Evaluation of oral meloxicam in beef calves with neonatal calf diarrhea

by

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Abstract

Objective

To examine behavioral and physiologic outcomes associated with the expression of pain in calves with naturally occurring neonatal calf diarrhea (NCD) and determine the impact of meloxicam administration on reducing these pain-related behaviors and outcomes.

Methods

Beef calves 3 to 30 days old with NCD and no comorbidities or prior NSAID or steroid administration were enrolled as clinical cases, and healthy beef calves were enrolled as controls. Clinical cases received either meloxicam (MEL group) or placebo (PLBO group) treatment; all controls received placebo. Over 7 time points, outcomes measured were substance P, prostaglandin E metabolite, mechanical nociceptive threshold, gait analysis, behavior analysis, physical examinations, and intake bloodwork.

Results

20 calves with NCD and 8 healthy calves were enrolled. Prostaglandin E metabolite was significantly higher in calves with NCD than controls. Control calves were more reactive to pressure over the hip than calves with NCD, particularly those treated with meloxicam. Calves with NCD had shorter stride length and slower gait velocity compared to controls. Calves with NCD spent more time lying and more time lying with their head up compared to controls. Calves in the MEL group spent more time lying with their head down compared to PLBO calves.

Conclusions

Differences in mechanical nociceptive threshold, gait analysis, and lying behavior possibly indicated the presence of abdominal pain. Differences in head posture between MEL and PLBO calves suggest that calves treated with meloxicam may have been more comfortable.

Clinical Relevance

Calves with NCD may experience pain, and treating them with meloxicam may relieve that pain.

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Introduction

Neonatal calf diarrhea (NCD) is a significant cause of morbidity and mortality of beef calves. According to data from the National Animal Health Monitoring System, morbidity in unweaned beef calves in the US is about 19% and mortality can be as high as 14% of all deaths in calves < 3 weeks of age.[1] Common etiologic agents responsible for NCD include rotavirus, coronavirus, *Escherichia coli*, Salmonella, Cryptosporidium, and Clostridium.

Diarrhea has been associated with abdominal pain in other species. Abdominal pain in dogs with infectious diarrhea, like that caused by rotavirus and coronavirus, is characterized by changes in posture or gait, anorexia, tachycardia, depression, restlessness, and sensitivity on abdominal palpation.[2] Abdominal pain is also a consistent symptom in human children and adults with rotavirus,[3,4] coronavirus,[5] Salmonella,[6,7] and Cryptosporidium.[8,9] Knowledge regarding the effect of pain management in calves with NCD is limited, with only 1 study[10] reporting that meloxicam was an effective adjunctive therapy for NCD on the basis of improved milk consumption and rate of gain in male Holstein calves raised in a research facility.

Pain has been objectively measured in calves undergoing surgical procedures such as castrations[11–13] and dehorning[14–16] and in experimentally induced bovine respiratory disease.[17] Parameters commonly utilized for pain assessment include biochemical values like cortisol, substance P (SP), and prostaglandin E metabolite (PGEM); mechanical measures like gait analysis, thermography, and mechanical nociceptive threshold; and behavioral metrics using novel or established behavior scoring systems[18,19] and approach scores.[20] These methods have all been validated for quantifying pain in calves and have been used to evaluate the effect of NSAIDs on pain response.

The objectives of this study were to examine and characterize behavioral and physiologic outcomes associated with the expression of pain in calves with naturally occurring NCD and to determine the impact of meloxicam administration on reducing these pain-related behaviors and outcomes. We hypothesized that calves experience pain associated with NCD and that meloxicam would be an effective treatment to alleviate this pain.

Methodology

Study procedures were approved by Kansas State University's IACUC (protocol No. 4763). A total of 28 calves were enrolled with client consent.

Clinical calf intake and treatment assignment

Twenty calves between the ages of 3 and 30 days old presented to the Kansas State University Health Center for management of NCD. To be enrolled, calves had to have had no prior NSAID or steroid administration, required IV fluid resuscitation, and were free of concurrent disease. Calves were housed individually in 4 X 7-foot calf stalls. If the dam was brought in with the calf, the dam was housed separately in a 12 X 12-foot stall out of sight of the calf. Grass hay and free-choice water were provided to all animals, and calves were either allowed to nurse milk replacer (Country Lane; Canadian Nurs-ette Distributors LLC) from a bottle or brought to nurse on their accompanying dam 2 to 4 times a day. Calves were on study for 3 days; all calves returned to normal metabolic status prior to discharge.

Calves with NCD were randomly assigned to 2 treatment groups with use of the RAND function in Excel (Microsoft Corp). Ten calves received meloxicam (MEL group), and 10 calves received placebo (PLBO group). Calves in the MEL group received meloxicam (7.5-mg tablets) at 1 mg/kg PO once a day for 2 days. Calves in the PLBO group received lactose monohydrate powder (lactose powder; Medisca) PO once a day for 2 days. Opaque capsules were used by the Kansas State University Veterinary Health Center pharmacy to create treatment boluses with either meloxicam tablets or lactose monohydrate powder. All study personnel were blinded to treatment groups during data collection and outcome analysis.

A 3-inch, 16-gauge polyurethane catheter (MILA International) was sterilely placed in the jugular vein to be used for treatment of NCD and study blood draws for MEL and PLBO calves.

