



The effects of dexamethasone administration on physiological, behavioral, and production parameters in dairy cows after a difficult calving

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ABSTRACT

Dairy cows are predisposed to diseases during the postpartum period. Dystocia has been associated with increased risk for disease, which is likely the result of increased tissue trauma and stress during the prolonged parturition. To attenuate the inflammatory response seen in dystocic animals and improve well-being, we assessed the effects of a glucocorticoid, dexamethasone administered within 12 h after calving. Dystocia was defined as a difficult birth resulting in a prolonged calving (≥ 70 min after the amniotic sac appears) and was monitored through 3 video cameras in the close-up dry-cow pen. Cows meeting the dystocia definition were randomly assigned to receive a single intramuscular injection of either dexamethasone (DEX; 0.1 mg/kg of body weight; $n = 43$) or saline (CON, $n = 44$) within 12 h following a dystocic calving. Serum haptoglobin, blood β -hydroxybutyrate (BHB) concentrations, body temperature, and several behaviors were measured for the first 7 d postpartum. Additionally, milk production and components for the first 120 d were recorded. Using a mixed model, the fixed effects of treatment, parity, calving assistance, and time, along with 2- and 3-way interactions, were analyzed with cow as a random effect. We observed that primiparous DEX cows had greater serum haptoglobin concentrations on d 3 and d 7 postpartum compared with primiparous CON cows. There was no difference between treatment groups for blood BHB concentrations and body temperature. Behavior was altered between treatments, with DEX cows having reduced activity for the first week postpartum, as well

as less restlessness and increased lying times on some of the days following calving. Treatment interacted with time for milk yield, such that DEX cows produced 2.7 kg/d less milk than CON cows for the first month following calving. The administration of dexamethasone resulted in changes in behavioral measurements, which could suggest a reduction in discomfort; however, due to the reduction in milk yield for the first month following calving, DEX administration may not be applicable for typical farm use. Additional research is needed to investigate treatments for cows experiencing dystocia without detrimental effects on milk yield.

Key words: dystocia, anti-inflammatory, well-being, haptoglobin

INTRODUCTION

Parturition is a necessary process to produce milk from dairy cattle to be used in the human food supply. Difficulty of calving can affect the dam's health and productivity (Meyer et al., 2001; Lombard et al., 2007). Dystocia is defined as a difficult or extended calving event (Mee, 2008); it predisposes cows to postpartum diseases such as retained placenta, metritis, and left displaced abomasum, and increases the risk for ketosis (Correa et al., 1993; Duffield et al., 2009). Moreover, dystocia negatively affects milk yield and fertility, while increasing veterinary costs (Dematawewa and Berger, 1997). These negative effects from dystocia may be due in part to the increased inflammatory state during the first week postpartum, as noted by greater haptoglobin concentrations (Pohl et al., 2015; Shin et al., 2018). Haptoglobin, a commonly measured acute phase protein, is used as an inflammatory marker due to its rapid increase in response to inflammation (Van Leeuwen and Van Rijswijk, 1994; Petersen et al., 2004). Indeed, elevated haptoglobin concentrations have been associated with poorer health and performance in postpartum dairy cows (Bradford et al., 2015).

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Previous studies have shown that the administration of nonsteroidal anti-inflammatory drugs (NSAID) can increase milk yield in postpartum cows (Farney et al., 2013; Carpenter et al., 2016), although this is not always the case. In fact, administration of flunixin meglumine immediately following calving increased the risk for metritis and reduced milk yield (Newby et al., 2017). In contrast, meloxicam administration increased milk yield in postpartum cows that experienced eutocia but not dystocia (Newby et al., 2013; Swartz et al., 2018). It is possible that administration of a more potent anti-inflammatory agent could enhance milk yield in dystocic cows. Because of their potent anti-inflammatory action, glucocorticoids have historically been used in the veterinary field to treat a wide variety of ailments such as ketosis and udder edema, and for induction of calving (Black, 1974). The anti-inflammatory effects of glucocorticoids are broader than those of NSAIDs, as glucocorticoids inhibit more inflammatory pathways (Becker, 2013). Dexamethasone is a synthetic glucocorticoid with one of the most potent anti-inflammatory properties, being 30 times more potent than cortisol (Dluhy et al., 1973). In addition to its anti-inflammatory effects, dexamethasone has been used as an adjunctive therapy to propylene glycol for cows with clinical ketosis, resulting in reductions in blood BHB concentrations (van der Drift et al., 2015).

Because cattle are stoic, monitoring for signs of discomfort is crucial. Numerous behavioral changes in activity and lying behaviors occur both before and after calving (Jensen, 2012). More specifically, cows experiencing a difficult calving exhibited more lying (Swartz et al., 2018) and standing bouts (Proudfoot et al., 2009) on the day of calving than cows that calved easily, likely indicating restlessness. In response to either pre- or postcalving meloxicam treatment given to dystocic cows, Swartz et al. (2018) observed decreased locomotion in meloxicam-treated animals compared with untreated control animals. Therefore, the use of these behaviors may have value in assessing treatment efficacy from glucocorticoid therapy in postpartum dairy cows.

The objective of this study was to determine whether dexamethasone is an appropriate treatment for cows experiencing a difficult calving. Specifically, our objectives were to determine whether dexamethasone treatment attenuates inflammation, alters lying behaviors and activity, and enhances lactational performance in postpartum cows following a difficult calving. We hypothesized that dexamethasone administration to postpartum dystocic animals would increase milk yield and decrease serum haptoglobin and blood BHB concentrations, and decrease the postpartum restlessness behavior exhibited by dystocic cows.

