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Optimal lameness induction model development using amphotericin B in meat goats

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ABSTRACT:

Lameness continues to be a critical health and welfare concern associated with goat production. Amphotericin B (amp B) is an antimicrobial successful in inducing transient lameness for research purposes previously in livestock animals. The objectives of this study were to (1) identify which of three varying doses of amp B would be most effective in inducing lameness in meat type goats and (2) develop a facial grimace scale for goats. Lameness was produced by an intra-articular injection of amphotericin B into the left hind lateral claw distal interphalangeal joint with either a 5 mg/0.25 mL (high–low, 5 mg of amphotericin B in a volume of 0.25 mL), 5 mg/0.5 mL (high–high, 5 mg of amphotericin B in a volume of 0.5 mL), or a 2.5 mg/0.25 mL (low–low, 2.5 mg of amphotericin B in a volume of 0.25 mL). A saline treatment of 0.5 mL was used as control (0.9% sterile saline solution). Lameness response was analyzed by infrared thermography (IRT) at the induced joint, mechanical-nociception threshold (MNT), visual lameness scoring (VLS), a visual analogue scale (VAS), kinetic gait analysis (KGA), plasma cortisol (CORT), substance P (Sub P), and behavior scoring. The IRT and MNT values differed by timepoint ($P \leq 0.0001$). Results from VLS showed the HL treatment was the most effective at inducing lameness (6/6 goats became lame compared to HH 4/6 and LL 2/6). At 24, 48, and 72 h, VAS scores were significantly higher when comparing HL to all other treatment groups ($P = 0.0003$). Both behavior observers (1 and 2) reported a significant time effect ($P = 0.05$), with goats exhibiting more facial grimacing at 24 h post-lameness induction. From these data, an optimal dose for a repeatable lameness induction model in goats was aquired. An effective Goat Grimace Scale (GGS) was also developed to evaluate pain responses in goats.

Keywords: amp B, goat, grimace scale, lameness, pain

Introduction

Lameness has been established as one of the most serious concerns amongst ruminant species (Christodoulopoulos, 2009). Sole ulcers, subsolar abscesses, and interdigital dermatitis are all hoof lesions associated with causing goat lameness (Hill et al., 1997; Christodoulopoulos, 2009; Crosby-Durrani et al., 2016). The economic impact of lame animals is reflected by decreased productivity, cost of treatment and premature culling of animals (Christodoulopoulos, 2009). Treatment of lame goats is challenging due to an incomplete understanding of physiologic and behavioral responses to lameness induced pain.

Amphotericin B (Amp B) has been shown to successfully induce acute transient synovitis and arthritis (McIlwraith et al., 1979; Coetzee et al., 2014; Reppert et al, 2020). Amphotericin B is a polyene antimicrobial that has been used in previous lameness models in horses and cattle (McIlwraith et al., 1979; Schulz et al., 2011). Lameness severity and clinical onset differs between species using Amp B. Duration of lameness in horses lasted from 3 d to 2 wk, while cattle peaked from 6-12 h; ending at or before 72 h (McIlwraith et al., 1979; Schulz et al., 2011). Little is known about lameness duration from associated amp B injection in small ruminants.

In a pilot study comparing Amp B and kaolin-carrageenan in goats, both treatment protocols produced lame goats, showing the effectiveness of Amp B as a lameness inducing agent (Reppert et al., 2020). Unfortunately, goats in both treatment groups became severely lame necessitating rescue analgesic intervention. These results insuninated the need for further dose refinement.

The meat goat industry is one of the fastest growing sectors of the U.S. livestock industry, indicating a need for a clearer understanding of lameness concerns in goat production (USDA, 2010). Mixed breed meat type goats were utilized during this study due to their increasing prevalence in the United States (USDA, 2010). This study's objectives were to optimize an experimental lameness induction model using Amp B, along with developing a facial grimace scale for pain assessment in goats.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (Protocol #4387).

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Animals and Study Design

Twenty-four intact male and female (21 male, 3 female) crossbred meat type goats were enrolled on this study. All goat were between 4 and 6 months of age and averaging a body weight of 19 kg (range 15 to 25 kg). Prior to study enrollment, all animals were found to be free of lameness, as determined by a trained individual using a visual lameness scoring (VLS) system. Goats were housed individually in raised pens with a grated floor and fed a complete mixed ration pellet, along with free access to water. This study was conducted over a 3-week period, with 1 week dedicated to acclimation and the following week focused on lameness induction, data collection, and the last week dedicated to post-study monitoring. Following the one-week acclimation period, a random number generator (random.org) was used to randomly assign each goat to one of four treatments:

- high dose-low volume (HL; $n = 6$): 5 mg of amphotericin B in a volume of 0.25 mL
- high dose-high volume (HH; $n = 6$) 5 mg of amphotericin B in a volume of 0.5 mL
- low dose-low volume (LL; $n = 6$): 2.5 mg of amphotericin B in a volume of 0.25 mL
- control (CNTL; $n = 6$): 0.5 mL of 0.9% sterile saline solution

Lameness Induction

Goats were separated into two groups to facilitate lameness induction. Groups 1 and 2 consisted of 12 individuals per group (2 females were in group 1, and 1 female was in group 2). An intravenous catheter (16 g × 7.5 cm, Mila International Inc., Florence, KY, USA) was aseptically placed in the right jugular vein of each goat. Catheters were used to facilitate the administration of Xylazine hydrochloride (0.1 mg/kg IV, Akron Inc., Lake Forest, IL, USA) to induce sedation as well as subsequent blood collections. Once sedated, hair on the lateral aspect of the distal limbs was clipped from hoof to fetlock joint. After clipping, the left lateral distal digit/claw was surgically scrubbed from the proximal interphalangeal joint to the coronary band. A 21 g × 2.54 cm needle was placed into the distal interphalangeal joint of the left lateral claw. All intra-articular injections were performed by a veterinarian (ER), blinded to treatment group, and skilled in arthrocentesis of small ruminant joints. Intra-articular injection was confirmed by lack of resistance to injection by the veterinarian. Resistance in this study was defined as the inability to distribute the full amount of treatment into the distal interphalangeal joint of the left lateral claw. If resistance was present during injection administration, the treatment was periarticular and recorded ($n = 3$). To ensure distribution of treatment throughout the joint after injection, the digit was flexed and extended five times. After injection completion, sedation was reversed using a single intramuscular injection of atipamezole hydrochloride (Zoetis INC, Kalamazoo MI, USA) at a dosage of 0.04 mg/kg. Each goat was monitored hourly for behavioral signs of distress or pain out to 10 h post-induction, and twice daily thereafter for 5 d.

Amphotericin B (X-Gen Pharmaceuticals Inc., Big Flats, NY) was prepared by adding 2.5 mL or 5 mL of sterile water for injection (Baxter Healthcare Corporation, Deerfield, IL, USA) to achieve the needed concentrations for the high dose-low volume (HL) (20 mg/mL), high dose-high volume (HH) (10 mg/mL) or low dose-low volume (LL) (10 mg/mL) treatments.