Control calves

Eight beef calves aged 8 to 9 days old (median, 8 days) with no prior history of illness were enrolled as healthy controls (CTRL group). To be enrolled, controls needed to have had a normal physical examination and no prior treatment with NSAIDs or steroids. Calves in the CTRL group were housed in 12 X 12-foot stalls with their dams, with free-choice hay and water available, and received lactose monohydrate PO once a day for 2 days.

Timeline and intake measures

A total of 7 time points for data collection were used over the course of 3 days. Times of 7:00 am, 12:00 pm, and 7:00 pm were used to avoid interfering with regular clinical activity. The first time point for each calf was dependent on their arrival time at the hospital. The first time point for data collection was chosen as the time point closest to each calf's arrival at the clinic. Treatment was administered at the third time point. A total of 7 time points were used. At each 7:00 am and 7:00 pm time point, a physical examination consisting of temperature, heart rate, and respiratory rate was performed and a fecal score (0 to 3) was assigned, as follows: 0 = normal stool, 1 = semiformed pasty stool, 2 = loose stool that remains on top of bedding, and 3 = watery stool that sifts through bedding.[21]

On intake of clinical calves (MEL and PLBO), fecal samples were collected for a calf enteric PCR panel to test for rotavirus, coronavirus, *E coli*, Salmonella, and *Cryptosporidia* spp (Kansas State Veterinary Diagnostic Laboratory). A blood sample was taken to run an i-STAT CG8+ (Abbott Point of Care) or Nova panel (Nova Biomedical) to determine blood pH, sodium, potassium, ionized calcium, glucose, and bicarbonate deficit. A manual PCV and total protein were also determined from this blood sample. After the final time point, the catheter was removed and the calf was discharged to the client **(Table 1)**.

Table 1: Outline of measures taken throughout the course of the study. Physical exam, iStat (chemistry), packed cell volume, total protein, and sample submission for fecal PCR were performed on calf intake. Treatment (meloxicam or placebo) was administered at timepoints 3 and 6 (24 hours apart). Fecal scoring occurred two times a day at 7am and 7pm. At all seven timepoints, blood was obtained for substance P (Sub P) and prostaglandin E metabolite (PGEM) analysis, kinetic gait analysis was performed on a pressure mat, and mechanical nociceptive threshold (MNT) was obtained. An accelerometer was placed on the left lateral metatarsus on intake and was left on for continuous monitoring throughout the study period.

Timepoint:	**Intake**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Physical Exam	X	X	X		X	X		X
Treatment				X			X	
Fecal PCR	X							
Fecal Score		X	X		X	X		X
Blood								
iStat	X							
Packed Cell Volume/Total protein	X							
Sub P		X	X	X	X	X	X	X
PGEM		X	X	X	X	X	X	X
Behavior score		X	X	X	X	X	X	X
Gait analysis		X	X	X	X	X	X	X
MNT		X	X	X	X	X	X	X
Accelerometer	Continuous							

Accelerometers

All calves (MEL, PLBO, and CTRL) had a 3-axis accelerometer (HOBO; Onset Brands) attached to their left hind limb that continuously monitored movement and posture (standing vs lying) for the duration of the study. Because of the continuous nature of data collection, accelerometer data were analyzed by day (days 1 to 3). Data were offloaded from accelerometers with compatible software (HOBOware; Onset Brands) and was transformed into standing or

lying data[22] in R studio (Posit Software PBC). At the final time point, accelerometers were removed.

Behavior

Video cameras (HDR-CX405 Handycam; Sony Electronics Inc) were mounted on a tripod and placed outside each pen to record calf behavior. Thirty minutes of data was collected at each of the 7 time points, prior to handling calves. Each video was scored continuously by a trained observer (HMN) blinded to treatment, time point, and health status of the calves with Behavioral Observation Research Interactive Software (BORIS, version 8.27; University of Torino) and a detailed ethogram[18,23] **(Table 2)**. For all behaviors except vocalization, behavior was analyzed as the percentage of time spent performing that behavior in the observed 30 minutes. Vocalization was analyzed as the total number of times calves vocalized rather than a percentage. Immediately following the recording session, an adapted approach test[20] was conducted and scored on the basis of the following scale: 0 = calf is already standing or stands with minimal verbal encouragement; 1 = calf rises after researcher makes contact with the calf; 2 = calf rises after researcher makes repeated contact with the calf, may be slow/reluctant; and 3 = calf will not stand.

Table 2: Ethogram used to score calf behavior and activity (adapted from Molony et al., 1995 and Borderas et al., 2008).

Behavior	Description
Walking	Taking two or more steps forward. Calf is steady on its feet, maintaining balance and stability.
Abnormal walking	Taking two or more steps forward. Calf is unsteady on its feet and may appear wobbly or unable to maintain balance.
Standing	Four limbs under body and in contact with the floor. Even weight distribution across each limb and a straight back.
Abnormal standing	Four limbs in contact with the floor. Uneven weight distribution across each limb ± an arched back. One or more limbs may be splayed out.
Lying head up	Lying in sternal position with head held upright. No part of head is in contact with the ground.
Lying head down	Lying in sternal or lateral position with head resting on the ground or tucked into body.
Grooming	Tongue moved over body, licking.

Eating	Ingesting hay provided in feed pan.
Drinking	Consuming water from a bucket.
Vocalization	Any sound emitted from the calf's mouth (e.g., moo, bellow, grunt)
Nursing	Time spent actively nursing dam. Head under dam with mouth in contact with the udder.
Off camera	Calf is completely obstructed from view and it cannot be reliably scored.

