was more than enough to detect significant positive outcomes when using PG to treat cows with SCK.

In addition to the health benefits found with PG administration on all farms, the economic implications of increased milk production within the first 30 DIM on farm A and farm B are not trivial. Further investigation is needed to determine the best testing and dosing scheme that is economical, beneficial to the cows, and practical for management and labor purposes.

CONCLUSIONS

These results show the positive effects of oral PG administration in fresh cows with SCK in helping resolve SCK as well as preventing clinical ketosis. In addition, cows diagnosed with SCK that received oral PG had improved milk yield during early lactation in some herds.

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- treated cow milk, and averaged 41.8 kg of milk per cow per day during the study period. Close-up dry heifers and cows were housed together in a 3-row freestall barn with an average of 44 cm of bunk space per cow and approximately 20% more stalls than cows. The close-up diet consisted of wheat straw and corn silage; both cows and heifers freshened in group pens bedded with straw. The fresh cow pen was located in a 3-row barn with an average of 52 cm of bunk space per cow and approximately 10% fewer stalls than cows; stalls were covered with water mattresses and bedded with recycled manure solids. Cows remained in the fresh pen until approximately 40 DIM. The farm's prestudy fresh cow management involved physical examination of all cows from 1 to 10 DIM using rectal temperature recording and urine ketosis dipsticks (Ketostix, Bayer Animal Health, Shawnee, KS). Cows with results of moderate or large on the urine dipsticks were recorded by the farm as having ketosis and were given 300 mL of oral PG and 500 mL of 50% dextrose intravenously. Lactating cows were fed a TMR consisting of approximately 60% forage (corn silage, alfalfa and grass haylage, alfalfa silage, alfalfa hay, and wheat straw) and 40% concentrate (cornmeal, soybean meal, and canola meal) with a standard vitamin and mineral pack that contained 16 g of monensin per tonne of diet DM.
- Farm B was a 1,800-cow Holstein herd that milked 3 times daily in 1 of 2 parlors, a double-22 parallel parlor used for the majority of cows or a single-10 parallel parlor used for collection of colostrum and antibiotic-treated cow milk, and averaged 41.8 kg milk per cow per day during the study period. Close-up dry heifers and cows were housed separately in a 2-row freestall barn with an average of 50 cm of bunk space per animal in each pen. Both pens contained 30 to 40% more cows than stalls and cows freshened in a group pen bedded with straw. The close-up diet consisted of wheat straw, canola meal, and corn silage. The fresh pens were located in a 6-row barn with a separate pen for heifers and cows, and contained an average of 56 and 62 cm of bunk space per animal, respectively. Stalls were covered with mattresses and bedded with recycled manure solids, and both pens were maintained with equal stall and cow numbers. Heifers remained in their pen until approximately 60 DIM and cows until approximately 40 DIM. The farm's prestudy fresh cow management involved physical examination of all animals listed as "deviation" on the farm's Dairy Comp 305 program; ketosis status was tested using Ketostix urine dipsticks. Cows with results of trace or small on the urine dipstick received 300 mL of oral PG, those with a result of moderate received 300 mL of PG orally and 500 mL of dextrose and 10 mL of vitamin B₁₂ intravenously, and those with a result of large received 300 mL of PG

APPENDIX

Farm A was a 1,900-cow Holstein herd that milked 3 times daily in 1 of 2 parlors, a double-28 parallel parlor used for the majority of cows or a double-12 parallel parlor used for collection of colostrum and antibiotic-

orally and 500 mL of 50% dextrose, 10 mL of vitamin B complex, and 10 mL of dexamethasone intravenously. Both fresh pens were fed a TMR consisting of approximately 53% forage (corn silage, and alfalfa and grass haylage) and 47% concentrate (cornmeal, canola meal, and high-moisture shelled corn) with a standard vitamin and mineral pack that contained 12 g of monensin per tonne of diet DM.