Outcome Variables

Parameters for data collection included: behavioral scoring, facial grimace (GGS), infrared thermography (IRT), mechanical-nociception threshold (MNT), kinetic gait analysis (KGA), plasma cortisol (CORT) concentrations, plasma substance P concentrations (Sub P), visual lameness score (VLS), and visual analogue score (VAS) (Fig. 1). All outcomes

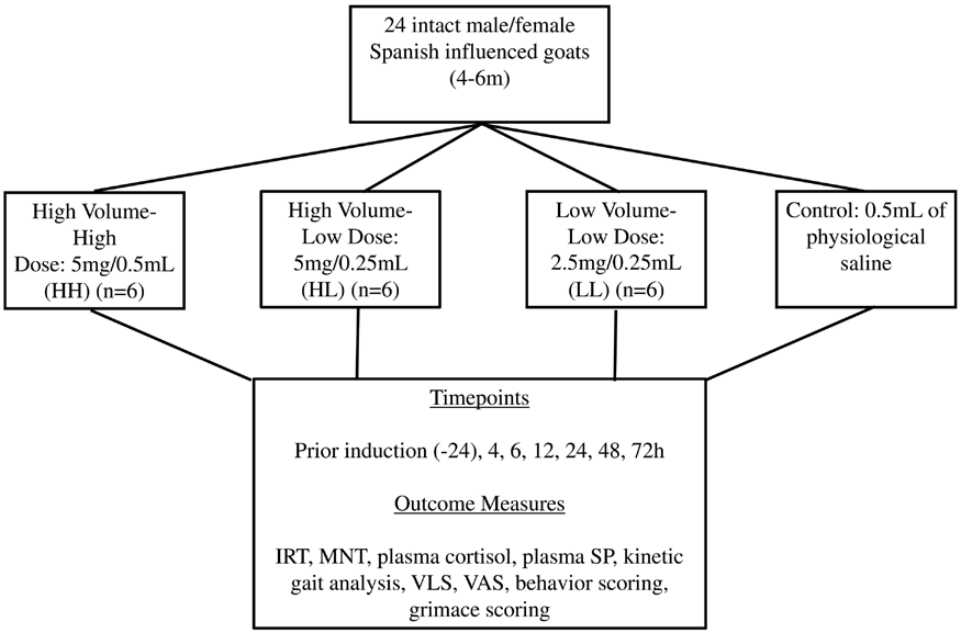

Figure 1. Flow chart of study events.

were collected at the following timepoints; baseline (–24 h prior to induction), 4, 6, 12, 24, 48, and 72 h post-induction of lameness. All observers were blinded to treatment during the study.

Behavior Scoring

High-definition video cameras (Sony Handycam HDR-CX405, Sony USA Inc., NY, USA) mounted on tripods were positioned in front of the goat pens, with goats in direct view. Every goat on study was video recorded for 30 min at each study time point (prior to induction (–24), 4, 6, 12, 24, 48, and 72 h). BORIS (Behavioral Observation Research Interactive Software v 7.7.3, Torino, Italy) was used to score goat behavior, along with a detailed ethogram adapted from Reppert et al. (2020) (Table 1). Raters had to obtain an ICC of 0.80 or higher in order to score behavior data. A total of 5,040 min (84 h) of continuous behavior scoring was included in the final analysis.

Facial Grimace Score

A high-definition video camera mounted on a tripod was placed at the end of the pressure mat walkway to video record goat faces when they walked across the pressure mat at every study timepoint (prior to induction (–24), 4, 6, 12, 24, 48, and 72 h). A maximum of three facial images were pulled from each video recording by an individual blinded to treatment and time point. A total of 351 images (pre- and post-lameness induction) were assessed by an expert for facial grimace assessment. This person was not blinded to timepoint to ensure every goat had facial images to score for the two trained individuals. Four facial action units (FAUs) that changed in response to lameness (i.e., pain) in goats were identified: ear position, nostril shape and dilation, orbital tightening, and cheek tightening. Ear position was scored on a three-point scale (0 = symmetrical/forward ears, 1 = asymmetrical ears, and 2 = ears pulled back). Nostril shape and dilation were also scored on a three-point scale (0 = U-shaped nose and nostril dilation, 1 = intermediate

Table 1. Ethogram used to score goat behavior. Behaviors were grouped into the following: maintenance, locomotion, oral behavior, social interaction, and pain behavior

Behaviors	Description
Maintenance	
Eating	Ingesting food provided at feed bunk
Drinking	Consuming water from nozzle
Defecating	Passing fecal matter in standing or lying position
Urinating	Passing urine in standing or lying position
Sleeping	Lying down, eyes closed
Scratching	Using horns or rear hoof to scratch the body
Ruminating	Regurgitating, chewing, and swallowing food
Pawing	Moving front limb in a digging motion against the ground or feed bunk
Grooming	Licking or rubbing body or head against pen
Locomotion	
Walking	Moving forward at a normal pace
Limping	Walking with one or more legs not supporting body weight
Standing	Body weight supported by four legs, no forward movement
Standing on 3 legs	Body weight supported by three legs; non-weight bearing on one limb. No forward movement
Sitting	Body weight supported by hindquarters and front legs
Lying	Recumbent, body on ground
Rearing	Body weight supported by back legs. Front legs on fence or feed bunk
Kneeling	Body weight supported by front carpal joints and hind legs
Oral behavior	
Licking	Moving tongue over surfaces or pen mates
Chewing	Nibbling at substrates or conspecific in nearby pen
Sniffing	Inhaling air close to object or conspecific in nearby pen
Social interaction	
Playing	Running, trotting, galloping, or springing
Butting	Head-to-head or head-to-body contact with conspecific in nearby pen
Allo-grooming	Licking or rubbing body against conspecific in nearby pen
Agonistic	Biting or fighting other littermates (e.g., head-knock)
Pain behavior	
Restlessness	Repeated sitting, standing, or walking for short durations, unsettled
Attention to hoof	Guarding or constant attention to injured foot or limb
Tail wagging	Tail movement from side to side (or up and down)
Foot flicking	Moving back limb in a flicking/kicking motion