Mechanical nociceptive threshold

During each time point, a handheld algometer (FPX 100; Wagner Instruments) was used for mechanical nociceptive threshold (MNT) determination. The left hip was used as a control point and approximately 4 cm caudal to the umbilicus on midline as the experimental point. For each location, a force was applied perpendicularly at the rate of approximately 1 kg for force (KgF) per second. Pressure was applied to each MNT location 3 times, and the average was taken. The same observer performing the threshold test was blinded to the results as they were obtained.

Kinetic gait analysis

A commercial pressure mat (Strideway; Tekscan Inc) was used to analyze gait and weight distribution of calves at each of the 7 time points. The pressure mat was calibrated with the use of a known mass to ensure the accuracy of the measurements at each time point. Calves were walked across the pressure mat to obtain 8 continuous footfalls. An invalid run was defined as a run in which the calf stopped, ran, slid, or was pushed by an assistant on the mat. Video synchronization was used to ensure consistent gait and accurate designation of the footfall. Data were recorded and analyzed with research-specific software (Walkway, version 7.7; Tekscan Inc) as previously described.[24]

Blood sampling

Blood samples were obtained to measure SP, PGEM, and meloxicam concentrations. All samples for clinical cases were drawn off a catheter by use of a 3-syringe technique to obtain 5 to 6 mL of blood. For control calves, 5 to 6 mL of blood was drawn by venipuncture with a 20-gauge needle and 12-mL syringe. Blood samples were collected at each of the 7 time points. Samples were immediately placed on ice after collection. Samples were collected into EDTA tubes with 1mM benzamidine additive for SP samples and lithium heparinized tubes for PGEM

and meloxicam. All samples were spun down in a refrigerated centrifuge at 4 °C at 1,500 X *g* for 10 minutes within 30 minutes of collection, and plasma was frozen at –80 °C until testing.

Substance P measurement

Substance P concentrations were determined with a radioimmunoassay as described by Martin et al.[17] Radioactivity was counted on a Wizard^2 gamma counter (PerkinElmer Inc) for 1 minute. The raw data file was then uploaded onto MyAssays desktop software (version 7.0.211.1238; MyAssays Ltd) for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a coefficient of variation ≥ 18% were reanalyzed. The standard curve ranged from 20 to 1,280 pg/mL. The coefficient of variation for the intra-assay variability was 7.45%, and the interassay variability was calculated to be 13.03%. The average R2 for the calibration curve was 0.998.

Prostaglandin E metabolite ELISA

A commercially available ELISA (PGEM ELISA kit; Cayman Chemical) was used to determine PGEM levels in plasma with the use of methods described by Martin et al,[14] adapting the methods in the manufacturer instructions. Experimental samples were initially diluted by a factor of 5 in the PGEM assay buffer. The plate was read at 405 nm on a SpectraMax i3X plate reader (Molecular Devices), and raw data were inputted to MyAssays for analysis. Additional sample dilutions were performed as needed (dilution factor range, 1 to 10). The coefficient of variation for the intra-assay variability was 8.33%, and the interassay variability was calculated to be 8.97%. The average R2 for the calibration curve was 0.998.

Liquid chromatography–mass spectrometry

Meloxicam was detected in serum by ultraperformance liquid chromatography with tandem mass spectrometry.[23] Ultraperformance liquid chromatography was performed at 40 °C with a 50 X 2.1-mm, 1.8-µm column (C18 HSS T3 column; Waters Corp) equipped with a 10-nm, 1.8-µm, 2.1 X 5-mm precolumn (Acquity UPLC C18 HSS T3 VanGuard precolumn; Waters Corp), at a flow rate of 0.4 mL/min. The mobile phases A and B were composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. A gradient program was used for the separation of the analytes; the percentage of solvent A and B (A/B) was changed as follows: 0 minutes, 95%/5%; 4 minutes, 5%/95%; 5 minutes, 5%/95%; 5.01 minutes, 95%/5%; and 7 minutes, 95%/5%. The total run time of the analysis was 7 minutes, and the injection volume was 2 µL.

A triple quadrupole mass spectrometer (Xevo TQ-S; Waters Corp) was used for quantification. Quantification was performed with the use of meloxicam-d3 as the internal standard. The lower limit of quantification was 1.00 ng/mL.

Statistical analysis

Prior to statistical analysis, SP and PGEM outcomes were log transformed for normality. Statistical analyses of activity, MNT, SP, PGEM, and kinetic gait analysis were completed with JMP software (version 17.0; SAS Institute Inc). Individual calf was considered the experimental unit. Outcome responses were analyzed using linear mixed models with the calf ID with treatment nested as the random effect. Treatment, time, treatment by time interaction, and enrollment date were fixed effects. *F* tests were utilized for testing the significance of main effects and interactions. Pairwise comparisons with significant overall differences were performed with the Tukey honestly significant difference test. Planned contrasts included comparing MEL and PLBO treatment (scours) groups. Statistical significance was determined a priori at $P \leq .05$.

Behavior results were analyzed with a mixed-model procedure, including time, treatment, health status (healthy or scouring), time by treatment, and treatment by health status interactions, in SAS (version 9.4; SAS Institute Inc). Time was a repeated measure with calf as the experimental unit. Post hoc tests were conducted on significant factors with the Tukey-Kramer adjustment.