MATERIALS AND METHODS

Animals, Housing, and Management

All experimental procedures were approved by the Virginia Tech (VT) Institutional Animal Care and Use Committee (#19-100). A total of 264 dairy cows at the VT Dairy Center (Blacksburg) were evaluated for the study between November 2019 and March 2021, beginning 10 d before their expected calving. Cows were only eligible for study enrollment once. Starting approximately 21 d before expected calving and lasting until 21 d following parturition, dry cows and pregnant heifers were housed in a bedded pack that was aerated daily following standard herd management protocols. Group size varied but space was maintained at approximately 9.3 m² (100 ft²) per cow. After calving, cows were moved into a separate pen that was also a compost-bedded pack with similar management. At approximately 21 DIM, lactating cows were moved to a freestall barn, which was bedded with sand. Cows were fed a TMR daily at 0830 h. Cows were milked twice a day in a double-12 parallel parlor at 0100 and 1300 h.

Determination of Dystocia

Close-up dry cows and pregnant heifers were monitored 24 h/d using 3 video cameras (Axis P1353-E, Axis Communications AB) to allow sight of all areas of the pen. The videos were saved in 3-h intervals on a computer connected through an Ethernet cord (Media Recorder, Noldus Information Technology Inc.). Videos were examined for each calving to determine the occurrence of dystocia by examining the amount of time that elapsed between amniotic sac appearance at vulva and calf expulsion. Dystocia was defined as a difficult birth resulting in a prolonged calving (≥ 70 min after the amniotic sac appears; Schuenemann et al., 2011; Funnell and Hilton, 2016), assisted calving (any assistance), or twin births. Calving assistance was performed only by farm staff who were blinded to treatments. Calving ease scoring was recorded by the farm staff using a 1 to 5 scale (1 = unassisted, 2 = slight problem, 3 = needed assistance, 4 = considerable force, and 5 = extreme difficulty).

Design and Treatments

Ten days before expected calving date, animals began study evaluation, and blood samples were collected. Within 12 h of calving, dystocia was determined using the aforementioned definition. Dystocic cows were randomly assigned to 1 of 2 treatments using a random number generator. Using milk yield as our primary out-

come, a power analysis was conducted using $\alpha = 0.05$, $\beta = 0.80$, SD of 12 kg, and a 15% expected difference in peak milk yield (60 to 90 DIM); based on this analysis, 42 cows should be allotted per treatment group. Body weights were obtained from an automated scale (AfiWeigh, Afimilk Ltd.) located outside the parlor. The most recent BW values obtained at dry-off were then used to determine the approximate dexamethasone dosage (**DEX**; 0.1 mg/kg of BW, administered intramuscularly in the neck; Vet One, Sparhawk Laboratories) with an equal volume of saline used for control treatment (**CON**). Administration was performed in headlock restraint.

Health and Inflammatory Markers

Body condition scores (Wildman et al., 1982) ranging from 1 to 5 were recorded on the day of calving. Rectal temperatures were obtained each morning for the first 7 d after calving to monitor health using an automated thermometer (Dual Scale Digital Thermometer, Vet One). Blood samples were taken on d -10, 0, 1, 3, and 7 relative to calving through coccygeal venipuncture with a 10-mL evacuated tube coated with silicon (Monoject Blood Collection Tube, Covidien). Whole-blood BHB concentrations were determined on d 0, 3, and 7 using a previously validated BHB meter (Precision Xtra, Abbott Diabetes Care Inc.; Iwersen et al., 2009) and ketone test strip (Blood β -Ketone Test Strip, Abbott Diabetes Care Inc.) for each blood sample. Results (mmol/L) were recorded. Whole blood samples were transported immediately to the laboratory, where serum was collected after centrifugation ($2,000 \times g$ for 15 min at 4°C), transferred to 1-mL Eppendorf tubes, and stored at -20°C until further use. Haptoglobin was analyzed on d 0, 1, 3, and 7 using an ELISA (cat. no. HAPT-11, Life Diagnostics Inc.). Serum samples were thawed and serially diluted (1:10, 1:100, 1:1,000, 1:2,000, and up to 1:8,000). The diluted samples and standards were pipetted (100 μ L) in duplicate into the precoated bovine antibody plate provided with the kit and placed in a shaking incubator (45 min, 25°C, 150 rpm). Then, the plate was washed 5 times with the provided wash buffer. Horseradish peroxidase (HRP) conjugate was added (100 μ L) and the plate returned to the shaking incubator (45 min, 25°C, 150 rpm). The plate was washed 5 times and 3,3',5,5'-tetramethylbenzidine (TMB; 100 μ L) was added to each well and placed in a shaking incubator (20 min, 25°C, 150 rpm), at which point the reaction was halted using the stop solution (100 μ L). The resulting color reaction was read immediately (450 nm; μ Quant Universal Microplate Spectrophotometer, Bio-Tek Instruments Inc.). Samples were rerun if the intra-assay coefficient of variation was >10%.

Behavior

Cows were brought to the close-up dry pen by farm staff approximately 21 d before expected calving and fitted with a previously validated accelerometer (AfiTag II, Afimilk Ltd.; Borchers et al., 2016; Swartz et al., 2016), allowing activity (average daily steps/h), lying time (min/d), and rest per bout (average number of minutes for duration of a lying bout) to be measured continuously. The restlessness ratio was calculated using a proprietary equation (AfiMilk) involving lying time, activity, and the number of lying bouts as a measure of cow restlessness. These movements were transmitted to the farm office computer and were accessible using the farm management software (AfiAct II, Afimilk Ltd.). Behavior measurements were recorded for d 0 (calving) to 7 after parturition. Daily steps were calculated (steps/h $\times$ 24) and used in subsequent analyses.

Sampling

Quarter milk samples were collected from a single milking on d 1, 7, 14, 21, 35, 49, 63, 77, 91, 105, and 119 relative to calving in sterile 15-mL tubes (VWR Centrifuge Tube, VWR). These samples were transported to the laboratory where a composite sample was created by combining 2 mL from each quarter. Somatic cell count was determined on each composite milk sample with a DeLaval cell counter (DeLaval). Somatic cell count was transformed into SCS using the formula from Ali and Shook (1980): $SCS = \log_2 (SCC/100,000) + 3$.

