COMPARISON OF BEHAVIORAL TO PHYSIOLOGICAL RESPONSE OF COWS EXPOSED TO ELECTRIC SHOCK

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ABSTRACT

A series of experiments were performed to measure behavioral responses and changes in blood cortisol concentration of cows exposed to 60 Hz electrical current applied from front to rear hooves. Increased activity level was not a consistent indicator of response to current, whereas a flinch or startle response was a consistent and repeatable behavioral indicator. Cows responded at lower current levels to the one-front to two-rear-hoof pathway than to muzzle to four-hooves pathway. Cortisol levels did not increase in response to short-term current exposure at levels up to 1.5 times the flinch reaction level. Cortisol concentrations were found, however, to increase in response to hoof trimming. These results indicate that behavioral changes are a more sensitive indicator of response to short-term electrical current exposure than blood cortisol levels.

INTRODUCTION AND LITERATURE REVIEW

Behavioral observations have been used extensively as an indicator of dairy cow response to electrical current as cited in the review by Aneshansley and Gorewit (1991) and more recent studies (Reinemann et al., 1999; Aneshansley et al., 1997). Several studies investigating the concentration of blood cortisol and other hormones in cows exposed to electrical current have also been performed. Several of these studies have also measured changes in cow behavior in response to electrical exposure.

Lefcourt et al. (1986) reported that in a study in which seven lactating cows were subjected to 2.5 mA to 12.5 mA rms of 60 Hz electrical current, prolactin, glucocorticoids, and norepinephrine were unaffected by shock. At lower levels, cows showed limited movement and became more agitated as the current level increased. Heart rate immediately after shock increased significantly from baseline at 10 mA (+17 beats/min) and 12.5 mA (+30 beats/min). Behavioral responses were not correlated with physiological responses. Lefcourt et al. (1985) also reported that subjecting cows to 3.6 and 6.0 mA of electrical current from one front to one rear leg during milking produced minimal physiological response but noticeable behavioral changes. There was no change in milk yield or milking time, but milk flow rates increased slightly.

Henke et al (1982) noted behavioral reactions in cows when between two and four mA rms of 60 Hz current was applied from udder to four-hooves and concluded that these behavioral reactions were more sensitive indicators than endocrine response. In a study by Henke et al. (1985), cows exposed to zero, four, and eight mA of electrical current from udder to hooves during milking showed some behavioral responses that decreased with time. Changes of milking performance and milk composition were not significant. However, changes of milking-related cortisol responses during application of eight mA of current were significant.

Alternating currents were applied through the milk during milking in a study by Aneshansley et al. (1992). They reported that first lactation cows kicked at the milking unit when current exceeded 5 mA (8 V), while multiple lactation cows began kicking at currents above eight mA (16 V). There were no undesired behaviors or consistent significant differences in milking duration, milk yield, or composition

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for primary or residual milk for current application below these levels. Application of constant currents of 5 mA for first lactation cows and eight mA for multiple lactation cows produced no undesired behaviors but did result in some differences in production variables. Milking duration decreased during application of constant current to first lactation cows. Serum cortisol concentrations increased from 5 ng/mL before milking to 15 ng/mL 10-min after milking. Cows exposed to eight mA of current had slightly reduced serum cortisol concentration at two and 6-min after milking than did control cows.

In an overview of farm animal behavior Rushen (1995) stated: "*Behavioral measures might be useful in indicating that an animal is in a state of stress, but behaviors can be caused by a wide range of physiological disturbances. It cannot be assumed that behavioral measures of stress will always be correlated with physiological ones. This may result from the fact that behavioral and physiological reactions may be alternative ways that animals have of reacting to stress, or that behavioral responses actually serve to reduce the physiological responses to stress. It is advised, therefore, to combine both behavioral and endocrine responses to better understand animal response to potential stressors.*"

In most past studies, groups of cows have been exposed to a prescribed voltage or current level with no attempt to account for individual animal sensitivity. Reinemann et al. (1999) reported on methods developed to apply electrical stimuli to cows relative to their individual behavioral response levels. This method of exposure produced more consistent aversive response than previous studies that did not take individual animal sensitivity into account. It was speculated that taking individual animal sensitivity into account when applying current might produce a more consistent relationship between behavioral and physiological responses.