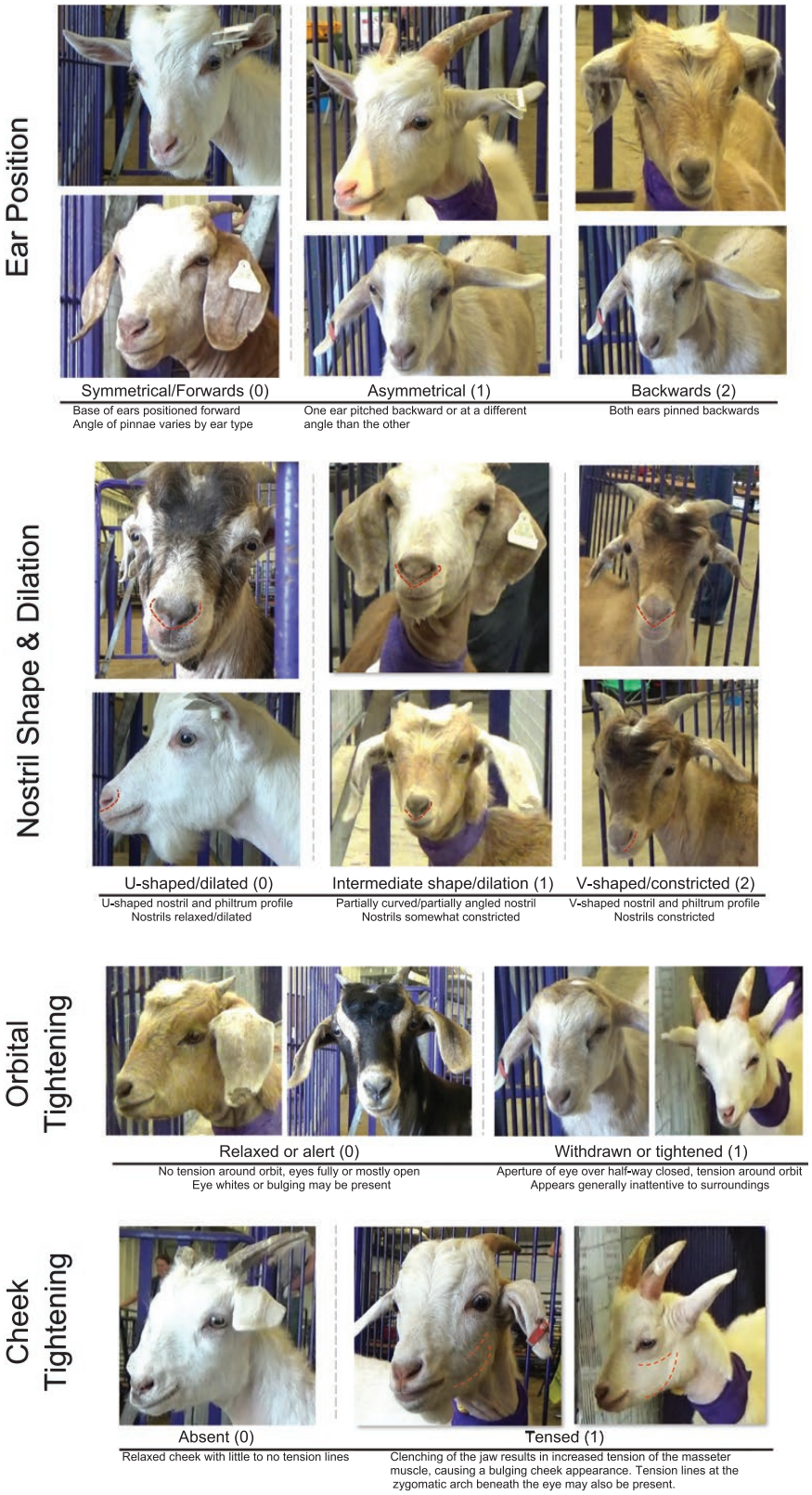

Figure 2. The Goat Grimace Scale with four Facial Action Units (FAUs), ear position, nose shape and dilation, orbital tightening, and cheek tightening.

nose shape, and 2 = V-shaped nose and nostril constriction). Orbital tightening and cheek tightening were scored on a two-point scale (0 = absent and 1 = present). Therefore, the maximum score on the Goat Grimace Scale (GGS) was 6. Facial images from goats enrolled in this study were used to create the GGS (Fig. 2).

Two individuals blinded to treatment and time point, with extensive experience in facial grimace assessment, scored

all 351 images using the GGS. If an image could not be reliably scored, the individuals were instructed to exclude it from scoring. The GGS score for each image was calculated by summing the scores allotted to each FAU. The intraclass correlation coefficient (ICC) was assessed for the two scorers prior to analysis to determine rater reliability.

Infrared Thermography

Thermographic images of the dorso-lateral aspect of the lateral rear feet were taken at each timepoint; one of the left distal limb (affected) and one of the right distal limb (control) prior to induction (–24), 4, 6, 12, 24, 48, and 72 h. The infrared camera (FLUKE Ti580 IR Imager, Fluke Corp., WA, USA) was positioned at a 45° angle from the dorso-lateral aspect 1 m from the coronary band of each limb. Images were analyzed to obtain the maximum temperatures for each distal limb with a research grade computer software (SmartView 4.3, Fluke Corp., Everett, WA, USA). The difference between the left distal limb (induced) and right distal limb (control) foot were determined as described by Kleinhenz et al. (2019). These differences were used for statistical analysis.

Mechanical-Nociception Threshold

All MNT measurements were taken with a handheld algometer (FPX 100, Wagner Instruments, Greenwich, CT) prior to induction (–24), 4, 6, 12, 24, 48, and 72 h. The MNT measured the lateral digit at the level of the coronary band at a point halfway between midline and the heel bulb, by applying slow, steady pressure until the goat responded by moving away from pressure or displaying a flinch response. The MNT measurements were taken on both the left (affected) distal limb and right (control) distal limb. The average of three readings for each distal limb at each time point was used for analysis as described by Kleinhenz et al. (2019). The difference between left (induced) and right (control) distal limbs MNT measurements (left distal limb minus right distal limb) was also determined for each time point. These differences were used for statistical analysis. The investigator measuring MNT was blinded to treatment and the algometer readings to prevent bias. A second investigator recorded algometer readings, also to prevent testing bias.

Kinetic Gait Analysis

A commercial pressure/force mat system (Strideway, Tekscan, Inc.) was used to record and analyze the gait of each goat at each timepoint (prior lameness induction (–24), 4, 6, 12, 24, 48, 72 h post-induction) using methods described by Reppert et al. (2020). Video analysis was used to ensure synchronization of each goat's gait while walking across the pressure mat system. A research grade software (Strideway v 7.7, Tekscan, Inc., South Boston, MA, USA) was used to analyze different parameters of gait motion using methods described by Reppert et al. (stance time, stride length, contact force, impulse, contact pressure, and contact area).

Plasma Cortisol

Blood samples were collected from the intravenous catheter in each goat prior to induction (–24), and at 4, 6, 12, 24, 48, and 72 h post-lameness induction. Plasma cortisol concentrations from each sample were determined in duplicate using a radioimmunoassay (RIA) system using methods adapted from Kleinhenz et al., (2017). Plasma cortisol concentrations were determined using a commercially available RIA kit (MP Biomedicals, Irvine, CA) following manufacturer specifications with minor modifications as previously described (Martin et al., 2022); the standard curve was extended to include 1 and 3 ng/mL by diluting the 10 and 30 ng/mL manufacturer-supplied standards, 1:10, respectively. The standard curve ranged from 1 to 300 ng/mL. A low (25 ng/mL) and high (150 ng/mL) quality control (QC) was run at the beginning and end of each set to determine inter-assay variability. Plain 12 × 75 mm polypropylene tubes were used as blank tubes to calculate non-specific binding. Input for standards, QCs, and samples was adjusted to 50 µL. Samples were incubated at room temperature for 30 min before adding I-125. Manufacturer instructions were then followed. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software (version 7.0.211.1238, 21 Hampton Place, Brighton, UK) for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a coefficient of variation (CV) > 18% were re-analyzed. The intra- and inter-assay CV were determined to be 21.3% and 24.5%, respectively.

Plasma Substance P

Substance P (SP) concentrations were determined through RIA using methods described by Van Engen et al. (2014). The standard curve, ranging from 20 to 1,280 pg/mL, was created by diluting synthetic SP (Phoenix Pharmaceuticals, Burlingame, CA) with RIA Buffer (50 mM sodium phosphate dibasic heptahydrate, 13 mM disodium EDTA, 150 mM sodium chloride, 1 mM benzamidine hydrochloride, 0.1% gelatin, 0.02% sodium azide; pH 7.4). For analysis, 100 µL of sample, standard, or QC were aliquoted into plain 12 × 75 mm conical bottom tubes followed by 100 µL of Rabbit anti-SP primary antibody (1:20,000; Phoenix Pharmaceuticals). Iodine-125-SP tracer (custom iodination by PerkinElmer) was diluted with RIA buffer to 20,000 cpm, then 100 µL was added to the sample, standard, and QC tubes. Samples were then covered and stored at 4 °C for 48 h. At the end of the 48 h incubation, samples were placed on ice and 100 µL of normal rabbit plasma (1:80) and goat anti-rabbit secondary antibody (1:40; Jackson ImmunoResearch, West Grove, PA) were added to each tube. Samples were then incubated at room temperature for 10 min, placed back on ice, and 100 µL of blank bovine plasma was added to the standards and QCs. All tubes then had 1 mL of 12% polypropylene glycol in 0.85% sodium chloride added. Samples were centrifuged at 3,000 × *g* for 30 min at 4 °C and the supernatant aspirated. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a CV > 18% were re-analyzed. The intra- and interassay CV were determined to be 18.8% and 30.02%, respectively.

