

Blood Cortisol as an Objective Tool to Measure Painful and Non-painful Hoof Lameness States in Multiparous Sows

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Summary and Implications

The objective of this study was to compare blood plasma cortisol concentrations from sows in painful and non-painful hoof lameness states. Ten, clinically healthy, mixed-parity, crossbred sows were used. The sow was the experimental unit and a cross-over design with a 2 (legs) x 3 (days) factorial arrangement of treatments were compared. On D 0, Ten mg of amphotericin B were injected in the distal interphalangeal joint space in both claws of one rear foot. All sows served as their own control and treatment. After completion of the first round, sows were given a 10-d rest period and then the trial was repeated. Blood cortisol was measured on *sound* (D-1; 1 day before induction), *most lame* (D+1; first day after injection of amphotericin B to induce lameness) and *resolved day* (D+6; 6th day after the induction of lameness). Cortisol sample analysis was completed with the IMMULITE® 1000 cortisol assay. All data were statistically analyzed using the PROC MIXED procedure in SAS. A *P* value of ≤ 0.05 was considered to be significant. No differences were observed for foot (*P* = 0.78) or round (*P* = 0.86). There was no difference in cortisol levels between D -1 and D +6 (*P* = 0.35), but sows expressed higher cortisol concentrations on most lame day (D +1; Figure 3; *P*=0.0013). In conclusion, lameness induced with amphotericin B was associated with elevated plasma cortisol levels relative to baseline (day-1) and resolution (day+6).

Introduction

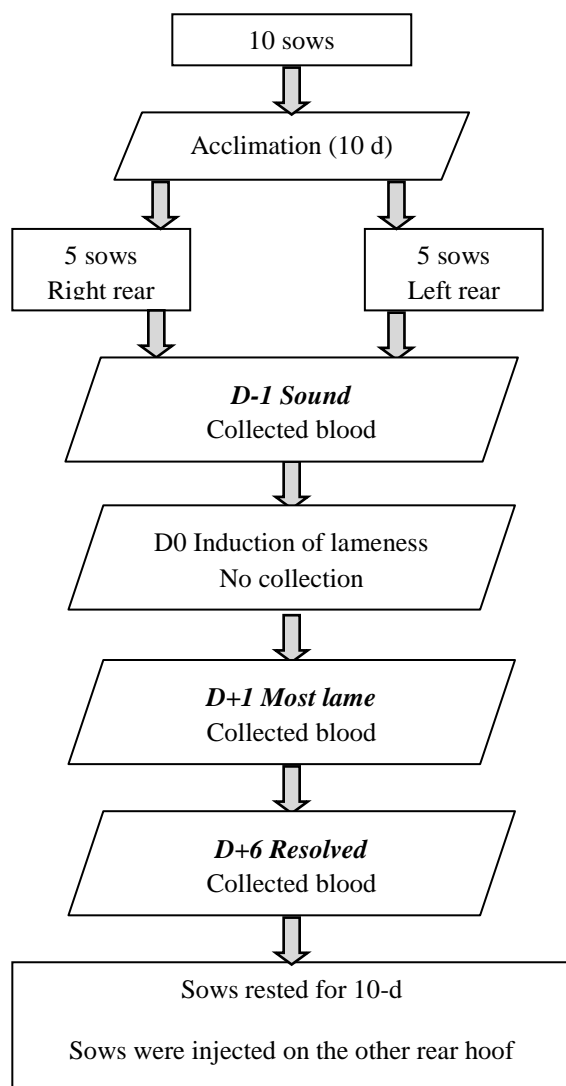
There are currently no analgesic drugs specifically approved for pain relief in livestock by the U.S. Food and Drug Administration (FDA). The FDA Guidance Document 123 for the development of effectiveness data for non-steroidal anti-inflammatory drugs (NSAIDs) states that “*validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in the target species.*” As a result, identification and validation of robust, repeatable pain measurements are essential for the development and approval of analgesic drug regimens for use in food animals. Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. In previous studies, cortisol concentration has been used to assess stress status in livestock. The objective of this study was to compare concentrations of blood cortisol in multiparous sows in painful and non-painful hoof lameness states.