The muzzle-hoof pathway is the most likely exposure pathway for cows in the housing environment as waterers and feeders are potential sources of neutral to earth voltage exposure. Another potential pathway is from hoof to hoof. This exposure condition may occur when cows are entering a milking parlor or perhaps during milking. Most previous experiments have used the muzzle-hooves pathway for electrical current exposure. The only controlled exposure study of the hoof-hoof pathway was reported by Currence et al. (1990). It was therefore desired to do further testing on the hoof-hoof pathway and compare muzzle-hoof sensitivity to hoof-hoof sensitivity using the same methods.

The specific objectives of this study were to:

- Investigate the relationship between behavioral responses and plasma cortisol concentration in cows,
- Compare dairy cow sensitivity to current applied from hoof-hoof with the muzzle-hoof pathway, and,
- Compare cortisol reactions to current exposure with another common stressor, hoof trimming.

MATERIALS AND METHODS

Cortisol Assay

A cortisol specific ELISA was developed to quantify the extremely low concentrations of cortisol during non-stressful conditions (0.5 ng/mL to 20 ng/mL; Munksgaard and Simonsen, 1996; Ley et al., 1996). The ELISA used the monoclonal antibody (P01-92-92M; Biostride Inc, Redwood CA) that had some cross-reactivity with other glucocorticoids (corticosterone = 22%; cortisone = 26%). These compounds are present in low amounts in bovine serum and therefore this cross-reactivity was not considered a major problem. Unlike other antibodies it was found to have less than 0.01% cross-reactivity with progesterone, 17ß-estradiol, estrone, estriol, and with other steroids that were tested. This was important because the mid-lactation cows that were used in our studies were expected to have substantial concentrations of progesterone (4 ng/ml) and estrogens (10-100 pg/ml).

Serum (500 µl) was extracted twice using diethyl ether (2.5 ml each time). Diethyl ether was added to the serum, vortexed for 1 min, snap frozen in a methanol/dry ice bath, and extracted diethyl ether poured into a second glass tube. Following evaporation of the diethyl ether the extract was resuspended in 250 µl of assay buffer for use in the ELISA. The extraction efficiency was found to average approximately 80% in each extraction and with the 2 extractions it was found to be about 95%. The ELISAs were performed in 96-well plates (Nunc-immuno plate, Maxisorp, Roskilde, Denmark). The plates were coated first with an attachment antibody (goat anti-mouse Ig-G antibody from Calbiochem, San Diego, CA) by incubation for 18 h at 4˚C with 100 µl of a 1-µg/ml antibody solution in coating buffer (.05 *M* sodium bicarbonate; pH 9.6), washed four times with wash buffer (.02 *M* 3-[N-morpholinopropane sulfonic acid with .025% Tween 20; pH 7.2), and the anti-cortisol antibody (P01-92-92M) was added at a 1:20,000 dilution and incubated overnight. Plates were washed 4 times and 100 µl of standards (0.5-64 ng/ml) or unknowns were added to the plates. The plates were incubated for 1.5 h and 50µl of cortisol conjugated to horseradish peroxidase (Biostride, Inc.; P91-92-91H; 1:500,000 dilution) was added and incubated for another 1.5 h. Plates were washed four times and 3,3'5,5'-tetramethylbenzidine was used to quantify horseradish peroxidase activity. The intra- and inter-assay coefficients of variation were 6% and 11% respectively using a quality control sample containing 5 ng/ml.