Results

Clinical and control calves

Twenty-eight calves (11 Simmental, 3 Wagyu, 2 Angus, 10 Angus crosses, 1 Red Angus, and 1 Braunvieh) were enrolled between January 2023 and March 2024. Ages of calves in the MEL group ranged from 7 to 14 days old (median, 12 days), and PLBO calves' ages ranged from 7 to 25 days old (median, 10.5 days). Clinical calf weights ranged from 30.4 to 46.5 kg (mean, 38.2 kg) in the MEL group and 27.8 to 62.3 kg (mean, 42.8 kg) in the PLBO group. Control calf weights ranged from 36.6 to 54.5 kg (mean, 42.25 kg).

Fecal PCR results for clinical calves revealed 10 calves with rotavirus, 3 calves with coronavirus, 2 calves with both rotavirus and coronavirus, and 1 calf with Cryptosporidium. Four calves were PCR negative for etiologic agents.

An i-STAT was performed on all 8 CTRL calves and on 12 clinical (MEL or PLBO) calves on intake, and a NOVA was run on the other 8 calves **(Table 3)**. There were significant differences between CTRL calves and MEL calves in pH ($P < .001$), Pco_2 ($P < .001$), base deficit ($P < .001$), bicarbonate ($P < .001$), total CO_2 ($P < .001$), and PCV ($P = .003$). There were significant differences between CTRL and PLBO calves in pH ($P < .001$), Pco_2 ($P < .001$), base deficit ($P < .001$), bicarbonate ($P < .001$), total CO_2 ($P < .001$), glucose ($P = .02$), and PCV ($P = .001$). There was a significant difference in pH between MEL and PLBO groups ($P = .016$).

Table 3: Mean ± SD for i-STAT and NOVA values for healthy control calves (CTRL; n = 8) and calves with neonatal calf diarrhea (NCD) treated with meloxicam (MEL; 10) or placebo (PLBO; 10). Reference ranges are not available for calves or cattle on the i-STAT CG8+; provided values are from Van Metre et al.[25]

BEEcf = Base excess in the extracellular fluid compartment. Glu = Glucose. iCa = Ionized calcium. N/A = Not applicable. sO_2 = Oxygen saturation. TP = Total protein.

	CTRL		MEL		PLBO		Reference Range
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	
pH	7.46	±0.03	7.07	±0.07	7.17	±0.10	7.31-7.53

PCO2 (mmHg)	46.05	±2.5	28.54	±7.17	28.99	±8.85	35-44
PO2 (mmHg)	47.13	±12.61	74.27	±32.46	61.22	±20.71	N/A
Beecf (mmol/L)	8.75	±1.58	-21.78	±3.85	-17.51	±6.34	N/A
HCO3 (mmol/L)	32.75	±1.46	8.41	±2.85	11.11	±4.97	17-29
TCO2 (mmol/L)	34.13	±1.36	9.4	±3.02	12.14	±5.26	21-32
sO2 (%)	81.75	±7.7	84.6	±12.84	81.4	±13.74	
Na (mmol/L)	136.5	±1.6	138.51	±4.97	132.76	±6.77	132-152
K (mmol/L)	4.68	±0.32	4.84	±0.91	5.61	±2.25	3.9-5.8
iCa (mmol/L)	1.36	±0.04	1.28	±0.22	1.26	±0.13	N/A
Glu (mg/dL)	124	±27.86	101.2	±24.95	93.8	±18.15	80-120
PCV (%)	30	±2.62	37.3	±5.17	40.9	±7.64	32-38
TP (g/dL)	5.98	±0.35	6.7	±0.71	7.6	±1.17	6.7-7.46

Physical examinations were performed on all calves (MEL, PLBO, and CTRL groups) twice a day at 7:00 am and 7:00 pm. Temperature, heart rate, and respiratory rate were recorded. No significant difference was found at any time between controls and calves with NCD, nor between MEL and PLBO calves. Fecal scores improved over time, though there was no significant difference between MEL versus PLBO calves **(Table 4)**.

Table 4: Fecal scores for calves and pathogens found on PCR for calves presented with NCD scored on a scale of 0 to 3, where 0 = normal stool; 1 = soft, pasty stool; 2 = loose stool that sits on top of bedding; and 3 = watery stool that sifts through bedding, based on Garcia et al.[21] Scoring occurred at 7:00 am and 7:00 pm each day.

Table 4: Fecal scores for calves and pathogens found on PCR for calves presented with NCD scored on a scale of 0 to 3

	Day 1		Day 2		Day 3		Pathogen
	AM	PM	AM	PM	AM	PM	
Calf 1		3	3	2	2	2	Rota/Corona
Calf 2		3	2	2	1	1	Rota/Corona
Calf 3		2	1	1	1	1	Rotavirus
Calf 4		2	2	1	1	1	Negative
Calf 5	3	3	2	2	1		Coronavirus
Calf 6		3	3	2	2	2	Cryptosporidium
Calf 7		3	2	2	no fecal	2	Coronavirus
Calf 8		2	1	1	1	1	Rotavirus
Calf 9		2	3	3	3	3	Rotavirus
Calf 10	3	3	3	3	3	3	Coronavirus
Calf 11		2	2	1	1		Negative
Calf 12		2	2	2	1	1	Rotavirus
Calf 13		3	2	2	2	2	Rotavirus
Calf 14		3	3	3	3	3	Rotavirus
Calf 15		3	3	3	3	3	Negative
Calf 16		3	3	2	1		Rotavirus
Calf 17		3	3	2	2	1	Rotavirus
Calf 18		3	2	2	1	1	Rotavirus
Calf 19		3	2	2	1	1	Negative
Calf 20	2	2	3	3	3		Rotavirus