Materials and Methods

Animals and housing: This project was approved by the Iowa State University IACUC. Ten, apparently healthy, mixed-parity, crossbred sows were purchased from a commercial producer in Iowa. To avoid injury due to aggression, sows were housed in individual pens, measuring 3.7 m length x 1.4 m width x 1.2 m height. A rubber mat was provided for sow comfort. All sows were fed twice daily to meet their dietary requirements. Sows had *ad libitum* access to water via one nipple waterer. Metal fences were affixed at the end of each home pen and lights were on a 12:12 light dark cycle (light hours were between 0600 and 1800). Sows were acclimated for 10 days before any treatments were applied. The research was conducted October-November 2011.

Experimental design and treatments: The sow was the experimental unit. A cross over design with a 2 (feet) x 3 (days) factorial arrangement of treatments were compared. Three day treatments: *sound* (D-1 baseline), *most lame* (D+1 after induction of lameness occurred with amphotericin B) and *resolved* (D+6 after the induction of lameness) and two feet treatments: *left* vs. *right*. All sows served as their own control. After completion of the first round, sows were given a 10-day rest period and then the trial was repeated (Figure 1).

Figure 1. Example of a round



Induction of Lameness: All sows were restrained in a standing position using a humane pig snare and then anesthetized using a combination of Xylazine (4.4 mg/kg), Ketamine HCl (2.2 mg/kg), and Tiletamine HCL and Zolazepam in combination (Telazol[®]; 4.4 mg/kg) administered IM. The assigned claws to be injected were washed with mild soap and water to remove obvious fecal contamination, scrubbed for 3 min with iodine based surgical scrub using 10 x 10 cm sterile gauze pad, and rinsed with 70% isopropyl alcohol until no evidence of the surgical scrub remains. Ten mg of amphotericin B were injected in the distal interphalangeal joint space in both claws of one rear foot. All sows were monitored continuously until, fully recovered.

Blood Collection: Sows were restrained in a standing position using a humane pig snare. Blood was collected from the jugular vein between the hours 1500 and 1700 on -1 day

+1 and day +6, respectively. A total of 5ml of blood was collected and immediately placed into a glass vacutainer plain serum blood collection red top tube, centrifuged at 1,500xg for 15 min at 10°C. After centrifugation, plasma was removed and stored at -80°C prior to analysis.

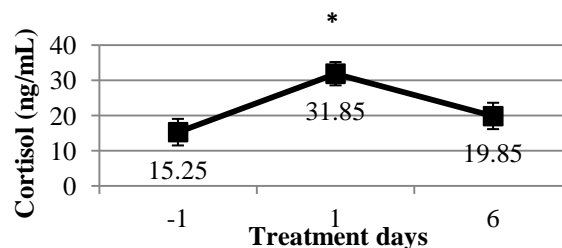
Cortisol Assay: Cortisol sample analysis was completed with the IMMULITE[®] 1000 cortisol assay. The IMMULITE assay is a solid phase, competitive chemiluminescent enzyme immunoassay.

Statistical analysis: Data were analyzed using the PROC MIXED procedure in SAS for parametric data. The main effects of day, foot, round and the interactions were compared. The interactions were not significant and were removed. A *P* value of ≤ 0.05 was considered significant.

Results and Discussion

No differences were observed for sows that had lameness induced in the left leg vs. right leg (*P* = 0.78) or between rounds (*P* = 0.86). There was no difference between D -1 and D +6 (*P* = 0.35), but cortisol concentration was greatest on the day when sows were most lame (D +1; Figure 2; *P*=0.0013). In conclusion, lameness induced with amphotericin B was associated with elevated plasma cortisol levels relative to baseline (day-1) and resolution (day+6).

Figure 2. Cortisol concentrations (ng/ml) when sows were sound (D-1) most lame (d+1) and resolved (D+6). Differences P = 0.0013



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