This assay was performed on 4 cows that were canulated and had blood samples drawn periodically over a 48 hour interval. Blood samples were taken immediately after canulation (approximately noon) and then again on the following day every hour from 8 am until 5 pm with additional samples taken immediately before and after the evening milking and then again from 8 am to 12 pm the next day. Two of these cows were moved into the test stalls used for subsequent behavioral measurements while the other 2 cows were not moved.

Electrical Exposure and Behavioral Observations

The stalls used for these experiments consisted of two concrete pads with embedded steel reinforcing bars suspended by a wooden framework (Figure 1). The entire stall assembly was suspended about 3 cm from the floor of the barn. The front and rear concrete pads were separated by a 9 cm air gap. The only physical connection between the front and rear concrete pads was a wooden framework along the sides of the stalls. These wooden components were treated with a rubber compound to keep the wooden components dry. The test stall was suspended on a PVC pipe in the center of the front and two load cells on the rear corners. A schematic of the circuit to deliver and monitor the current applied to cows is shown in Figure 2. A source voltage of 220 V was developed using a controlled voltage source and step-up transformer. The current delivered to cows was controlled by adjusting the source resistance and was measured as the voltage across a 1000-Ohm resistor in series with the cow circuit and confirmed using a precision current clamp.

For muzzle-hoof current application, current was applied to a ball-end, non-piercing nose ring used in previous experiments (Reinemann et al., 1999). The four-hooves contact point was created by bonding the metal reinforcing bars in the front and rear concrete pads (Figure 1). For hoof-hoof current application current a one-front to two-rear hooves pathway was created by placing a wooden plate covered with two pieces of expanded metal mesh on the front half of the stall. This front plate was divided in half with two sections of wire mesh separated by a raised wooden divider down the center. The rear hooves were in contact with the rear concrete pad. Current was applied alternately to the right and left side of the front pads so that current flowed through only one front hoof at a time. Exposure to the front hooves was alternated between front right and front left hooves every two seconds. The return path for current was the two rear hooves in contact with the rear concrete pad. For all experiments the concrete surfaces were wetted before testing to reduce the variability of contact resistance.

Trained observers counted the number of steps (lifting hoof off the pad) and monitored other cow behaviors during current exposure. One of the load cells at the rear of the stall was monitored using a computer-based data acquisition system. A movement of the cow from side to side could be detected by monitoring the change in force measured by the load cell over time. The threshold used to count activity events was a rate of change exceeding 9 kg/s. The number of activity events recorded in this way was slightly greater than the number of steps as counted by human observers. The electronic recording system was thus able to record weight shifts not accompanied by a visible lifting of a hoof.

Muzzle-Hoof Compared to Hoof-Hoof Sensitivity

An experiment was performed to determine the relationship between muzzle-hoof and hoof-hoof exposure pathways. Eight Holstein cows in second to fourth lactation, from 51 to 192 days in milk, and producing from 29 to 46 kg (65 to 103 pounds) of milk per day were exposed to an ascending series of currents. Each series began with a five-min observation, during which no current was applied, followed by one-min exposure periods separated by one-min periods with no exposure. The series began with application of 0.25 mA and then 0.5 mA with 0.5 mA increments up to 4 mA and then 1 mA increments. The current exposure was increased in this way until a behavioral reaction (startle reaction, or flinch) was first observed, as described below. Cows were then exposed to one or two additional current increments to gather activity data at higher exposure levels and to confirm the flinch observation.

Cortisol and Behavioral Studies

A second experiment was performed to measure cortisol responses at current exposures relative to each cow's behavioral threshold. The same group of eight cows used in the first experiment described above was used for this second experiment. Blood samples were taken for cortisol assay in 5-min intervals beginning 20 minutes before the first current exposure. Cows were exposed to current for 5-min intervals using the alternating front-hoof to two-rear-hooves method described above. The current exposure levels used were 0.5, 0.75, 1.0 and 1.5 times the behavioral reaction level for each cow as determined in the first experiment (e.g., for cow 4106 the 0.5R level is 1.5 mA, the 0.75R level is 2.25 mA, the 1.0R level is 3 mA, and the 1.5R level is 4.5 mA). The time between current exposures was 10-min.