Daily milk weights and components were obtained at each milking in the parlor starting 3 d after calving until 120 d postpartum. Daily milk weights were averaged by month, where mo 1 was the average milk weight from d 3 through 30, mo 2 averaged d 31 through 60, mo 3 averaged d 61 through 90, and mo 4 averaged d 91 through 120. Components were analyzed at each milking and averaged for daily percentage. Energy-corrected milk was calculated according to the formula described by (Orth, 1992). The formula used to calculate ECM was as follows:

$$ECM = (0.327 \times \text{milk kg}) + (12.95 \times \text{fat kg}) \\ + (7.65 \times \text{protein kg}).$$

Statistical Analyses

All data were compiled in Excel (Excel 2013 for Windows, Microsoft Corp.) and imported into SAS (SAS 9.4, SAS Institute Inc.). Data were analyzed using mixed models (PROC GLIMMIX) with fixed ef-

Table 1. Descriptive statistics (mean $\pm$ SD, where applicable); treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX) and a saline control (CON)

Treatment	Enrolled (no.)	Finished study (no.)	Parity	BCS at calving	BW (kg)	Assistance given (%)	Calving assistance score	Calving length (min)
DEX	43	41	2.0 $\pm$ 1.4	3.3 $\pm$ 0.2	693 $\pm$ 99	30.2	1.9 $\pm$ 1.2	92 $\pm$ 46
Primiparous	23	22	—	3.4 $\pm$ 0.2	627 $\pm$ 57	34.8	2.0 $\pm$ 1.2	97 $\pm$ 47
Multiparous	20	19	3.3 $\pm$ 1.2	3.3 $\pm$ 0.3	768 $\pm$ 80	25	1.8 $\pm$ 1.3	85 $\pm$ 46
CON	44	42	1.8 $\pm$ 1.2	3.4 $\pm$ 0.2	682 $\pm$ 121	45.4	1.8 $\pm$ 1.2	84 $\pm$ 41
Primiparous	28	28	—	3.4 $\pm$ 0.2	618 $\pm$ 76	46.4	1.6 $\pm$ 1.1	85 $\pm$ 48
Multiparous	16	14	3.1 $\pm$ 1.1	3.4 $\pm$ 0.2	794 $\pm$ 101	43.8	1.9 $\pm$ 1.3	81 $\pm$ 24

fects of treatment (DEX or CON), parity (primiparous or multiparous), calving assistance (unassisted or assisted), and time (day relative to calving), as well as all biologically plausible 2- and 3-way interactions. Time was used as a repeated measure with the random effect of cow. The first-order autoregressive error structure was used unless sampling time points were uneven, in which case the spatial power error structure was used instead. Data collected just before treatment application were tested as a covariate in their respective models. For milk yield, milk component yields, and ECM, covariates included BCS at calving, genetic traits (PTA for milk, for fat, and for protein in their respective models), and the season (winter, spring, summer, fall) in which the cow calved. For haptoglobin and BHB concentrations, BCS recorded on the day of calving was tested as a covariate. Additionally, serum haptoglobin and BHB concentrations from blood samples collected prior to treatment administration (d 0) were used as covariates in their respective models. Stepwise backward elimination, from highest to lowest significance, was used to remove nonsignificant terms from the model; however, treatment and time were forced into the model. If there was a statistically significant interaction, treatment means were separated using the SLICE option. Residuals were assessed for normality and outliers (PROC UNIVARIATE). Rest bout duration, restlessness ratio, and blood BHB concentrations were not normally distributed and consequently the natural logarithmic transformation was used. If an observation had a studentized residual greater than an absolute value of 4, the data point was removed. Statistical significance was declared at $P \leq 0.05$. Least squares means (**LSM**) $\pm$ standard errors are provided in the text; figures illustrate LSM $\pm$ 95% CI.

RESULTS

Descriptive Results

Throughout the study period, a total of 264 dairy cows were monitored starting 10 d before expected calving. Of those, 146 cows experienced eutocic calv-

ings. Due to technological issues including camera or computer crashes, calving duration could not be assessed for 31 cows. A total of 87 animals (DEX, $n = 43$; CON, $n = 44$) that experienced dystocia were enrolled into the study. Four cows did not complete the study, with 2 being euthanized before study completion and 2 being externally removed; none of these cases were study related. As a result, sample sizes at the conclusion of the study included 41 cows for DEX and 42 cows for CON. General descriptive statistics are shown in Table 1.

Physiological Parameters

For serum haptoglobin, there was a 3-way interaction between treatment, time, and parity ($P = 0.02$, Figure 1). Although there was no difference in haptoglobin concentration between treatment groups for primiparous cows on d 1 ($P = 0.40$), haptoglobin concentration was greater in primiparous DEX cows on d 3 (DEX vs. CON: 710.2 $\pm$ 55.4 vs. 203 $\pm$ 51 μ g/mL; $P < 0.001$) and on d 7 (DEX vs. CON: 395.1 $\pm$ 55.4 vs. 214.9 $\pm$ 51 μ g/mL; $P = 0.02$; Figure 1A) compared with primiparous CON cows. No difference in serum haptoglobin concentration was seen on any of the days for multiparous cows (Figure 1B).