Visual Lameness Scoring

A previously described VLS (Deeming et al., 2018) was used by an observer trained in lameness identification and severity. Each goat was scored at the following timepoints: prior to induction (–24), 4, 6, 12, 24, 48, and 72 h. Animals were walked through an alley system with non-slip flooring while traveling to the pressure mat system. Lameness was graded on a scale from 1 to 5 (1-normal gait, 2-uneven gait, 3-mildly lame, 4-moderately lame, and 5-severly lame).

Visual Analogue Scale

A VAS for pain was used to score each goat at each timepoint (prior to induction (–24), 4, 6, 12, 24, 48, and 72 h). An observer skilled in recognizing pain indicators scored each goat as they walked across the pressure mat walkway. Pain was graded on a scale from 1 to 5 (1-no pain, 2-slight pain, 3-mild pain, 4-moderate pain, and 5-severe pain). Pain was also graded with a 100-mm (10 cm) line anchored at each end by descriptors of "No Pain" or "Severe Pain". The evaluator marked the line between the 2 descriptors to indicate pain intensity. A millimeter scale was used to measure the score from the zero-anchor point to the evaluator's mark. Seven parameters were used to assess pain: depression, tail swishing or flicking, stance, head carriage, spinal alignment, movement, and ear carriage adapted from Martin et al. (2020). No pain was characterized by being alert and quick to show interest, no tail swishing, a normal stance, head carriage above spine level, a straight spine, moving freely around the pen and ears forward. Severe pain was characterized by being dull and showing no interest, more than three tail swishes per minute, legs abducted, head held below spine level, a curved spine, reluctant to move, and ears down.

Statistical Analysis

Plasma cortisol was log transformed for normality prior to statistical analysis. The outcome responses of IRT, KGA, and plasma cortisol were analyzed using linear mixed models with goat as the experimental unit. Goats nested in a treatment group were designated as a random effect, with treatment, time, treatment by time interaction, and replicate designated as fixed effects.

Visual lameness scores were treated as categorical data and Fisher's exact test was used to compare scores. All statistics, except for behavior data, were performed using statistical software (JMP Pro 14.0, SAS Institute, Cary, NC, USA). Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment. Statistical significance was set at $P \leq 0.05$.

Behavior results were analyzed using a generalized linear mixed model (GLIMMIX) with a beta distribution, including timepoint, treatment, group, and treatment by timepoint interaction in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC, USA). Time was a repeated measure and group was designated as a random effect, with goat as the experimental unit. The same parameters were used to analyze results of the GGS, using a mixed model in SAS. Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment. Statistical significance was set at ($P \leq 0.05$).

Results

Behavior Scoring

Goats in the HH and LL groups spent significantly more time grooming across the observation period than goats in the HL group ($P = 0.02$) (Table 2). Similarly, goats in the HH and CNTL groups scratched their bodies using their horns or rear hooves more than HL goats ($P = 0.01$). Control goats were also observed licking the surfaces of their pen significantly more than HL goats ($P = 0.02$). All Amp B treatments were successful in inducing behavioral changes associated with lameness. Notably, the incidence of limping and postural alteration (standing on three legs) across the Amp B treatment goats did not differ significantly ($P > 0.05$).

Irrespective of treatment, goats spent significantly more time standing and drinking at baseline than at all other post-lameness induction time points ($P < 0.05$). Comparing only the post-lameness induction time points, goats spent significantly less time standing at 6 and 12 h than at 72 h ($P = 0.02$). Likewise, all goats spent significantly more time lying at 6 h post-lameness induction compared to 72 h ($P = 0.008$). The duration of standing and lying behavior did not return to baseline levels until 72 h post-lameness induction. Less maintenance behavior (grooming) was observed 4 h post-lameness induction compared to 24 and 72 h ($P < 0.0001$). Goats also ate significantly less at 24 h compared to 72h post-lameness induction ($P = 0.02$). None of the goats were seen exhibiting restless behavior, limping, or standing on three legs prior to lameness induction.

A few behavioral differences were found between the two groups of goats in this study. Compared to the goats in group 1, group 2 goats spent more time standing ($P = 0.001$), standing on three legs ($P = 0.02$), walking ($P = 0.03$), less time lying ($P = 0.008$), scratching (P = 0.01), and rearing up on their home pen ($P = 0.02$). Group 1 goats spent significantly

Table 2. Pre- and post-treatment proportional mean durations (presented in seconds ± SE) of behavioral analysis outcomes across all time points for goats with experimentally induced lameness using Amp B in the following dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 ml (LL: $n = 6$), or a control induction using 0.5 mL of physiological saline (CNTL; $n = 6$)

Behavior[c]	Pre-lameness induction		Post-lameness induction					
	Trt *P*-value	Pre-reatment	Trt *P*-value	Time *P*-value	HH ($n = 6$)	HL ($n = 6$)	LL ($n = 6$)	CNTL ($n = 6$)
Grooming	0.47	108.8 ± 22.2	0.02	0.0004	120.4 ± 13.8[a]	60.9 ± 14.7[b]	113.0 ± 13.6[a]	111.7 ± 13.6[ab]
Scratching	0.51	49.7 ± 16.1	0.01	0.43	55.9 ± 7.2[a]	22.8 ± 7.8[b]	47.5 ± 7.3[ab]	52.2 ± 7.0[a]
Lying	0.60	734.6 ± 436.5	0.15	0.005	1132.0 ± 115.7	1137.0 ± 107.0	914.2 ± 106.2	858.6 ± 110.8
Standing	0.70	1634.6 ± 90.8	0.20	< 0.0001	797.5 ± 120.4	680.7 ± 129.2	868.5 ± 109.8	1035 ± 108.1
Licking	0.88	33.3 ± 8.7	0.02	0.03	16.6 ± 11.1	—	15.9 ± 12.7	38.0 ± 7.7
Eating	0.69	132.1 ± 53.1	0.12	0.02	342.7 ± 48.4	234.3 ± 49.2	321.0 ± 45.7	210.5 ± 43.3
Drinking	—[d]	135.5 ± 22.6	0.09	0.001	21.9 ± 7.0	—	11.3 ± 4.3	23.0 ± 4.2

[a,b]Means with different superscripts in the same row differ significantly ($P < 0.05$).
[c]Only behavior variables that were significant post-treatment are presented.
[d]Dash indicates behavior was not observed.

more time attentive to their affected hoof than group 2 goats ($P = 0.02$).

Infrared Thermography

Maximum IRT temperatures did not differ by treatment ($P = 0.08$) or treatment over time interaction ($P = 0.14$) but did differ by timepoint ($P \leq 0.0001$) (Fig 3). Differences in temperature were consistently higher between LH (affected limb) and RH (control limb) when compared with baseline across all treatment groups at the 48 h timepoint only (HH: 5.03 °C [95% CI: (2.65 to 7.41) °C], (HL: 4.10 °C [95% CI: (1.72 to 6.48) °C], (LL: 6.08 °C [95% CI: (3.70 to 8.47) °C], and (CNTL: 2.48 °C [95% CI: (0.10 to 4.87) °C]. There were no treatment effects observed for the difference in the maximum temperature between the left distal limb and right distal limb ($P = 0.08$).