Hoof Trimming Study

A third experiment was performed to examine the cortisol response to the common animal stressor of hoof trimming. Blood samples were taken for cortisol assay from eight cows before and after hoof trimming. These cows were scheduled for routine hoof trimming at the UW Arlington experiment station and were in the first to fourth lactation and producing between 18 kg and 40 kg (40 lb. and 90 lbs.) of milk per day. The cows are restrained in the trimming stall to avoid injury to the hoof trimmer or the cow. Straps are run under the cow to hold it up while one leg is lifted and held in place during trimming. The hoof trimming procedure took from 10-min. to 30-min. Blood samples were taken with cows in their housing stalls prior to moving the cows to the trimming stall. Another sample was taken immediately after trimming procedure was completed with the cow still restrained in the trimming stall.

RESULTS AND DISCUSSION

Behavioral Responses

Human observers noted that a startle response (flinch) at the beginning of the exposure period was the most consistently observable behavioral change. The first current exposure level at which two human observers observed a flinch for each individual cow was taken as that cow's behavioral response threshold and is indicated as 1.0 R. Box plots of the change in activity events for each cow from the 5-min preceding the current exposure to the 5-min of current exposure for the cortisol/behavioral experiment are shown in Figure 3. On average there was a small but significant ($p<0.05$) increase in activity associated with the 1.0 R current exposure. None of the other exposure levels had a significant ($p>0.05$) change in activity. Not all cows showed an increase in activity at the 1.0 R reaction level. The range of responses at the 1.5R level of exposure shows more clearly that some cows became more active and some became less active during current exposure.

Muzzle-Hoof Compared to Hoof-Hoof Sensitivity

The results of this experiment are summarized in Table I. A paired t-test showed that the difference between the reaction levels was significantly different for the two exposure pathways ($p < 0.01$) with cows being more sensitive (responded at a lower total current) to the one-front to two-rear-hooves pathway. These results for the one-front to two-rear-hooves pathway are in good agreement with the study by Currence et al. (1990), who reported an average behavioral response level of 3.5 mA for 24 cows using a similar exposure pathway.

Table I. Reaction level (mA, rms) for one-front to two-rear-hooves compared to muzzle-four-hooves pathways.

Cow Number	3963	4102	4106	4145	4169	4192	4205	4243	Mean	SD
One-front to two-rear-hooves	3.5	2	3	3	3.5	3	2.5	3.5	3.0	0.53
Muzzle-4-hooves	5	5	8	8	5	5	3.5	3.5	5.4	1.7

Cortisol and Behavioral Studies

The cortisol measurements from 4 lactating Holstein cows that had blood samples taken periodically during a 48 h period and were not exposed to electrical current are summarized in Table II. The cortisol concentrations varied between 0.5 and 17.8 ng/ml with periodicity of several hours. There was no discernible effect of canulation, milking, feeding or movement to new stalls. The range and periodicity of cortisol concentrations in cows not exposed to stress in our study showed remarkable agreement with the radioimmunoassays reported by Lefcourt et al (1976) who reported cortisol concentrations ranging from 1 to 17 ng/ml and also saw no relationship between cortisol concentration and milking or feeding.

Table II. Circulating cortisol concentrations (ng/mL) in four lactating Holstein cows not subjected to stress that had blood samples taken over a 48 h period.