Accelerometers

The measures analyzed were standing time, number of standing bouts, average standing duration per bout, lying time, number of lying bouts, and average lying duration per bout. There was no treatment effect on any parameter. For standing time, there was an effect by day ($P <$ .001) and by treatment X day ($P = .011$). Standing time on day 2 was significantly longer (313.48 ± 21.62 minutes) than on days 1 (176.35 ± 21.62 minutes) and 3 (194.23 ± 21.62 minutes). Calves in the MEL group spent significantly more time standing on days 2 (306.44 ± 34.31 minutes) and 3 (219.11 ± 34.31 minutes) than on day 1 (106.78 ± 34.31 minutes). Calves

in the CTRL group stood significantly longer on day 2 (371.13 ± 38.91 minutes) than on days 1 (243.29 ± 38.91 minutes) and 3 (188 ± 38.91 minutes). There was no significant difference between standing time by day in PLBO calves.

There was a significant effect of day ($P < .001$) and treatment X day ($P = .01$) for standing bouts. On day 2 there were significantly more standing bouts (22.28 ± 1.19 bouts) than on days 1 (9.77 ± 1.19 bouts) and 3 (13.87 ± 1.19 bouts). Calves in the MEL group had significantly more standing bouts on days 2 (20.11 ± 1.89 bouts) and 3 (15.33 ± 1.89 bouts) than on day 1 (4.89 ± 1.89 bouts). Calves in the CTRL group had significantly more standing bouts on day 2 (25.86 ± 2.14 bouts) than on days 1 (15.43 ± 2.14 bouts) and 3 (12.00 ± 2.14 bouts). Calves in the PLBO group had significantly more standing bouts on day 2 (20.86 ± 2.14 bouts) than on day 1 (9.00 ± 2.14 bouts), with no significant difference between day 3 (14.29 ± 2.14 bouts) and day 1 or 2.

There was a significant effect by day ($P = .01$) on average standing duration per bout. Day 1 (21.50 ± 2.37 min/bout) had a longer duration per bout than days 2 (14.48 ± 2.37 min/bout) and 3 (14.87 ± 2.37 min/bout).

There was a significant effect of day ($P < .001$) and treatment X day ($P = .02$) for lying time. Lying time was significantly longer on day 2 (1,084.19 ± 41.19 minutes) than on days 1 (514.58 ± 41.19 minutes) and 3 (766.52 ± 41.19 minutes). Calves in the MEL group spent significantly less time lying on day 1 (398.44 ± 65.38 minutes) than on days 2 (1,133.56 ± 65.38 minutes) and 3 (946 ± 65.38 minutes). Calves in the CTRL group spent significantly more time lying on day 2 (1,068.86 ± 74.14 minutes) than on days 1 (632 ± 74.14 minutes) and 3 (603.57 ± 74.14 minutes). Calves in the PLBO group spent significantly more time lying on day 2

(1,050.14 ± 74.14 minutes) than on day 1 (513.29 ± 74.14 minutes); there was no significant difference between day 3 (750 ± 74.14 minutes) and day 1 or 2.

There was a significant effect of day ($P < .001$) and treatment X day ($P = .01$) for lying bouts. There were significantly more lying bouts on day 2 (22.86 ± 1.18 bouts) than on days 1 (10.41 ± 1.18 bouts) and 3 (14.44 ± 1.18 bouts). Calves in the MEL group had significantly more lying bouts on days 2 (21.00 ± 1.87 bouts) and 3 (16.33 ± 1.87 bouts) than on day 1 (5.67 ± 1.87 bouts). Calves in the CTRL group had significantly more lying bouts on day 2 (26.14 ± 2.12 bouts) than on days 1 (15.71 ± 2.12 bouts) and 3 (12.29 ± 2.12 bouts). Calves in the PLBO group had significantly more lying bouts on day 2 (21.43 ± 2.12 bouts) than day 1 (9.86 ± 2.12 bouts); there was no significant difference between lying bouts on day 3 (14.71 ± 2.12 bouts) and day 1 or 2.

There was no significant effect of treatment, day, or treatment X day on average lying duration per bout.

Behavior

There was no significant difference between approach scores of MEL and PLBO calves at any time point **(Table 5)**.

Table 5: Approach score frequencies for calves with neonatal calf diarrhea treated with meloxicam (MEL) and placebo (PLBO). 0 = calf is already standing or stands with minimal verbal encouragement; 1 = calf rises after researcher makes contact with the calf; 2 = calf rises after researcher makes repeated contact with the calf, may be slow/reluctant; 3 = calf will not stand (adapted from Cramer et al 2016).

APPROACH SCORE:	**0**	**1**	**2**	**3**
TIMEPOINT 1				
MEL	10	10	50	30

	(1/10)	(1/10)	(5/10)	(3/10)
PLBO	10 (1/10)	20 (2/10)	50 (5/10)	20 (2/10)
TIMEPOINT 2				
MEL	60 (6/10)	0 (0/10)	40 (4/10)	0 (0/10)
PLBO	20 (2/10)	30 (3/10)	50 (5/10)	0 (0/10)
TIMEPOINT 3				
MEL	20 (2/10)	20 (2/10)	60 (6/10)	0 (0/10)
PLBO	20 (2/10)	50 (5/10)	30 (3/10)	0 (0/10)
TIMEPOINT 4				
MEL	10 (1/10)	70 (7/10)	20 (2/10)	0 (0/10)
PLBO	30 (3/10)	60 (6/10)	10 (1/10)	0 (0/10)
TIMEPOINT 5				
MEL	40 (4/10)	40 (4/10)	20 (2/10)	0 (0/10)
PLBO	10 (1/10)	80 (8/10)	10 (1/10)	0 (0/10)
TIMEPOINT 6				
MEL	20 (2/10)	70 (7/10)	10 (1/10)	0 (0/10)
PLBO	10	80	10	0