Mechanical-Nociception Threshold

MNT differed by treatment ($P = 0.0023$) and timepoint ($P \leq 0.0001$), but a treatment over time interaction was not observed ($P = 0.40$; Fig 4). The HL, HH, and LL groups were significantly higher when compared with the CNTL group when evaluating difference in MNT response between LH (affected limb) and RH (control limb) (HH: −1.14 KgF [95% CI: −1.37 - (−0.91) kgF], (HL: −1.18 KgF [95% CI: −1.42 - (−0.94) KgF], (LL: −1.09 KgF [95% CI: −1.34 - (−0.86) KgF], and (CNTL: −0.54 KgF [95% CI: −0.78 - (−0.31) KgF]. All animals across all treatment groups had higher measurements across 24, 48, and 72 h timepoints when compared with baseline measurements (−24 h; $P < 0.05$).

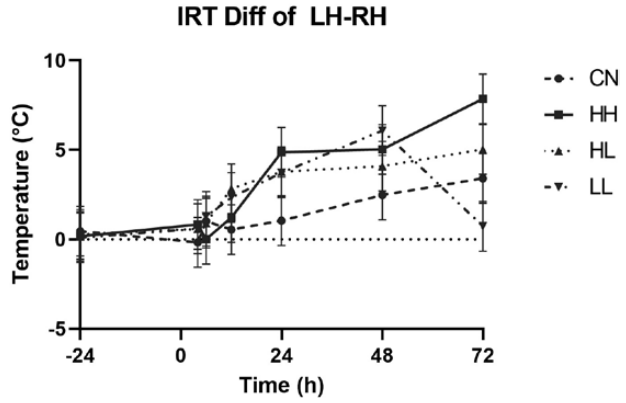

Figure 3. Mean (±SE) maximum temperatures (°C) from infrared thermography for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 mL (LL: $n = 6$) or a control induction using 0.5 mL of physiological saline (CNTL; $n = 6$).

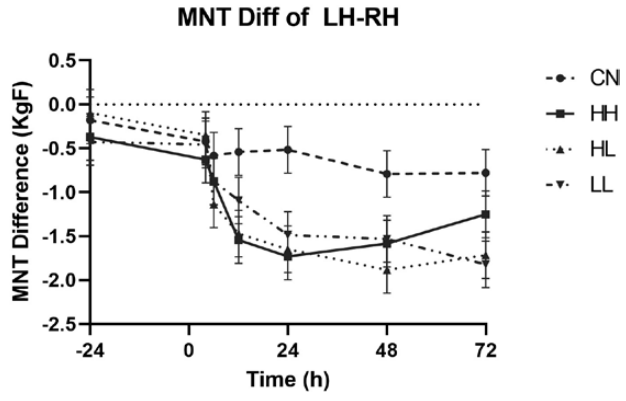

Figure 4. Mean (±SE) mechanical-nociception threshold differences between LH–RH for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 mL (LL: $n = 6$) or a control induction using 0.5 mL of physiological saline (CNTL; $n = 6$).

Plasma Cortisol

Plasma cortisol did not differ by treatment ($P = 0.87$) but did differ by timepoint ($P = 0.01$) and treatment over time interaction ($P = 0.04$) (Fig 5). Cortisol levels were highest at 12 h [10.28 ng/mL (95% CI:7.60 to 12.95 ng/mL)] and at 24 h [7.77 ng/mL (95% CI:5.10 to 10.45 ng/mL)] when compared with baseline [6.09 ng/mL (95% CI: 3.41-8.76 ng/mL)].

The HH treatment group had the highest cortisol levels at 12 and 24 h [13.19 ng/mL (95% CI: 7.96 to 18.41 ng/mL)] and [10.55 ng/mL (95% CI: 5.33 to 15.78 ng/mL)] compared to the HL treatment group [7.01 ng/mL (95% CI: 1.78 to 12.24 ng/mL)] and [7.04 ng/mL (95% CI: 1.82 to 12.27 ng/mL)], the LL treatment group [12.71 ng/mL (95% CI: 7.48 to 17.93 ng/mL)], and [6.04 ng/mL (95% CI: 0.33 to 11.76 ng/mL)], and the CNTL group [8.20 ng/mL (95% CI: 2.50 to 13.91 ng/mL)] and [7.45 ng/mL (95% CI: 2.23 to 12.68 ng/mL)] at the same timepoints (12 and 24 h).

Plasma Substance P

Plasma substance P did not differ by treatment ($P = 0.50$), timepoint ($P = 0.14$), or treatment over time interaction ($P = 0.76$; Fig 6). The overall mean for plasma substance P by treatment was reported to be CNTL [390.33 pg/mL (95% CI: 233.39 to 547.27 pg/mL)], HH [429.49 pg/mL (95% CI: 272.55 to 586.43 pg/mL)], HL [390.73 pg/mL (95% CI: 233.78 to 547.67 pg/mL)], and LL [554.85 pg/mL (95% CI: 397.91 to 711.79 pg/mL)], respectively.

Kinetic Gait Analysis

Definitions of KGA parameters are in Table 3. KGA outcomes are shown in Table 4.

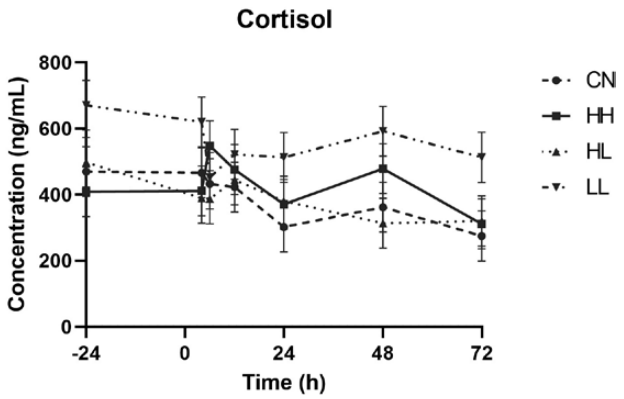

Figure 5. Mean (±SE) plasma cortisol concentrations (ng/mL) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 mL (LL: $n = 6$) or a control induction using 0.5 mL of physiological saline (CNTL; $n = 6$).

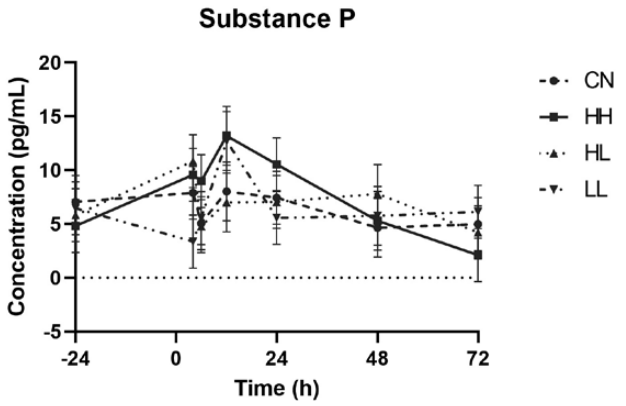

Figure 6. Mean (±SE) plasma substance P concentrations (ng/mL) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: *n* = 6), 5 mg/0.25 mL (HL: *n* = 6), 2.5 mg/0.25 mL (LL: *n* = 6) or a control induction using 0.5 mL of physiological saline (CNTL; *n* = 6).

Table 3. Definitions of KGA biomechanical markers

Outcome	Definition (unit)
Stance time	The time that passes in a gait cycle of one extremity (s)
Stride length	The distance between two successive placements of the same extremity (cm)
Force	The maximum force measured for a single step from a single extremity (kg)
Impulse	The maximum force applied per unit of time measured (kg × s)
Contact area	The peak pressure measured from a singular footfall (kg/cm^2)

Stance Time (s) Left distal limb stance time did not differ by treatment ($P = 0.17$) or treatment over time interaction ($P = 0.08$) but did differ by timepoint ($P = 0.01$). At 12 h, left distal limb stance time was reported as [25s (95%CI: 1 to 35 s)]. Stance time peaked at 72 h [32 s (95% CI: 15 to 88 s)] when compared to baseline measurements (–24) [18 s (95% CI: 13 to 24 s)]. Stance time increased among all treatment groups when compared to CNTL after lameness induction.