	Minimum	Average	Maximum	Std. Dev.
Cow 1	1.7	6.3	15.5	5.1
Cow 2	1.1	6.2	15.4	4.1
Cow 3	1.3	6.7	17.8	4.8
Cow 4	0.5	5.8	15.4	4.8
All Cows	0.5	6.3	17.8	4.6

The cortisol data for individual cows from the electrical exposure study are shown in Figure 4. Cortisol is excreted in pulses and has a half-life of about 20-min; consequently, sampling every 5-min will detect any release of cortisol. The 0.5R and 0.75R levels were chosen to determine if a cortisol response would occur at levels below those at which a behavioral reaction could be observed. The 1.0R level was chosen to determine if the level of stimulus required to produce an observable behavioral response would produce a cortisol response. The 1.5R level was chosen as a level of annoyance, which was shown in previous studies (Reinemann et al., 1995) to cause avoidance of water bowls.

The range of cortisol concentrations was similar to those recorded in the preliminary measurements reported in Table II. Two cows started toward the high end of the normal daily range, three toward the low end of the range, and three in mid-range. The three cows that started with low cortisol concentrations at the beginning of the experiment showed an increasing trend in cortisol concentration toward the end of the experiment. Box plots of the change in cortisol concentration for each cow from the 15-min immediately before each current exposure to the 15-min interval immediately after that exposure are shown in Figure 5. A positive value indicates that cortisol concentration is increasing after exposure, while a negative value indicates a decreasing cortisol concentration. A paired t-test indicated that none of the average changes in cortisol concentrations were significantly different from zero ($p>0.05$), although there is a suggestion of an increasing trend at the 1.5 R exposure level. Only one value was above the maximum cortisol concentration for unstressed cows, however, and this occurred at the beginning of the observation period, before any current exposure. The variation in cortisol concentrations during electrical exposures up to 1.5 times that required to elicit a behavioral response did not appear to differ from the normal periodic fluctuations in unstressed cows.

Hoof Trimming Experiment

The plasma cortisol concentrations measured before and after trimming are presented in Table III. The results of a paired t-test of the before and after hoof trimming data showed that the mean increase in cortisol concentration of 31.7 ng/mL (standard deviation of differences = 11.9 ng/ml) was significant ($p < 0.0001$)

Table III. Cortisol concentrations before and after hoof trimming.

Cow number	Blood Cortisol Concentration Before Hoof Trimming (ng/mL)	Blood Cortisol Concentration After Hoof Trimming (ng/mL)
4389	2.1	38.3
4230	16.7	41.1
4394	2.8	46.8
3966	1.1	52.2
4304	4.4	34.5
4350	7.2	24.4
4056	15.8	34.5
4428	2.5	34.6
Average	6.6	38.3
S.D.	6.3	8.5

Box plots of the data from the hoof trimming study along with the cortisol measurements taken immediately before and after hoof-hoof exposure to current at 1.5 times the behavioral reaction level are shown in Figure 6. The average cortisol concentration immediately before current exposure was 3.1 ng/mL (std. dev. = 1.3 ng/mL) and after exposure to 1.5R level of current was 4.6 ng/mL (std. dev. = 2.1 ng/mL). This difference was not significant at the 95% confidence level. A two-tailed paired test with alpha of 0.05 and 80 percent power and a sample size of 8 cows results in an estimated minimum mean detectable of 16.7 ng/mL, which would be sufficient to detect any changes outside the normal daily variation in blood cortisol concentration. It is clear from these results that the cortisol response to hoof trimming is far more severe than exposure to electrical current at 1.5 times the behavioral reaction level.

CONCLUSIONS

Dairy cows were more sensitive (reacted at lower current) to current applied from one-front to two-rear hooves than current applied from muzzle-4-hooves. No increase in cortisol level was observed for cows subjected to 5-min of 1.5 times the current required to produce a behavioral response. A cortisol increase was observed in response to hoof trimming. Behavioral responses were found to be a more sensitive indicator of perception or annoyance than cortisol levels in dairy cows in agreement with several previous studies.

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Figure 1. Diagram of experimental stall.