Blood BHB concentrations required logarithmic transformation to achieve normality. Although there was a treatment $\times$ time interaction for blood BHB concentrations ($P = 0.03$), there was no detected difference between treatment groups on either d 3 [DEX vs. CON: -0.39 ± 0.07 vs. -0.25 ± 0.07 BHB ln(mmol/L); back-transformed LSM (95% CI), DEX vs. CON: 0.68 (0.63, 0.72) vs. 0.78 (0.73, 0.83) mmol/L; $P = 0.13$] or d 7 [DEX vs. CON: -0.22 ± 0.07 vs. -0.32 ± 0.07 BHB ln(mmol/L); back-transformed LSM (95% CI), DEX vs. CON: 0.80 (0.75, 0.86) vs. 0.73 (0.68, 0.78) mmol/L; $P = 0.29$]. Multiparous cows had greater blood BHB concentrations than primiparous cows [primiparous vs. multiparous: -0.46 ± 0.049 vs. -0.13 ± 0.06 ln(mmol/L); back-transformed LSM (95% CI), primiparous vs. multiparous: 0.63 (0.60, 0.67) vs. 0.88 (0.83, 0.93) mmol/L; $P < 0.001$]. Body temperature

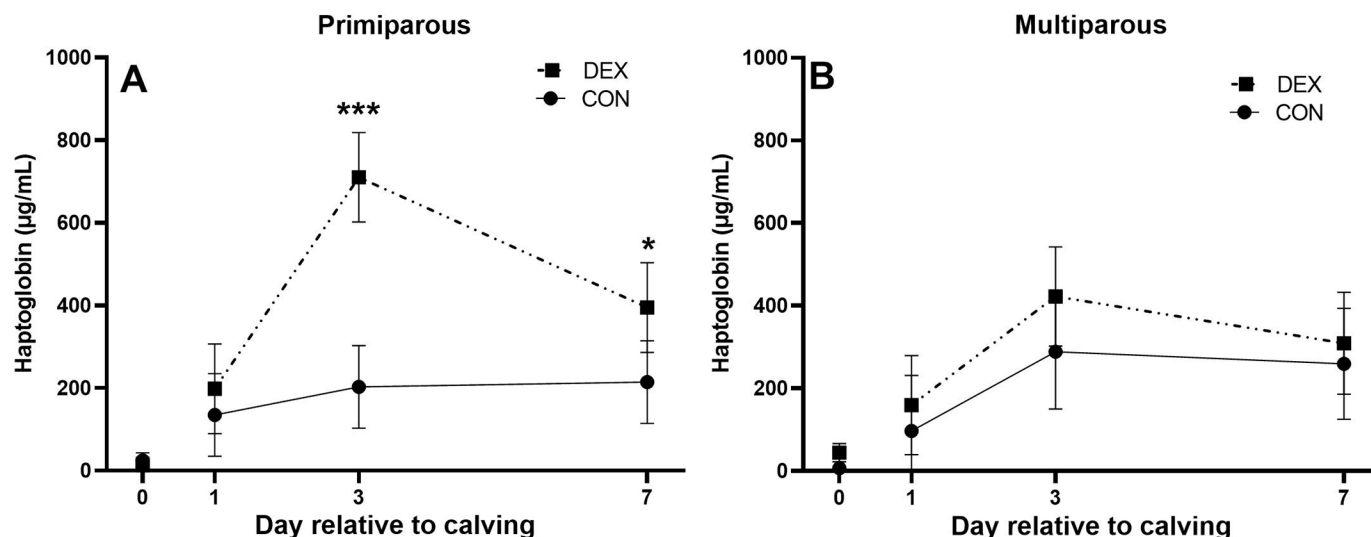


Figure 1. Treatment least squares means (95% CI) by parity (primiparous, A; multiparous, B) for serum haptoglobin concentrations ($\mu\text{g/mL}$) from blood samples collected on d 0, 1, 3, and 7 following calving. There was an interaction between treatment, parity, and time ($P = 0.02$). The blood sample from d 0 was collected before treatment administration and was used as a covariate in the statistical analysis. Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX; primiparous, $n = 23$; multiparous, $n = 19$) and a saline control (CON; primiparous, $n = 27$; multiparous, $n = 15$). Differences between treatments: * $P \leq 0.05$, *** $P < 0.001$.

for the first 7 d after calving was not different between treatment groups (DEX vs CON: 38.5 ± 0.04 vs. $38.5 \pm 0.04^\circ\text{C}$; $P = 0.64$) but was affected by parity (primiparous vs. multiparous: 38.6 ± 0.04 vs. $38.4 \pm 0.04^\circ\text{C}$; $P = 0.05$).

Behavioral Results

The probability level for the main effects and interactions for all behavioral data (activity, lying time, rest bout duration, and restlessness ratio) are provided in Table 2. Restlessness ratio and rest bout duration required logarithmic transformation to achieve normality.

Reduced activity was observed for DEX compared with CON in the first week postpartum (DEX vs. CON: $3,847 \pm 146$ vs. $4,315 \pm 145$ steps/d; $P = 0.02$; Figure 2). For lying time, a 3-way interaction between treatment, parity, and time was observed ($P = 0.05$;

Figure 3). Primiparous DEX showed greater lying time than primiparous CON on d 1 (DEX vs. CON: 698 ± 33 vs. 477 ± 29 min/d; $P < 0.001$) and d 2 (DEX vs. CON: 658 ± 33 vs. 428 ± 28 min/d; $P < 0.001$), and no difference was found on d 3 (DEX vs. CON: 520 ± 32 vs. 450 ± 27 min/d; $P = 0.10$; Figure 3A). For multiparous cows, dexamethasone treatment increased time spent lying compared with control animals on d 2 relative to calving (DEX vs. CON: 740 ± 35 vs. 573 ± 41 min/d; $P < 0.01$; Figure 3B).

A 3-way interaction was seen for rest bout duration between treatment, time, and parity ($P = 0.02$; back-transformed LSM $\pm$ 95% CI are provided in Figure 4). Primiparous DEX cows showed reduced rest bout duration on d 0 [DEX vs. CON: 3.2 ± 0.10 vs. 3.5 ± 0.10 ln(rest bout duration); $P < 0.01$], d 2 [DEX vs. CON: 3.7 ± 0.10 vs. 3.9 ± 0.10 ln(rest bout duration); $P = 0.02$], and d 3 [DEX vs. CON: 3.6 ± 0.10 vs. $3.9 \pm$

Table 2. Probability level for the main effects and interaction terms for activity (steps/d), lying time (min/d), rest bout duration (min/bout), and restlessness ratio measured from d 0 until d 7 after calving with cows receiving dexamethasone (DEX, $n = 37$) or a saline control (CON, $n = 40$) after dystocia