The right distal limb stance time did not differ by timepoint ($P = 0.13$), treatment ($P = 0.08$), or treatment over time interaction ($P = 0.43$).

Stride Length (cm) Left distal limb stride length did not differ by treatment ($P = 0.32$) or treatment over time interaction ($P = 0.29$) but did differ by timepoint ($P = 0.0065$). Stride length was shortest at 48 h [64.43 cm (95% CI: 54-74 cm)] and longest at 6 h [86.12 cm (95% CI: 76 to 95 cm)] when compared with baseline measurements (–24) [84.50 cm (95% CI: 74 to 94 cm)].

Right distal limb stride length did not differ by treatment ($P = 0.48$) or treatment over time interaction ($P = 0.26$) but did differ by timepoint ($P = 0.002$). Stride length was shortest at 12 h [68.64 cm (95% CI: 60 to 77 cm)], and longest at 48 h [81.52 cm (95% CI: 73 to 90 cm)] when compared to baseline measurements (–24) at [94.68 cm (95% CI: 86 to 103 cm)].

Force (kg) The left distal limb force did not differ by treatment over time interaction ($P = 0.91$) but did differ by treatment ($P = 0.0034$) and timepoint ($P = 0.0002$). HH treatment had the lowest force for left hind (treated limb) [1.91 kg (95% CI:1.47 to 2.35 kg)]. All treatments were lower in force when compared to CNTL [2.98 kg (95% CI: 2.54 to 3.41 kg)]. Force was lowest at 48 h [1.85 kg (95% CI: 1.27 to 2.42 kg)],

Table 4. Overall mean (95% confidence interval) outcome measures from KGA in goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: *n* = 6), 5 mg/0.25 mL (HL: *n* = 6), 2.5 mg/0.25 mL (LL: *n* = 6) or a control induction using 0.5 mL of physiological saline (CNTL; *n* = 6)

Parameter	HH (*n* = 6)	LS[1] means			*P*-value		
		HL (*n* = 6)	LL (*n* = 6)	CNTL (*n* = 6)	Treatment	Time	Treatment × time
Left hind foot							
Stance time, s	0.27 (0.23-0.31)	0.21 (0.17-0.25)	0.25 (0.21-0.29)	0.25 (0.21-0.29)	0.17	0.01	0.08
Stride length, cm	74.29 (66.87-81.92)	81.86 (74.44-89.29)	73.54 (66.12-80.96)	79.53 (72.11-86.95)	0.33	0.0065	0.29
Force, kg	1.91 (1.48-2.35)	2.87 (2.43-3.31)	2.70 (2.27-3.14)	2.98 (2.55-3.42)	0.003	0.0002	0.91
Impulse, kg × s	0.32 (0.24-0.41)	0.44 (0.35-0.53)	0.46 (0.37-0.55)	0.46 (0.38-0.55)	0.086	0.64	0.33
Contact pressure, kg/cm^2	1.26 (1.07-1.45)	1.47 (1.29-1.66)	1.61 (1.43-1.80)	1.47 (1.28-1.66)	0.07	0.036	0.71
Right hind foot							
Stance time, s	0.28 (0.22-0.33)	0.23 (0.18-0.28)	0.31 (0.26-0.37)	0.23 (0.18-0.28)	0.08	0.13	0.43
Stride length, cm	78.87 (72.44-85.29)	82.66 (76.24-89.08)	79.38 (72.96-85.81)	75.47 (69.05-81.90)	0.48	0.0021	0.26
Force, kg	2.10 (1.68-2.52)	2.77 (2.35-3.19)	2.24 (1.82-2.66)	2.45 (2.02-2.87)	0.14	0.43	0.032
Impulse, kg × s	0.39 (0.31-0.47)	0.46 (0.38-0.54)	0.44 (0.36-0.52)	0.38 (0.30-0.46)	0.38	0.56	0.01
Contact pressure, kg/cm^2	1.36 (1.22-1.51)	1.43 (1.29-1.57)	1.44 (1.30-1.58)	1.19 (1.05-1.33)	0.05	0.87	0.03

[1]Least squares.

but all timepoints were lower when compared to baseline measurements (–24 h) [3.55 kg (95% CI: 2.97 to 4.12 kg)].

The right distal limb force did not differ by treatment ($P = 0.14$) or timepoint ($P = 0.43$) but did differ by treatment over time interaction ($P = 0.03$). Right hind foot force for the HL group was significantly higher at 12 and 48 h [3.79 kg (95% CI: 2.68 to 4.91 kg)] and [3.48 kg (95% CI: 2.37 to 4.60 kg)], respectively) compared with baseline (–24), 4, 6, 24, and 72 h (≤3.06 kg; $P = 0.03$). Average force values for CNTL at 12 and 48 h were [1.83 kg (95% CI: 0.71 to 2.94 kg)] and [2.22 kg (95% CI: 2.36 to 4.60 kg), respectively] ($P = 0.03$).

Impulse (kg × s) Left hind hoof impulse did not differ by treatment ($P = 0.09$), timepoint (P = 0.63), or treatment over time interaction ($P = 0.33$).

Right hind hoof impulse did not differ by treatment ($P = 0.38$) or timepoint ($P = 0.56$) but did differ by treatment over time interaction ($P = 0.01$). Right hind hoof impulse for the HL group was significantly higher at 12 [0.76 kg × s (95% CI: 0.54 to 0.97 kg × s)], 24 [0.56 kg × s (95% CI: 0.34 to 0.77 kg × s)]and 48 h [0.60 kg × s (0.38 to 0.81 kg × s) compared with –24, 4, 6, and 72 h (≤0.42 kg × s, respectively; $P = 0.01$). Average impulse values for CNTL at 12, 24, and 48 h [0.21 kg × s (95% CI: 0.004 to 0.42 kg × s)], [0.31 kg × s (95% CI: 0.10 to 0.52 kg × s), and [0.45 kg × s (95% CI:0.24 to 0.67 kg × s)], respectively; $P = 0.01$).

Contact Pressure (kg/cm²) Left hind hoof contact pressure did not differ by treatment ($P = 0.07$) or by treatment over time interaction. ($P = 0.71$) but did differ by timepoint ($P = 0.03$). Contact pressure was highest at 6 h [1.77 kg/cm² (95% CI: 1.52 to 2.02 kg/cm²)] and was lowest at 48 h [1.15 kg/cm² (95% CI: 0.90 to 1.4 kg/cm²)].

Right hind hoof contact pressure did not differ by treatment ($P = 0.054$) or by timepoint ($P = 0.87$); however, treatment over time interactions were significant ($P = 0.03$). Right hind hoof contact pressure for the HL group was significantly higher at 12 [1.92 kg/cm² (95% CI:1.55 to 2.29 kg/cm²)] and 48 h [1.66 kg/cm² (95% CI:1.10 to 1.85 kg/cm²)] compared with –24 h, 4, 6, 24, and 72 h (≤1.60 kg/cm²) ($P = 0.03$). Average force values for CNTL at 12 and 48 h [1.02 kg/cm² (95% CI: 0.64 to 1.39 kg/cm²)] and [1.04 kg/cm² (95% CI:0.67 to 1.41 kg/cm²)], respectively ($P = 0.03$).