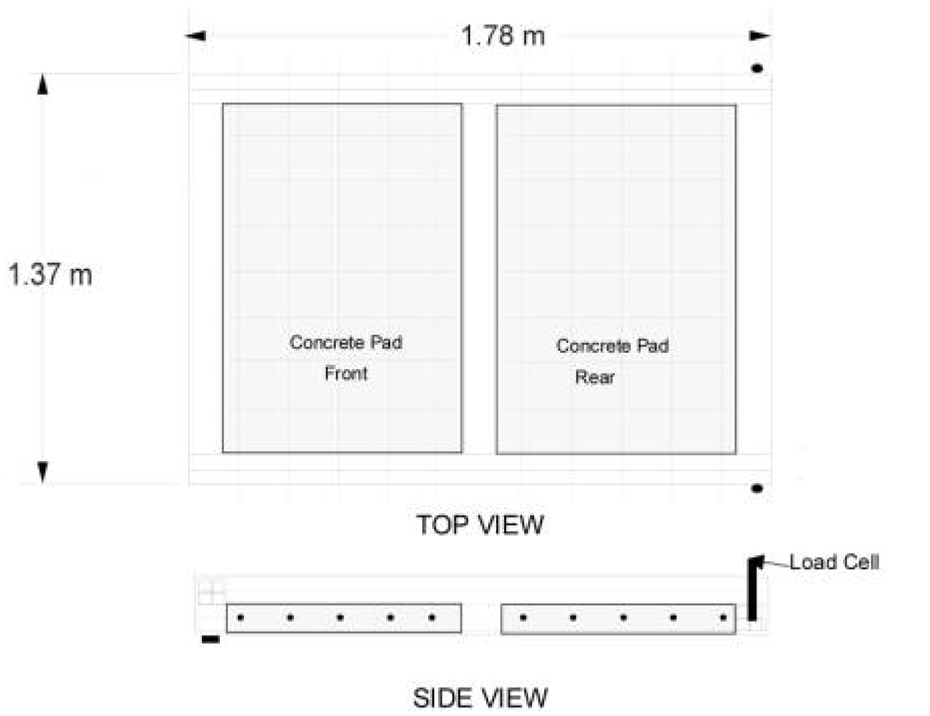

Figure 2. Schematic of current circuit for behavioral and cortisol studies.