		(8/10)	(1/10)	(0/10)
TIMEPOINT 7				
MEL	20 (2/10)	70 (7/10)	10 (1/10)	0 (0/10)
PLBO	20 (2/10)	80 (8/10)	0 (0/10)	0 (0/10)

Calves with NCD (MEL and PLBO) spent significantly more time lying than healthy calves ($P < .001$; **Table 6**). Calves with NCD also spent significantly more time lying with their head up compared to healthy calves ($P < .001$). Calves in the MEL group (43.95 ± 3.96%) spent more time lying with their head down than PLBO calves (30.05 ± 4.14%; $P < .02$; **Table 7**). Calves in the MEL group (2.82 ± 10.42%) on average vocalized 11 times less than PLBO calves (32.96 ± 7.46%; $P < .02$). There were no other significant behavioral differences observed.

Table 6: Least square mean percentage of time spent on measured behaviors in healthy control (CTRL) versus calves with neonatal calf diarrhea (NCD) which includes calves in both the meloxicam and placebo treatment groups. % time represents the total percent of time observed that the indicated behavior was observed.

		CTRL	NCD	P VALUE
LYING HEAD UP	***% time***	35.96	55.48	0.0001
	SEM	±4.33	±2.42	
LYING HEAD DOWN	***% time***	41.5	37	0.4624
	SEM	±5.34	±2.87	
TOTAL LYING	***% time***	66.5	85.31	<0.0001
	SEM	±4.04	±2.26	
STANDING	***% time***	27.52	27.39	0.9819

	SEM	±5.2	±2.97	
WALKING	***% time***	3.29	2.79	0.5698
	SEM	±0.77	±0.45	
GROOMING	***% time***	2.47	6.09	0.0768
	SEM	±1.79	±1.06	
EATING	***% time***	1.49	4.76	0.223
	SEM	±2.06	±1.4	
DRINKING	***% time***	1.41	1.69	0.6518
	SEM	±0.57	±0.39	

Table 7: Least square mean percentage of time spent on measured behaviors in healthy control (CTRL) versus calves with neonatal calf diarrhea (NCD) divided into calves treated with meloxicam (MEL) or placebo (PLBO). % time represents the total percent of time observed that the indicated behavior was observed. Numbers not connected by a letter in each row indicate a significant difference

		CTRL	MEL	PLBO
LYING HEAD	***% time***	40.55^{a}	50.89^{b}	60.06^{b}
UP	***SEM***	±3.59	±3.49	±3.35
LYING HEAD	***% time***	34.55^{ab}	43.95^{a}	30.05^{b}
DOWN	***SEM***	±4.53	±3.96	±4.14

Mechanical nociceptive threshold

Controls were more sensitive (1.30 ± 0.11 KgF) than both MEL (1.71 ± 0.10 KgF) and PLBO (1.77 ± 0.10 KgF) calves to pressure over the hip ($P < .007$). No significant difference between CTRL (0.76 ± 0.06 KgF) and MEL (0.96 ± 0.05 KgF) or PLBO (0.93 ± 0.05 KgF) calves to pressure over the umbilicus ($P < .053$). There was no significant difference between MEL and PLBO calves **(Table 8)**.

Table 8: Mean mechanical nociceptive threshold (MNT) values for healthy control calves (CTRL) and calves with neonatal calf diarrhea treated with either meloxicam (MEL) or placebo (PLBO).

Treatment	Hip		Umbilicus	
	Mean	***SD***	***Mean***	***SD***
CTRL	1.30	±0.11	0.76	±0.06
MEL	1.71	±0.10	0.96	±0.05
PLBO	1.77	±0.10	0.93	±0.05

Kinetic gait analysis

Left front ($P < .002$), left hind ($P < .01$), right front ($P < .017$), and right hind ($P < .007$) stance times were significantly shorter for CTRL calves (0.51 ± 0.05 seconds, 0.52 ± 0.05 seconds, 0.56 ± 0.05 seconds, and 0.50 ± 0.05 seconds, respectively) compared to MEL calves (0.75 ± 0.04 seconds, 0.75 ± 0.05 seconds, 0.78 ± 0.05 seconds, and 0.74 ± 0.05 seconds, respectively; **Table 9**).

Table 9: Least square means of left front limb (LF), left hind limb (LH), right front limb (RF), and right hind limb (RH) stance time, left front (LF) and left hind (LH) limb stride length, and gait velocity in healthy control calves (CTRL) and calves with neonatal calf diarrhea treated with either meloxicam (MEL) or placebo (PLBO). Values in a column not connected by a letter are significantly different.