Visual Lameness Scoring

Visual lameness scores differed only at 48 and 72 h post-lameness induction between all treatment groups (Fig 7). Differences included an increase or decrease in the number of

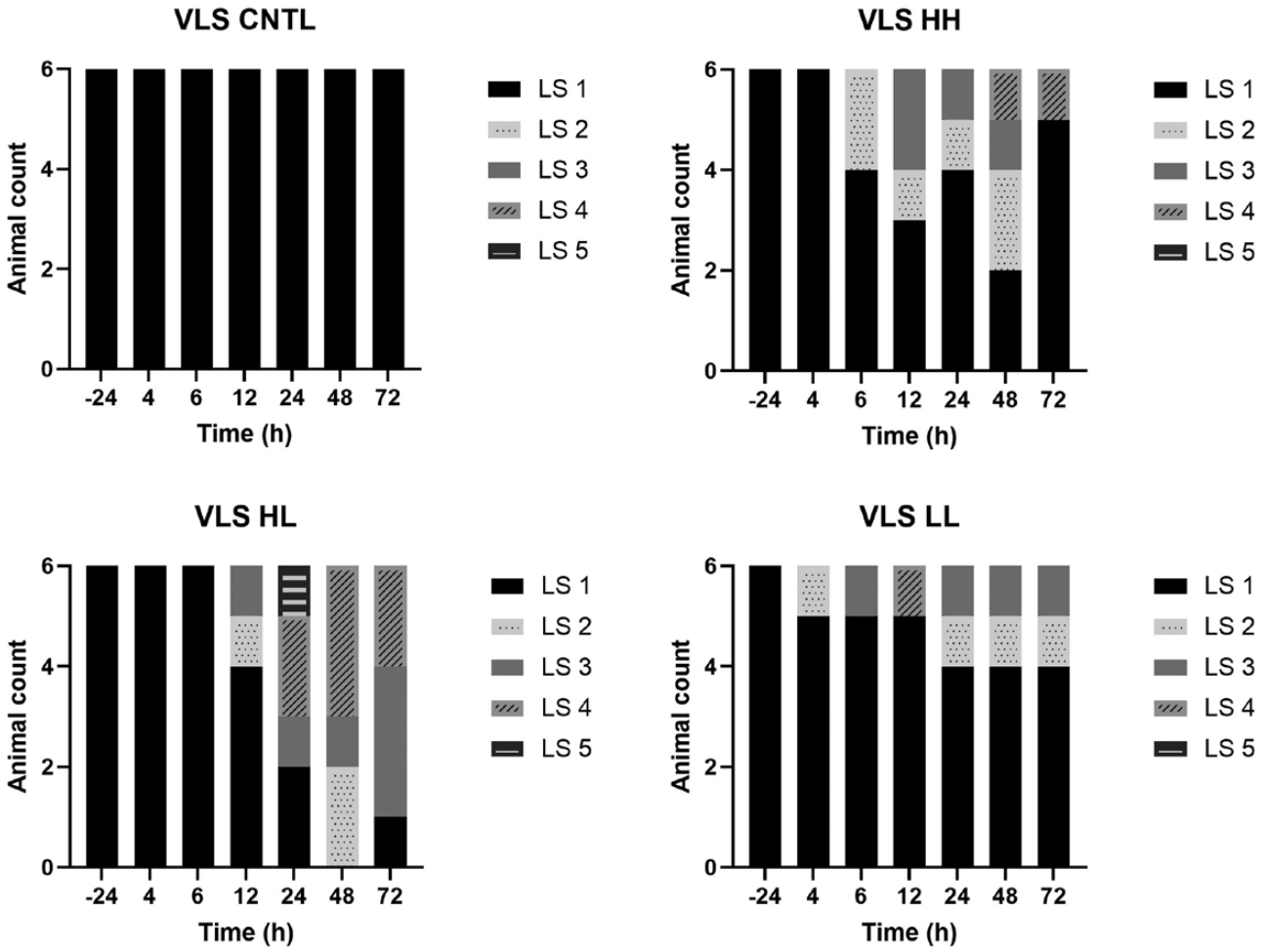

Figure 7. Visual lameness scores for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 mL (LL: $n = 6$) or a control induction using 0.5mL of physiological saline (CNTL; $n = 6$).

individuals at a lameness score of level 2 or above. The HH treatment group had four individuals at a level 2 or higher at 48 h. At 72 h, there was only one individual at a grade 2 or higher in the HH treatment group. The HL treatment group had six individuals at a grade 2 or higher at 48 h. At 72 h, there were five individuals at a grade 2 or higher in the same treatment group. The LL treatment group had two individuals at a grade 2 or higher at 48 and 72 h. The CNTL group had no individuals that scored above a grade 1 at any timepoint during the duration of the study.

Peak lameness severity was noted at 48 h post-induction across all three treatment groups (HH, HL, and LL). Five of the six individuals in the HH treatment group were sound (no lameness observed) by 72h. One of six individuals was sound by the 72 h mark in the HL group. Four of six individuals in the LL group were sound by the 72 h mark.

Visual Analogue Scoring

The VAS outcomes differed by treatment ($P = 0.04$), timepoint ($P < 0.0001$), and treatment over time interaction ($P = 0.0003$) (Fig 8). Treatment groups did not differ at 4, 6, and 12 h post-induction. The HL treatment group differed from all groups at 24, 48, and 72 h post-induction. All other treatment groups differed from the CNTL group only at the 24 h post-induction. The second replicate group (group 2) was less lame when compared to the first replicate (group 1) (1.5 vs 0.4 cm).

Facial Grimace Score

The ICC between the two observers was found to be extremely poor (0.19); therefore, the GGS results were not pooled between observers and were analyzed and interpreted independently. Observer 1 was much more experienced in facial grimace development and assessment across multiple livestock species than Observer 2.

Observer 1: There was a significant time effect ($P = 0.05$), with goats exhibiting more facial grimacing at 24 h post-induction (2.47 ± 0.22) compared to baseline (1.36 ± 0.23; $P = 0.01$). A significant treatment effect was also found ($P = 0.02$), with goats in the HH treatment group grimacing more (2.36 ± 0.17) than goats in the LL treatment group (1.63 ± 0.17; $P = 0.02$). Group 1 goats grimaced significantly more (2.14 ± 0.12) than group 2 goats (1.74 ± 0.12; $P = 0.02$).

Observer 2: There was a significant time effect ($P = 0.006$), with goats exhibiting more facial grimacing at 24 h post-induction (2.79 ± 0.21) compared with baseline observations (1.65 ± 0.20; $P = 0.003$).

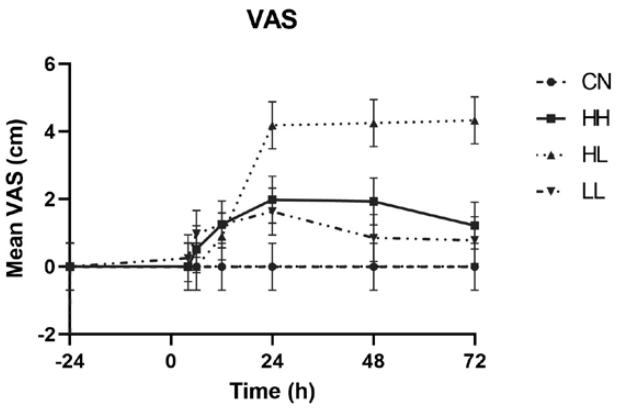

Figure 8. Visual analog scores (VAS) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: $n = 6$), 5 mg/0.25 mL (HL: $n = 6$), 2.5 mg/0.25 mL (LL: $n = 6$), or a control induction using 0.5 mL of physiological saline (CNTL; $n = 6$).