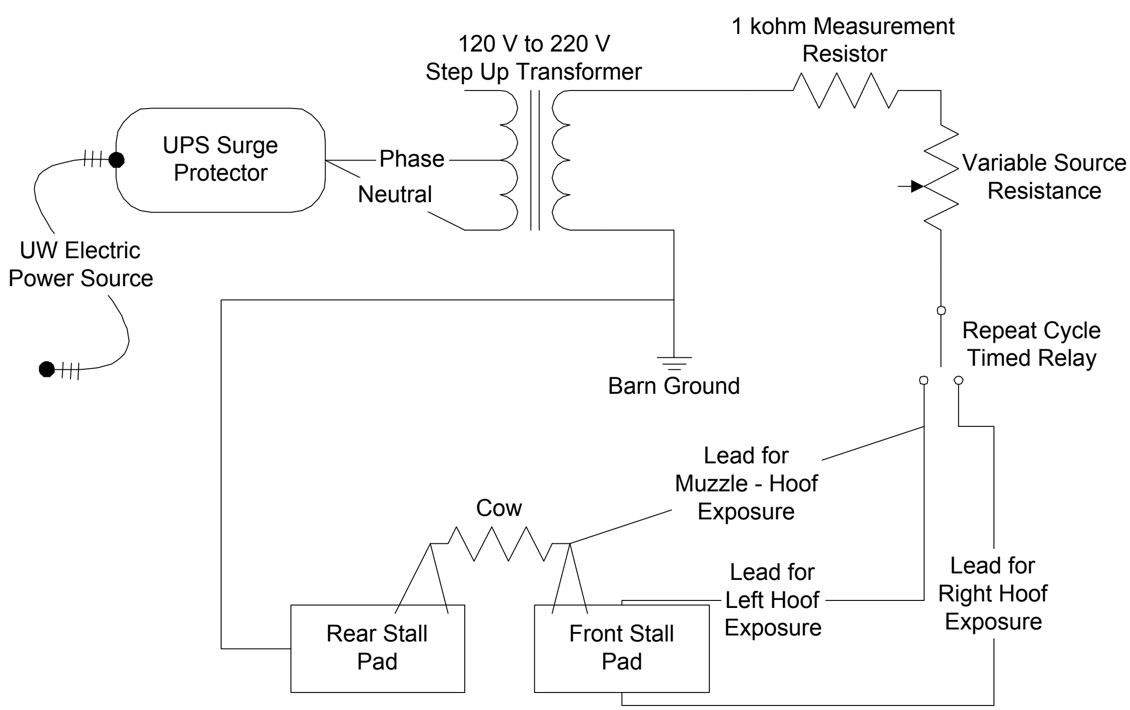

Figure 3. Box plot of 5-min average change in activity events of cows exposed to 0.5, 0.75, 1.0 and 1.5 times the current required to produce a behavioral response (R). (Horizontal white bar indicates mean

response, shaded boxes indicate +/- 25% of responses, and whiskers indicate maximum and minimum values.)

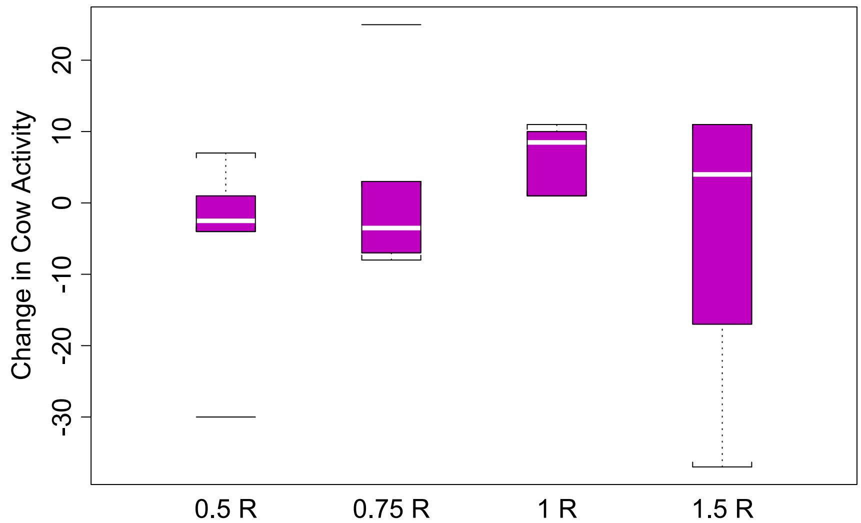

Figure 4. Blood cortisol concentration data for eight cows in cortisol/behavior study.