TREATMENT	LF STANCE TIME		LH STANCE TIME		RF STANCE TIME		RH STANCE TIME		LF STRIDE LENGTH		LH STRIDE LENGTH		GAIT VELOCITY	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
CTRL	0.51[a]	±0.04	0.52[a]	±0.05	0.56[a]	±0.05	0.5[a]	±0.05	76.42[a]	±2.64	75.79[a]	±1.79	92.77[a]	±6.68
MEL	0.75[b]	±0.04	0.75[b]	±0.05	0.78[b]	±0.05	0.74[b]	±0.05	67.18[b]	±2.40	68.73[b]	±1.68	63.05[b]	±6.14
PLBO	0.63[ab]	±0.04	0.64[ab]	±0.05	0.61[ab]	±0.05	0.59[ab]	±0.05	73.67[ab]	±2.38	71.87[ab]	±1.65	71.16[ab]	±6.04

Left front ($P < .041$) and left hind ($P < .022$) stride length was significantly shorter in MEL calves (67.18 ± 2.4 cm and 68.73 ± 1.68 cm, respectively) compared to CTRL calves (76.42 ± 2.64 cm and 75.79 ± 1.79 cm, respectively). Gait velocity was faster in controls (92.77 ± 6.68 cm/sec) compared to MEL calves (63.05 ± 6.14 cm/sec; $P < .008$).

There were no other significant differences in gait measures.

Substance P

Controls had significantly higher SP values at all time points (mean, 486.73 ± 60.33 pg/mL) compared to MEL (303.72 ± 15.36 pg/mL; $P < .008$) and PLBO (296.67 ± 14.78 pg/mL; $P < .003$) calves. There was no significant difference between MEL and PLBO calves.

Prostaglandin E Metabolite

Prostaglandin E metabolite concentrations were significantly higher in both MEL and PLBO calves compared to CTRL calves. The CTRL calf PGEM was a mean of 23.89 pg/mL (range, 21.99 to 29.94 pg/mL) across all time points. The PGEM of calves in the MEL and PLBO groups was significantly higher, at averages of 114.6 and 127.5 pg/mL, respectively, on intake. After treatment administration, PGEM concentrations in MEL calves dropped significantly more than PGEM in PLBO calves **(Figure 1)**.

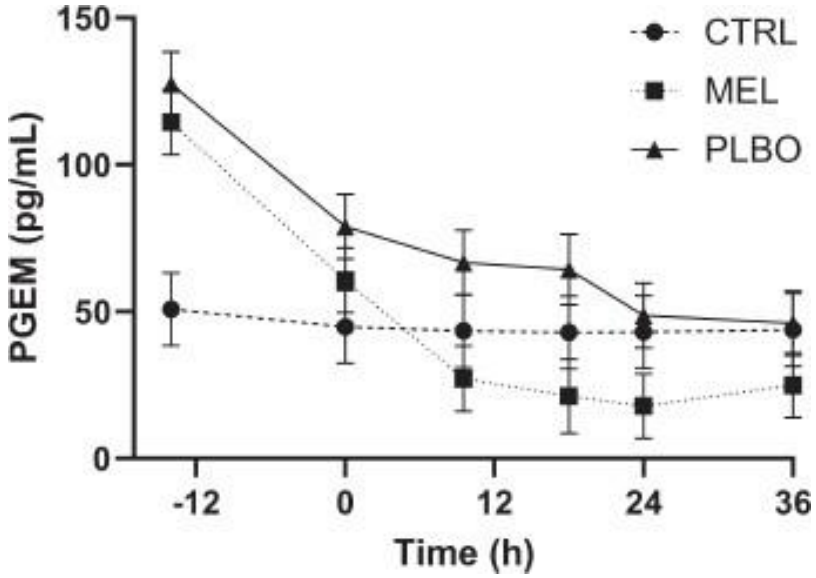

Figure 1: Mean ± SEM prostaglandin E metabolite (PGEM) over time in healthy control calves (CTRL; n = 8) and calves with diarrhea that were treated with meloxicam (MEL; 10) or placebo (PLBO; 10) at time 0 and 24 hours.

Meloxicam detection

No meloxicam was detected in PLBO calves. No meloxicam was detected during the 3 pretreatment time points for MEL calves, after which concentrations averaged at 3,678.33 ng/mL (range, 1,301.58 to 4,586.28 ng/mL), 4,456.2 ng/mL (range, 3,503.94 to 5,645.49 ng/mL), 4,412.52 ng/mL (range, 3,895.38 to 5,039.71 ng/mL), and 8,379.18 (range, 7,469.83 to 10,009.57 ng/mL) at time points 4, 5, 6, and 7, respectively.

Discussion

The results of this study demonstrated a difference in behaviors and physiologic values between healthy calves and calves with NCD. Additionally, behavioral differences were found between scouring calves treated with meloxicam versus a placebo.

The study population included beef calves from Kansas and Nebraska. Data from the Kansas State Veterinary Diagnostic Laboratory, which compiled data from 2023 based on fecal PCR from over 1,500 calves, showed a majority of calves with NCD were caused by rotavirus and/or coronavirus.[26] These data are consistent with our findings, in which the majority of calves were affected by rotavirus diarrhea. While human literature links abdominal pain with diarrhea, there is a paucity of data in calves with diarrhea. One study[10] described an increased milk intake in dairy calves with NCD that were treated with meloxicam versus those that were not. The authors speculated that meloxicam decreased abdominal discomfort, though no data related to pain measurement were provided to support this hypothesis. In the present study, changes seen in kinetic gait analysis and behavior are supportive of abdominal pain in calves with NCD.

Mechanical nociceptive threshold has been used to evaluate visceral abdominal pain in humans and has been reported to be a sensitive measure of visceral abdominal pain in children with appendicitis.[27] Several studies[28,29] report good interobserver correlation with the use of pressure algometry on the abdomen and consistently show that lower algometry values indicate abdominal pain, though no standard thresholds are established. In this study, no significant difference was seen in response to pressure over the abdomen. With MNT being a novel approach to identifying visceral abdominal pain associated with NCD, the abdominal location chosen may not have been adequate to obtain a significant response.