Farm C was a 2,800-cow predominantly Holstein herd that milked cows 3 times daily in a double-20 parallel parlor and averaged 39.4 kg of milk per cow per day during the study period. Cows and heifers were housed separately during the close-up dry period. Heifers were kept in a 3-row freestall pen that provided an average of 72 cm of bunk space per animal and contained 40% more stalls than heifers in the pen. Cows were housed in a 3-row freestall pen that provided an average of 61 cm of bunk space per cow and 25% more stalls than the number of cows in the pen. All stalls were deeply bedded with recycled sand over a packed gravel base. Close-up heifers and cows were fed a TMR consisting of approximately 71% forage (alfalfa hay, corn silage, grass hay, and wheat straw) and 29% concentrate (grain, protein supplements) with trace minerals, supplemental anions, and vitamins. Cows and heifers were moved to individual maternity pens deeply bedded with straw when they exhibited signs of active labor. After calving, both cows and heifers were moved to a 2-row hospital pen occupied by other recently fresh cows as well as sick cows with nonsaleable milk. This pen provided an average of 78 cm of bunk space per cow and 17% more stalls than the number of animals in the pen. After determining that the milk was saleable, heifers were moved to a 2-row fresh pen for first-lactation animals that provided 90 cm of bunk space per cow and 28% more stalls than the number of cows in the pen. Cows were moved to a different 2-row pen that provided an average of 65 cm of bunk space per cow and the same number of stalls as cows. After approximately 11 d in either fresh pen, healthy animals were moved to a large early lactation pen that housed both lactating cows and heifers. This pen provided 58 cm of eating space per cow and 7% fewer stalls than the number of cows in the pen. Lactating cows were fed a TMR consisting of approximately 68% forage (alfalfa hay, alfalfa silage, corn silage, and wheat straw), 32% concentrate (ground shelled corn and corn gluten feed), and whey permeate with a mix containing additional protein supplements,

minerals, vitamins, and 12 g of monensin per tonne of diet DM. Early lactation cows were monitored daily for attitude, appetite, and rumen fill. Animals suspected of having ketosis were tested using a nitroprusside milk powder (KetoCheck, Great States Animal Health, Lenexa, KS). Positive animals were treated with 335 mL of oral PG for 3 or more days. A small proportion of ketotic cows with strongly positive milk ketones or additional clinical signs were also treated with 500 mL of 50% dextrose intravenously.

Farm D was a 4,100-cow Holstein herd that milked cows 3 times daily on an 80-cow rotary parlor and averaged 35.3 kg of milk per cow per day during the study period. Cows and heifers were housed together before calving in a 2-row freestall pen that provided an average of 86 cm of bunk space per cow and 15% more stalls than the number of cows in the pen. All freestalls were deeply bedded with partially dried solids from an anaerobic digester over a packed clay base. Close-up animals were fed a TMR consisting of approximately 57% forage (alfalfa silage, corn silage, grass hay, and wheat straw) and 43% concentrate (ground shelled corn, corn gluten feed, dried distillers grains, and wet brewers grains) and a mix containing minerals, supplemental anions, and vitamins. Cows and heifers were moved to one side of a straw-bedded group maternity pen when they exhibited active signs of labor. Cows were kept on the other side of the maternity pen until their milk was determined to be saleable. Both fresh cows and heifers were then moved to a 2-row post-fresh pen that provided an average of 73 cm of bunk space per cow and 8% more stalls than cows in the pen. Healthy animals remained in the pen until approximately 30 DIM. Cows in early lactation were fed a TMR consisting of approximately 67% forage (alfalfa silage and corn silage) and 33% concentrate (ground shelled corn, corn hominy feed, corn gluten feed, dried distillers grains, wet distillers grains, wet brewers grains, blood meal, barley malt sprouts, and corn starch) with a mineral and vitamin mix not supplemented with monensin. Early lactation cows were monitored daily for attitude, appetite, rumen fill, and milk weights. Animals suspected of having ketosis were tested using KetoCheck nitroprusside milk powder. Positive animals were treated with 335 mL of oral PG for 4 or more days and given a 10-mL intramuscular injection of vitamin B complex once daily until the milk ketone test was negative.