Fixed effect	Activity	Lying time	Rest bout duration	Restlessness ratio
Treatment	0.02	<0.01	0.41	<0.001
Parity	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.01	<0.001	<0.001
Treatment $\times$ parity	—	0.78	0.05	0.88
Treatment $\times$ time	—	<0.001	0.19	0.07
Parity $\times$ time	—	<0.01	<0.01	0.15
Treatment $\times$ parity $\times$ time	—	0.05	0.02	0.03

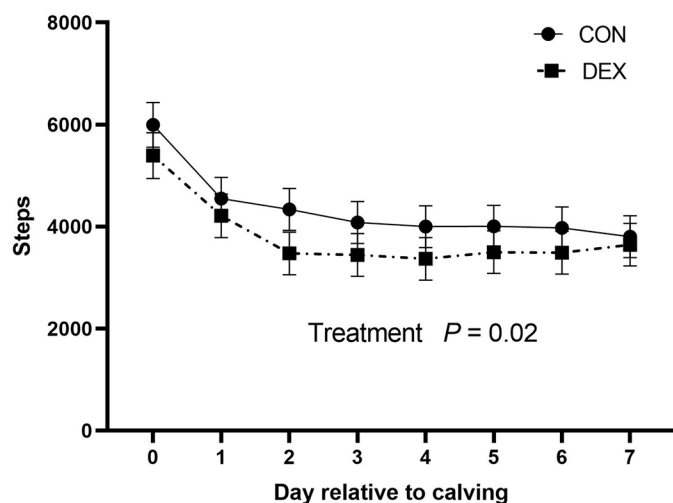


Figure 2. Treatment least squares means (95% CI) for activity (steps/d) measured from calving (d 0) until 7 d after calving. Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX, $n = 37$) and a saline control (CON, $n = 40$).

0.10 ln(rest bout duration); $P < 0.01$] compared with primiparous CON cows (Figure 4A). However, multiparous DEX cows had increased rest bout duration on d 0 compared with multiparous CON cows [DEX vs. CON: 3.4 ± 0.10 vs. 3.0 ± 0.10 ln(rest time/bout); $P < 0.01$; Figure 4B].

For restlessness ratio, a 3-way interaction was observed between treatment, parity, and time ($P = 0.03$; back-transformed LSM $\pm$ 95% CI are provided in Figure 5). In primiparous cows (Figure 5A), the restlessness ratio was greater for CON on d 1 [DEX vs. CON: 1.0 ± 0.10 vs. 1.5 ± 0.10 ln(restlessness ratio); $P < 0.001$] and d 2 [DEX vs. CON: 1.0 ± 0.10 vs. 1.4 ± 0.10 ln(restlessness ratio); $P < 0.001$], whereas no difference was found on d 3 [DEX vs. CON: 1.2 ± 0.10 vs. 1.4 ± 0.10 ln(restlessness ratio); $P = 0.10$] compared with DEX. In multiparous cows (Figure 5B), the restlessness ratio was greater for CON than DEX on d 0 [DEX vs. CON: 1.1 ± 0.10 vs. 1.6 ± 0.20 ln(restlessness ratio); $P = 0.02$] and d 2 [DEX vs. CON: 0.6 ± 0.1 vs. 1.0 ± 0.10 ln(restlessness ratio); $P = 0.03$].

Milk Production and Components

The probability level for the main effects and interactions for all milk yield and components data are provided in Table 3. Treatment interacted with time for monthly milk yield ($P = 0.05$; Figure 6). In the first month of lactation, cows in the DEX group produced 2.7 kg/d less milk than cows in CON ($P = 0.05$). No differences for milk yield were observed between treatment groups for mo 2, 3, or 4. No treatment effects

were found for milk protein yield (DEX vs. CON: 1.5 ± 0.03 vs. 1.5 ± 0.03 kg/d; $P = 0.34$), fat yield (DEX vs. CON: 1.7 ± 0.04 vs. 1.8 ± 0.04 kg/d; $P = 0.21$), ECM yield (DEX vs. CON: 47.3 ± 0.9 vs. 48.9 ± 0.09 kg/d; $P = 0.25$), or SCS (DEX vs. CON: 3.8 ± 0.2 vs. 3.6 ± 0.1 ; $P = 0.40$) recorded for 4 mo following calving.

DISCUSSION

Dystocia is associated with an increase in inflammatory markers during the postpartum period (Pohl et al., 2015; Shin et al., 2018). Despite that, very little research has been conducted assessing treatments specifically for cows experiencing difficult births. Moreover, research conducted thus far in dystocic cows has typically not yielded substantial improvements in well-being or performance. Indeed, previous work assessing meloxicam use during the peripartum period found an increase in milk yield in eutocic cows (Swartz et al., 2018); however, these effects were not seen in cows experiencing dystocia (Newby et al., 2013; Swartz et al., 2018). As a result, our objective was to assess the effects of a more potent anti-inflammatory agent, dexamethasone, on the inflammatory response following a difficult calving to improve well-being and performance in early lactation dairy cows. Here, we report that the administration of dexamethasone to cows experiencing a difficult calving increased serum haptoglobin concentrations in primiparous cows, altered activity and lying behaviors, and reduced milk yield for the first month following calving regardless of parity.