Discussion

This study demonstrated that the use of Amp B induces temporary, acute lameness in goats. Lameness among individuals was defined using parameters including GGS, VLS, VAS, IRT, MNT, KGA, CORT, and Sub P. Behavioral changes were also noted between the three Amp B treatment groups and the CNTL group.

Amphotericin B has been used to induce transient lameness in other species, including cattle and pigs (McIlwraith et al., 1979; Schulz et al., 2011; Coetzee et al., 2014; Muley et al., 2016). When compared with the CNTL group, Amp B produced consistent lameness in all three treatment groups when used in goats (18/24). No individuals on study received any type of rescue therapy (pain mitigation therapy (Meloxicam 1 mg/kg or pulled off study). Results from this study are consistent with severity and onset of lameness caused by Amp B when used in cattle with goats and cattle having shorter lameness durations compared to horses (Schulz et al., 2011; Coetzee et al., 2014). This provides justification for the need of species-specific lameness protocol treatments.

Notable behavioral changes are caused by lameness in goat and sheep species. Behaviors include, but are not limited to, longer lying bouts, a decrease in appetite, and abnormal social interactivity within a herd (Galindo and Broom, 2002; Bach et al., 2007; Ito et al., 2010; Blackie et al., 2011). In a previous lameness study in sheep, lame sheep and non-lame sheep displayed differences in walking, standing, and lying behaviors (Kaler et al., 2020). Goats displayed differences in walking, standing, and lying behaviors that were similar to behavior patterns shown in sheep in the aforementioned study (Kaler et al., 2020). Goats in this study also appeared restless in nature or vocalized more when compared to baseline (–24 h) behaviors. From an animal welfare standpoint, behavioral indicators in response to pain are crucial for establishing effective treatment protocols for lame animals.

This is the first paper to describe a facial grimace scoring system in goats. The facial grimace scoring system is a viable and novel method to better understand pain responses in goats. This is especially important in individuals that do not look similar due to breed differences or crossbreeding. A grimace scale for sheep has been characterized before to assess pain induced facial expressions and was used as a reference in developing the Goat Grimace Scale in this study (Hager et al., 2017, Viscardi et al., 2021). The sheep grimace scale developed by Hager et al. (2017) was a reliable method to evaluate pain responses in sheep after surgery. Higher SGS were seen after one day post op compared to baselines in sheep on study. Higher facial grimacing scores compared to baselines were also seen after lameness induction in goats on this study, supporting that grimace scoring systems are useful in helping determine pain response behaviors. Higher grimace scores are indicative of an individual experiencing a higher grade of pain. Facial grimacing scores in this study were highest across all treatment groups at 24 h when compared to prior to baseline (–24 h). Results from this

outcome measure supplied a novel way for researchers to quantify pain behavior in goats, leading to better identification and treatment of lame goats.

Objective measures of lameness detection used in this study included IRT and KGA. IRT was recently validated for detecting lameness associated with temperature differences in sheep with foot lesions (Byrne et al., 2019). Higher temperatures were noted in diseased hooves when compared to healthy hooves (Byrne et al., 2019). Similarly, higher temperatures have been reported in the hooves of dairy cattle with amphotericin induced lameness when compared to non-lame cattle (Kleinhenz et al., 2019; Warner et al., 2021). Our study identified similar higher thermal changes between lame (Amp B) hooves compared to CNTL, suggesting that IRT was a reliable method for detecting induced lameness in goats. Higher thermal changes are indicative of a greater inflammatory response in body tissue, which is commonly seen in lame animals.

KGA was recently evaluated as a tool for characterizing gait in healthy goats (Rifkin et al., 2019). KGA demonstrated a difference between the left hind (treated limb) and right hind (untreated limb) across timepoints for parameters including stance time, stride length, and force. When comparing KGA to a previous pilot study (Reppert et al., 2020), stance time in this study was overall shorter in time between both left hind and right hind. Stride length was overall shorter in length on the left hind when compared to the previous pilot study (Reppert et al., 2020). Force, impulse, and contact pressure were all smaller in value on both limbs (left affected and right control) when compared with previous results by Reppert et al. (2020).

Plasma cortisol has been used as a measure of stress and inflammation associated with lameness induction in other livestock species (Coetzee et al., 2014). In a previous pilot study (Reppert et al., 2020), there was no evidence of differences in mean cortisol concentrations between treatment groups. Evidence from this study suggests that there is a treatment by timepoint effect seen between all three Amp B treatment groups and CNTL ($P = 0.04$). This suggests that cortisol values were positively or negatively affected based on the lameness induction treatment randomly assigned to each individual on trial.

Substance P is a recognized neuropeptide shown to be involved with the pain integration within areas of neuroaxis (Devane, 2001). There is no published data regarding substance P in either naturally occurring lameness or following induction in lameness models in goats. Substance P is a neuropeptide that has been shown to be associated with nociception in cattle (Coetzee et al., 2008). In a previous lameness study in cattle (Kleinhenz et al., 2019), there was no evidence to suggest treatment differences concerning substance P concentrations. In horses, synovial concentrations of substance P were elevated in joints with osteoarthritis compared to non-diseased joints. Substance P levels have also been correlated to prostaglandin E_2 concentrations in arthritic joints (Kirker-Head et al., 2000). Substance P results from this study highlight that there were no treatment differences between all three Amp B treatments when compared to CNTL.

When comparing the previous pilot study (Reppert et al., 2020), results from this trial showed several lameness inducing similarities in goats. In the pilot study (Reppert et al., 2020), a single dose of Amp B (10 mg/mL) was prepared for injection to individuals. In the current study, three varying dosages of amphotericin B (5 mg/0.25 mL, 0.25 mg/0.5 mL, 2.5 mg/0.25 mL) were prepared to assess their associated levels of lameness. The objective of varying dosages of Amp B was to determine an optimal dose for inducing lameness, while avoiding making individuals severely lame (VLS Score of 4+) or lame for an extended period (48 h + duration). The pilot study conducted prior to this trial (Reppert et al., 2020) produced severely lame goats (VLS score of 4+), which was half of the individuals ($n = 3/6$) on study. Many of these individuals required rescue analgesia given the level of severity and duration of lameness (VLS > 2, 48 h + duration, prolonged lying bouts, depression, and reduced feed intake). When comparing varying doses of Amp B in our trial, no goats exhibited lameness to the degree that required rescue intervention procedures when compared with Reppert et al. (2020).

Varying dosages and volumes of Amp B allowed us to evaluate and compare which treatment protocol would best induce lameness in goats for a brief period of 3 d. While all three treatments produced lame individuals, the treatment that produced lame goats most consistently was the HL treatment group (5 mg/0.25 mL). This allowed researchers to induce lameness in all six goats with a smaller volume that needed to be administered into the intra-articular joint.

Conclusions

The first objective of this study sought to assess three different treatments of Amp B to determine which model was optimal for lameness induction in goats. Amphotericin B was able to induce acute lameness in meat goats across all three Amp B treatment groups, but only the HL treatment induced lameness in all six goats. The development of a goat grimace scale was also proven to be effective during this study to evaluate facial expressions in response to pain in goats. Lameness was characterized by using VLS, VAS, IRT, MNT, KGA, behavior, and facial grimace assessment. This study is one of only a few that has sought to investigate lameness concerns in growing goat populations. Limitations in this study include the number of individuals in each treatment group, and the overall number of individuals. A future study involving a larger population and a longer observation period is needed to further prove the efficacy of Amp B in consistently inducing acute lameness.

Acknowledgments

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Conflict of Interest Statement

The author and co-authors do not have any conflicts of interest to disclose.

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