Kinetic gait analysis has been used as a measure of abdominal pain associated with castration.[14,30] One of these studies[14] found that calves castrated without an NSAID had shorter forelimb stance times compared to those that were castrated with an NSAID. In the present study, however, healthy calves had shorter stance times compared to calves with NCD. Calves with NCD also had slower gait velocity compared to healthy controls, particularly between MEL and CTRL calves. Taken together, longer stance times and slower velocity may indicate a reluctance to move forward due to pain. Other castration studies have used video recordings to measure and analyze stride length. A shorter stride length was seen in 2 studies of calves that

were knife castrated versus banded[31] and calves undergoing castration versus shams.[12] In the latter study,[12] calves treated with flunixin meglumine had longer strides than those with untreated castrations. On the basis of this finding, shorter stride length could indicate a reluctance to move forward due to pain, which an NSAID may be able to mitigate. In our study, we did not appreciate a difference between MEL and PLBO calves, though the shortened stride length in calves with NCD could similarly be attributed to pain.

Prostaglandin E metabolite is a marker for inflammation and has been shown to negatively correlate with meloxicam concentrations. Elevated PGEM in clinical calves shows the physiologic difference in healthy versus sick calves, indicating a significant inflammatory process. In our study, PGEM concentration changed significantly over time in the MEL group, in correlation with the rise in plasma levels of meloxicam. Effective plasma concentrations of meloxicam when given by oral gavage to horses, dogs, and humans range from 0.13 to 0.93 μg/mL.[32] The meloxicam concentrations achieved in this study ranged from 1.3 to 10 μg/mL, well above the reported therapeutic range in other species. The significant decrease in PGEM over time once meloxicam was given in this study supports that this dosing threshold is effective.

Though no studies of calves have looked at vocalization in relation to diarrhea or other causes of visceral abdominal pain, there are studies showing increased vocalization with painful procedures like ear tagging[33] and castration.[11,12] Our study found that calves with NCD treated with meloxicam vocalized significantly less than those treated with a placebo. This decrease in vocalization may indicate that meloxicam provided some relief from diarrhea-associated pain. Vocalization may have been influenced by the presence of the dam (or lack thereof). Scouring calves were housed separately to safely facilitate IV fluid therapy. To have a true control, healthy calves would have needed to be removed from the dam for up to 48 hours. We felt it was in the best interest of the calves' well-being for them to stay with the dam.

Previous work has evaluated pain behaviors associated with NCD. Studies[34,35] have shown that calves with NCD and umbilical inflammation spend less total time lying down and less time lying per bout compared to controls, though not all studies have found significance in lying behavior.[36] Conversely, our study found that calves with NCD spent more time lying than healthy controls. The reason for the differences in lying time and bouts is not known, but differences in housing and bedding substrates may be factors. One study[34] housed calves individually on raised pens with coated metal flooring. Another study[35] group-housed calves, but

the differences in lying data between healthy and NCD subjects were prior to disease onset. Furthermore, calves in the present study were severely depressed and in need of fluid resuscitation. The calves in the papers discussed used fecal scoring, but calves were not as clinically depressed. Our study also indicated that the behavior of lying with the head down may be associated with increased comfort, as it was observed more frequently in CTRL calves compared to calves with NCD (MEL and PLBO) and in MEL compared to PLBO calves. One of the ethograms used as a model for this study[18] also differentiated between behaviors of lying with the head up versus head down to evaluate the behavioral response of calves to lipopolysaccharide stimulus, though no significant difference was found between the two behaviors. However, fever and sepsis were induced in calves of that study, whereas our study looked at naturally occurring disease of the gastrointestinal tract. There is likely a difference in the degree of pain associated with that study and the present study. The possibility that different lying postures may indicate different states of welfare warrants further study.

There were several limitations to this study. To accurately compare SP values in healthy calves and NCD calves, a catheter should have been placed in the controls. Because of the nature of housing calves with their dams, it was elected not to place catheters in controls and venipuncture was performed at each time point instead, alternating the side of the neck that was used each time. Substance P has been shown to increase with venipuncture, and it is expected that it was significantly elevated compared to NCD calves.

Another limitation was the time point assignment. To capture data in the beginning and during treatment for acidosis and dehydration, it was determined that the first time point would be the one closest to the intake of the calf. For example, for a calf presenting at 2:00 pm, the first time point in the study would be 7:00 pm. While most calves presented in the afternoon, there were some that came in the morning or overnight. Working time points were 7:00 am, 12:00 pm, and 7:00 pm, which were chosen to work around normal hospital operations. However, because of the irregular intervals between these time points, adjustments had to be made during data analysis for data to be appropriately compared and for some measures only the first 36 to 48 hours were of use.

The sample size was also a limitation of this study. While our sample size was appropriate for physiologic measures and gait analysis, it was not a significant sample size with regards to behavior analysis. While only a few behaviors showed statistically significant

differences, there were many other behaviors that were trending toward significance based on P values ($P < .1$ and $> .05$). A larger sample size may have elucidated more significant differences, and while our study provides a baseline for our proposed ethogram, additional studies would be needed to validate it with a larger sample size and additional observers.

Conclusion

In conclusion, on the basis of physiologic and behavioral outcomes, calves with NCD seemed to experience more pain compared to healthy calves. Differentiating behaviors of lying with the head down and head up may be a novel indication of animal welfare. Further research is needed to investigate the NCD pain in calves and the analgesic effect that meloxicam may provide for this associated pain.

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