Physiological markers are commonly measured during the postpartum period to monitor the onset of disease. Haptoglobin is an emerging marker in veterinary diagnostics as a nonspecific indicator of inflammation (Petersen et al., 2004). In the current study, DEX increased the serum haptoglobin concentration in primiparous cows on d 3 and d 7. Our results for primiparous cows contradict the results of Pascottini et al. (2020), who found a decrease in haptoglobin due to meloxicam treatment regardless of parity. However, fundamental differences in study design may have led to these conflicting results. In the current study, dexamethasone treatment was administered within 12 h of dystocic calvings, whereas Pascottini et al. (2020) administered NSAID treatment 10 to 13 d postpartum following unassisted calvings, which could explain the differences in results. Moreover, it should be noted that haptoglobin secretion can be induced by the administration of dexamethasone (Yoshino et al., 1993; Baumann et al., 1989); however, why this occurred in primiparous cows but not multiparous cows is unusual and may be related to parity differences in hepatocyte responsiveness to glucocorticoids. In support of

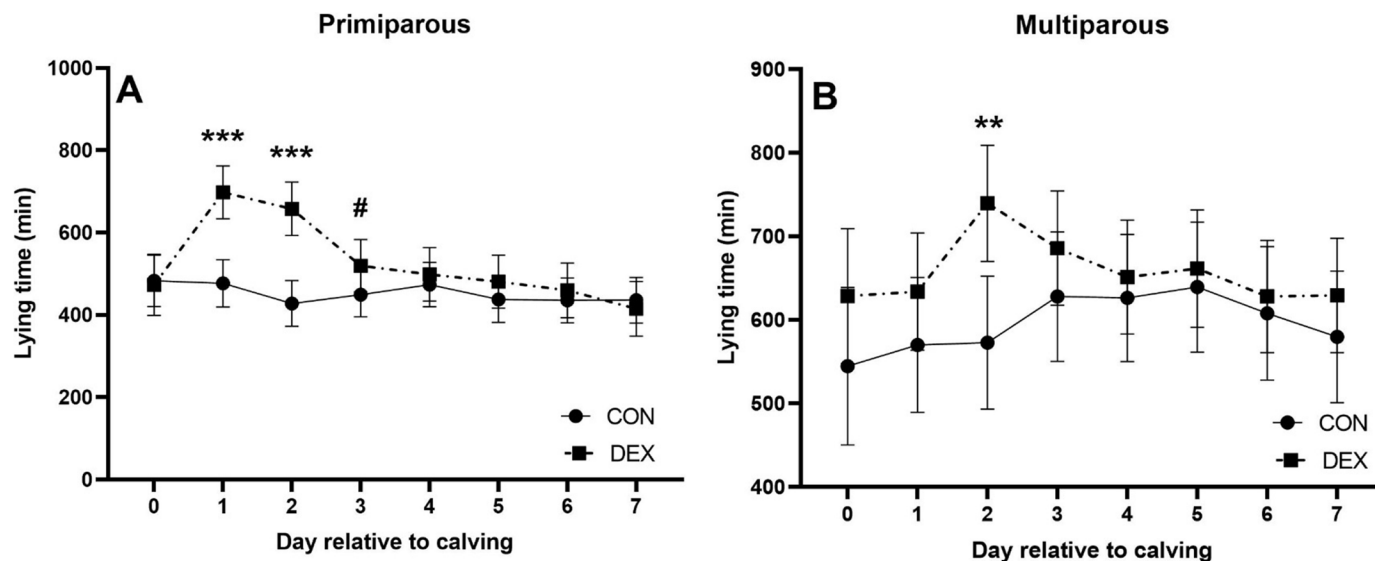


Figure 3. Treatment least squares means (95% CI) by parity (primiparous, A; multiparous, B) for lying time (min/d) recorded from calving (d 0) until 7 d following calving. There was an interaction between treatment, parity, and time ($P = 0.05$). Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX; primiparous, $n = 20$; multiparous, $n = 17$) and a saline control (CON; primiparous, $n = 27$; multiparous, $n = 13$). Differences between treatments: # $P < 0.10$, ** $P \leq 0.01$, *** $P < 0.001$.

this, other studies have found greater concentrations of haptoglobin in postpartum primiparous cows compared with multiparous cows (Crawford et al., 2005; Macmillan et al., 2020). As such, we speculate that the hepatocytes in the liver of primiparous cows are more responsive to glucocorticoids than those in multiparous cows. Nevertheless, the effect of increasing haptoglobin

concentrations because of dexamethasone administration to dystocic primiparous cows is still debatable, as the direct effects of this acute phase protein have not been elucidated in dairy cows.

Reducing inflammation can influence postpartum cow behavior, as seen in several studies (Newby et al., 2013; Mainau et al., 2014; Swartz et al., 2018). In