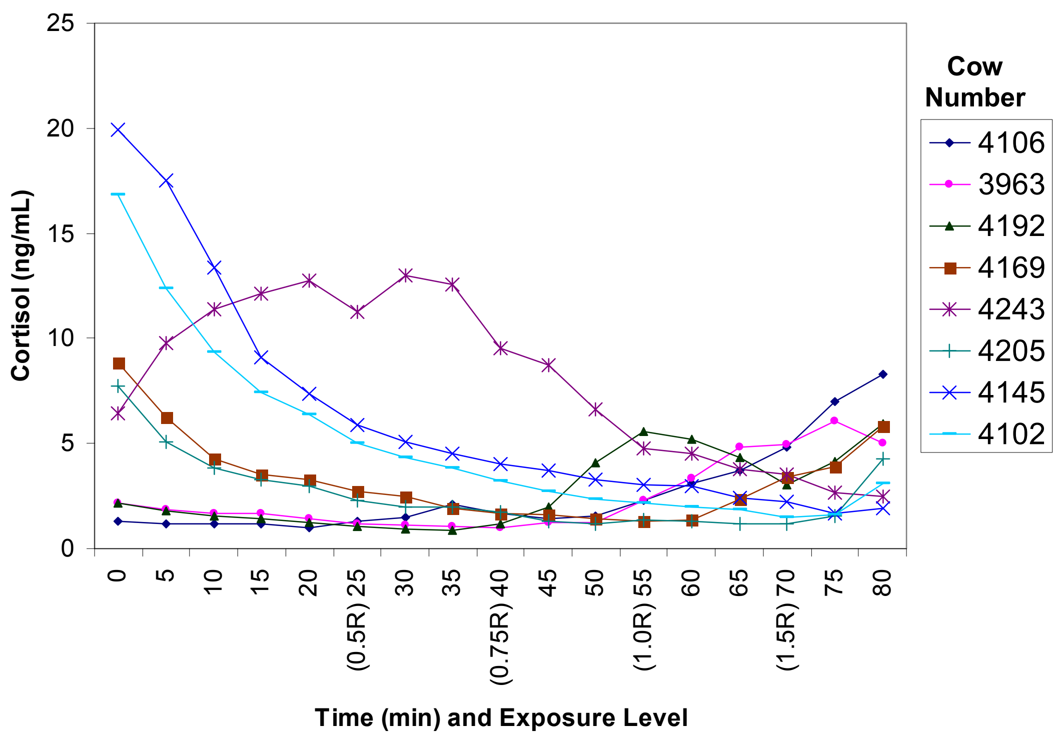

Figure 5. Box plot of the change in 15-min average cortisol concentration for cows exposed to 0.5, 0.75, 1.0 and 1.5 times the current required to produce a behavioral response (0.5R, 0.75R, 1R and 1.5 R respectively). (Horizontal white bar indicates mean response, shaded boxes indicate +/- 25% of responses, whiskers indicate maximum and minimum values.)

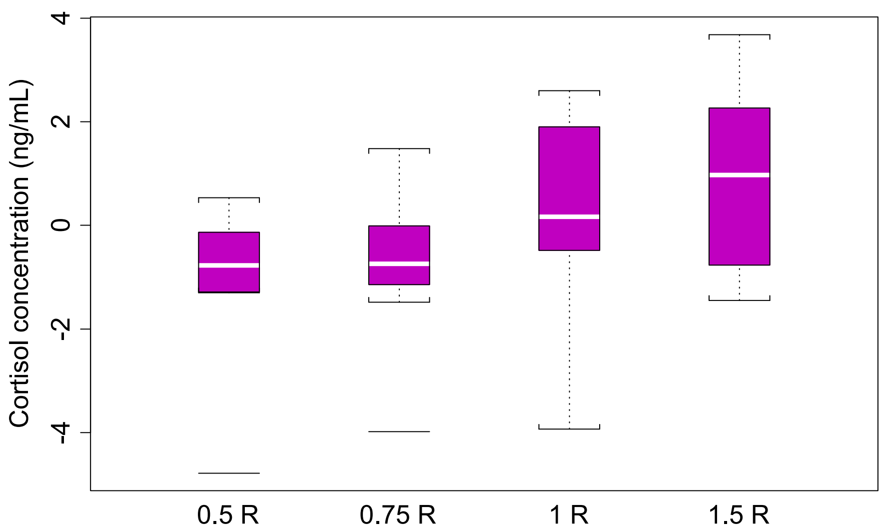

Figure 6. Box plot of cortisol concentrations of and eight cows before and after exposure to 1.5 times the current required to produce a behavioral response (1.5R) and eight cows before and after hoof trimming. (Horizontal white bar indicates mean response, shaded boxes indicate +/- 25% of responses, whiskers indicate maximum and minimum values.)