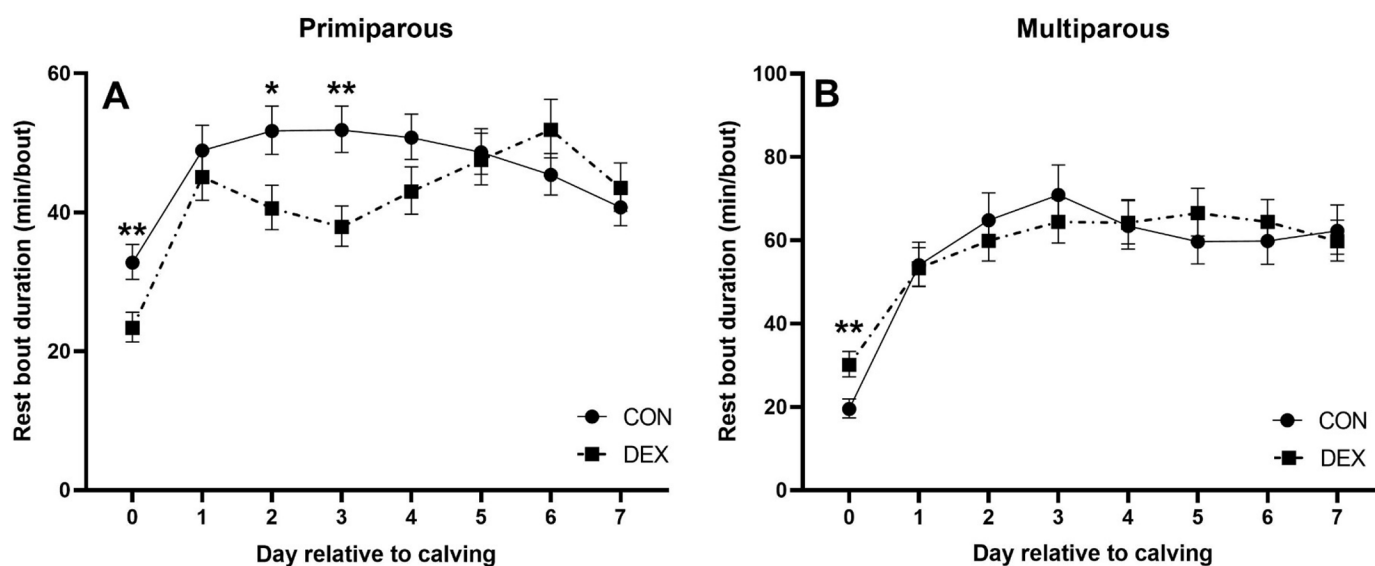


Figure 4. Back-transformed treatment least squares means (95% CI) by parity (primiparous, A; multiparous, B) for rest bout duration (min/bout) recorded from calving (d 0) through the first 7 d after calving. There was an interaction between treatment, parity, and time ($P = 0.02$). Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX; primiparous, $n = 20$; multiparous, $n = 17$) and a saline control (CON; primiparous, $n = 27$; multiparous, $n = 13$). Differences between treatments: * $P \leq 0.05$, ** $P \leq 0.01$.

Table 3. Probability level for the main effects and interaction terms for yields of milk, protein, fat, and ECM, and SCS measured from 3 d until 120 d after calving (averaged by month) with cows receiving dexamethasone (DEX, $n = 40$) or a saline control (CON, $n = 44$) after dystocia

Fixed effect	Milk yield	Milk protein yield	Milk fat yield	ECM yield	SCS
Treatment	0.45	0.34	0.21	0.25	0.40
Parity	<0.001	<0.001	<0.001	<0.001	—
Time	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment $\times$ parity	—	—	—	—	—
Treatment $\times$ time	0.05	—	—	—	—
Parity $\times$ time	<0.001	<0.001	<0.001	<0.001	—
Treatment $\times$ parity $\times$ time	—	—	—	—	—

the 48 h after parturition, the time spent in lateral recumbency was greater for animals who experienced an assisted parturition compared with animals with unassisted parturitions (Gladden et al., 2021). In the current study, dystocic cows treated with dexamethasone showed reduced activity for the first week postpartum and increased lying time (d 1 and 2 for primiparous, and d 2 for multiparous). The reduction in activity is similar to the results of Swartz et al. (2018), in which meloxicam administration (either before or after calving) reduced activity in dystocic cows. The greater lying time seen in the current study potentially contrasts with the reduced time spent in lateral recumbency seen in Gladden et al. (2021), in which ketoprofen was administered 3 h after parturition. However, in the current study, we did not measure lying positions (sternal vs. lateral recumbency) as was done by Gladden et al. (2021), thus the contrast may not necessarily be accu-

rate. As such, we speculate that the greater lying times, in addition to reductions in activity in DEX cows, could indicate that dexamethasone administration following a difficult calving alleviated discomfort and therefore allowed cows to rest more comfortably; however, future studies specifically assessing lying positions are needed to provide clarity.

Primiparous DEX cows spent less time resting per bout (on d 0, 2, and 3) compared with primiparous CON cows in the current study. However, the opposite was found on the day of calving (d 0) for multiparous cows. Moreover, DEX cows had a lower restlessness ratio than CON cows on d 1 and 2 for primiparous cows and d 0 and 2 for multiparous cows. Restlessness, an alternation between lying and standing, can be used in classifying cow discomfort or pain (Barrier et al., 2012). The restlessness ratio, however, accounts for lying behaviors as well as activity. As such, a decrease in

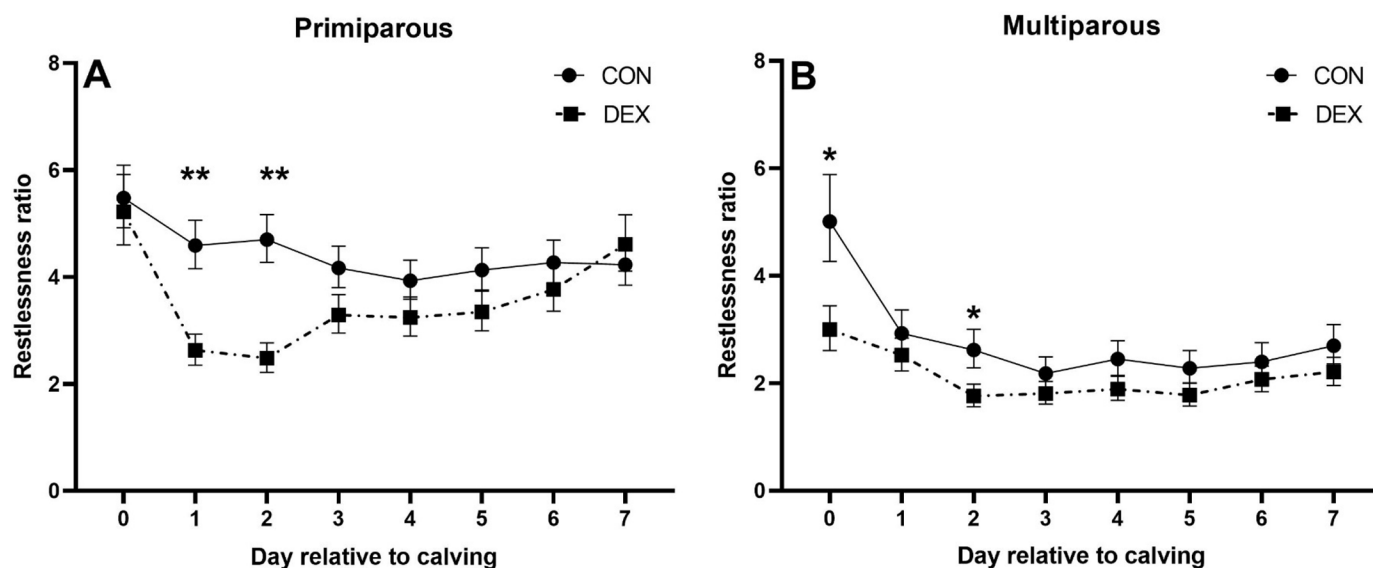


Figure 5. Back-transformed treatment least squares means (95% CI) by parity (primiparous, A; multiparous, B) for restlessness ratio recorded from calving (d 0) through the first 7 d after calving. There was an interaction between treatment, parity, and time ($P = 0.03$). Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX; primiparous, $n = 20$; multiparous, $n = 17$) and a saline control (CON; primiparous, $n = 27$; multiparous, $n = 13$). Differences between treatments: * $P \leq 0.05$, ** $P \leq 0.01$.

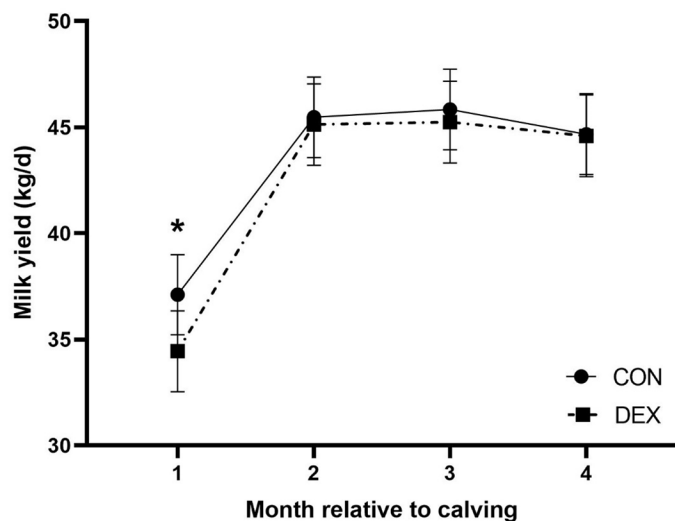


Figure 6. Treatment least squares means (95% CI) for milk yield (kg/d) recorded for the first 4 mo following calving. There was an interaction between treatment and time ($P = 0.05$). Treatments were dexamethasone (0.1 mg/kg of BW intramuscularly) after calving (DEX, $n = 40$) and a saline control (CON, $n = 44$). Differences between treatments: $*P \leq 0.05$.

rest per bout along with an increase in lying time would mean that primiparous DEX cows transitioned from lying to standing more often than CON cows. This could indicate greater restlessness from dexamethasone administration in primiparous cows; however, dexamethasone administration reduced activity levels (regardless of parity) and, likely as a result, the restlessness ratio was also reduced. Speculation aside, these data, although difficult to interpret, could be indicative of the DEX cows experiencing some alleviation of discomfort following dystocia compared with CON cows.

Previous research has investigated the effects of NSAID on milk yield. Swartz et al. (2018) observed an increase in milk yield and components with meloxicam treatment given either between 6 and 48 h before calving or within 6 h after calving, in cows that experienced eutocia. However, administration of meloxicam (within 25 h) after a difficult parturition yielded no difference in milk production (Newby et al., 2013; Swartz et al., 2018). Due to multiple studies exhibiting similar results for NSAID administration, we hypothesized that a more potent anti-inflammatory agent could improve milk yields in dystocic cows. Conversely, we found a reduction in milk yield for the first month in the current study. In past studies, dexamethasone treatment reduced milk yield in mid- to late-lactation cows (Shamay et al., 2000; Ponchon et al., 2017) for 2 (Ponchon et al., 2017) to 5 d (Shamay et al., 2000) following administration. The dose of dexamethasone in the aforementioned studies was 40 mg (Shamay et al., 2000)

and 20 mg (Ponchon et al., 2017) of dexamethasone intramuscularly, whereas the dose of dexamethasone in the current study (0.1 mg/kg) was chosen based on consultation with the attending herd veterinarian at the Virginia-Maryland College of Veterinary Medicine as a therapeutic, not immunosuppressive, dose. This resulted in a dose ranging from 42 to 96.8 mg. However, the recommended dexamethasone label dose is a range of 5 to 20 mg. Taken together, differences in stage of lactation and dose could have contributed to the differences observed in milk loss duration.

The current study did not evaluate the cause of milk loss. However, previous studies have examined the role of glucocorticoids in prolactin inhibition in healthy cows. Two experiments by Ponchon et al. (2017) used dexamethasone and observed reduced prolactin concentrations along with reduced milk yield in treated cows. Subsequently, Ponchon et al. (2017) deduced that prolactin inhibition may be a mechanism by which glucocorticoids affect milk yield. Another glucocorticoid mechanism potentially detrimental to production may be the effect of induced hyperglycemia potentially shunting glucose away from the mammary gland (Herdt and Emery, 1992). Hartmann and Kronfeld (1973) examined mammary flow with uptake of glucose after dexamethasone treatment and noted reduced glucose utilization. Thus, the effect of dexamethasone administration on milk yield is likely multifactorial.

We acknowledge a few limitations of the present study. First, although treatments were administered within 12 h following calving, we did not specifically measure the amount of time elapsed from calving until treatment was administered. Future studies should consider this because the amount of time between calving and treatment administration could affect treatment efficacy. Second, we identified interactions of treatment with parity for 4 of our outcomes. As such, future studies should consider parity when conducting a power analysis to determine the appropriate sample size. Last, we acknowledge that the equation to calculate the restlessness ratio is not publicly available and, to the best of our knowledge, has not been externally validated.

CONCLUSIONS

Dexamethasone treatment increased serum haptoglobin concentrations in primiparous dystocic cows. Moreover, dexamethasone treatment reduced activity for the first week postcalving. Because of the negative effects of dexamethasone on milk yield for the first month following calving, this treatment may not be advised for widespread farm application. Future studies assessing treatments for dystocic cows without detrimental effect on milk yield are still needed.

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