Dairy Cow Response to Electrical Environment

Final Report

Part I. Comparison of Behavioral to Physiological Responses

and

Part II. Comparison of Treatments Applied during Milking

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by
Douglas J. Reinemann, Ph.D., Associate Professor, Biological Systems Engineering,
University of Wisconsin-Madison
Morten Dam Rasmusssen, Ph.D., Senior Scientist, Danish Institute of Agricultural Sciences, Department of Animal Health and Welfare
Milo C. Wiltbank, Ph.D., Associate Professor of Dairy Science
University of Wisconsin-Madison
Lewis G. Sheffield, Ph.D., Associate Professor, of Dairy Science
University of Wisconsin - Madison
Steven D. LeMire, Research Assistant, .Biological Systems Engineering
University of Wisconsin-Madison

Abstract Part I. A series of experiments were performed to measure behavioral and blood cortisol concentration responses of cows exposed to current applied from front to rear hooves. Increased activity level was not a consistent indicator of response to current, whereas a startle response (flinch) was a consistent and repeatable indicator. Cows responded at lower current levels to the 1-front to 2-rear hoof pathway than to muzzle to 4-hooves pathway. Cortisol levels did not increase in response to current exposure at levels up to 1.5 times the behavioral reaction level. Cortisol concentrations were found, however, to increase in response to hoof trimming. It appears for these results that behavioral changes are amore sensitive indicator of response to current than blood cortisol levels. This result agrees with several past studies.

Abstract Part II. Experiments were performed to compare milking performance of cows subjected to electrical current exposure applied during milking to the response to two common milking machine problems. The electrical exposure of one mA, rms of 60 Hz electrical current was applied from front to back hooves during milking. The milking machine problems created were either a pulsation failure (no massage phase) or excessively aged milking machine liners. The response measures included milk yield, average milk flow rate, maximum milk flow rate, cow activity, and strip yield (hand stripping yield). There was no statistically significant main effect on any of these variables for current exposure. Pulsation failure produced a significant decrease in cow activity (5.8 fewer weight shifts during a milking). Aged liners produced a significant effect on milk yield (2.2 kg increase), average flow rate (0.77 kg/min reduction), maximum flow rate (1.2 kg/min reduction) and liner slips (21 more per milking).

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). The relationship between behavioral and endocrine response during electrical exposure has also been studied. Henke et al (1982) noted behavioral reactions in cows between 2 and 4 mA rms 60 Hz current applied from udder to 4-hooves and concluded that these behavioral reactions were more sensitive indicators than endocrine response. Lefcourt et al., (1986) reported the following based on a study in which seven lactating cows were subjected to 2.5 to 12.5 mA rms of 60 Hz electrical current.

> *At lower levels, cows became tense and showed limited movement. As the current level increased, cows became more agitated. Heart rate immediately after shock increased significantly from baseline at 10 mA (+17 beats/min.) and 12.5 mA (+30 beats/min.). Prolactin and glucocorticoids were unaffected by shock; however, both increased pronouncedly following a single recannulation prior to blood sampling. Norepinephrine was unaffected by shock or recannulation. Epinephrine doubled in two exceptional cows at 10 mA. The two exceptional cows showed consistent glucocorticoid responses, had consistently elevated baseline heart rates and prolactin, and were the only cows not shocked at 12.5 mA due to severe behavioral responses. The dramatic behavioral responses displayed by cows subjected to electrical shock were not correlated with significant or prolonged physiological responses.*

There have also been several studies of behavioral and endocrine response to electrical current applied to cows during milking. Lefcourt et al (1985) 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.

Cows exposed to 0, 4, and 8 mA of electrical current from udder to hooves during milking showed some behavioral responses that decreased with time (Henke et al., 1985). Changes of milking performance and milk composition were not significant, however, changes of milking related cortisol responses during 8 mA current stimulation 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 8 mA (16 V). There were no undesired behaviors or consistent significant differences in milking duration, milk yield, or composition for primary or residual milk for current application below these levels. Application of constant currents of 5 mA for first lactation cows and 8 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-m after milking. Cows exposed to 8 mA of current had slightly reduced serum cortisol concentration at 2 and 6-m after milking than did control cows.

In an overview of farm animal behavior Rushen (1995) stated:

> *"…a wide range of physiological disturbances that can result from behavioral problems or the emotional reactions of farm animals have been documented. Second, behavioral*

> *measures may be useful in indicating that the animal is in a state of stress. One of the main reasons to expect some link between behavioral and physiological responses to stress is that the same neuroendocrine systems have been found to control them. While the taking of behavioral measures may seem technically easier than taking physiological measures, we can not assume, unfortunately, that behavioral measures of stress will always be correlated with physiological ones. In fact, there are a number of cases where behavioral measures of stress have been found to be negatively correlated with physiological measures. 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."*

In the final report of the Science Advisors to the Minnesota Public Utilities Commission (1998) this panel of experts stated:

> *"Previous methods have relied primarily upon behavioral response as an indication of the sensitivity threshold to electrical exposure. Less subjective and more quantitative dairy cow behavioral response indicators and more reliable physiological response indicators are desired. … Recent studies indicate that behavior and performance are reliable indicators of stress. These reports provide evidence that behavioral, endocrine and immune system studies combined with studies on performance criteria are required to fully assess potential harmful impacts of stressors."*

The research reported in this paper, funded by the Minnesota Public Utilities Commission upon the recommendation of these science advisors, was undertaken to address these issues. Recent advances in the sensitivity of endocrine assays prompted a reinvestigation of the relationship between behavioral and physiological response. 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. One of the objectives of this study was to determine if this method of exposure would provide more consistent results with physiological responses.

Numerous controlled research studies have shown that behavioral responses to electrical current begin at current levels above about 2 mA of current flowing through cows. Anecdotes from the field have suggested that increased cow activity during milking (stepping and kicking) may be attributable to current exposure of less than 1 mA though cows. These reports have not been documented in a controlled study. Further objectives of this study were to compare hoof-hoof exposure, as may occur during milking, to cow's sensitivity to other current pathways, and to compare the responses to current during milking to other milking machine problems.

Part I. Sensitivity Testing and
Comparison of Behavioral to Physiological Responses

Objectives

The specific objectives of this part of the study were:

> To compare dairy cow sensitivity to current applied from hoof-hoof with the muzzle-hoof pathway, and,
>
> To investigate the relationship between behavioral responses and plasma cortisol concentration in cows.

Materials and Methods

Several pilot studies were conducted to develop methods to measure cortisol concentrations in cows, monitor normal daily cortisol concentrations, and measure cow behavior. An experiment was then performed to compare sensitivity of dairy cows to current applied between muzzle to 4-hooves and from 1-front to 2-rear-hooves. This was followed by an experiment to determine the relationship between behavioral response and cortisol concentration. A final study was done to examine the cortisol response to hoof trimming as a positive control.

Cortisol Assay Development

Three radio-immunoassay kits (DPC Coat-A-Count, DPC Double Antibody, and DSL Double Antibody), which are routinely used to assay cortisol in human serum, were tested for sensitivity of cortisol measurement in bovine serum. Concentrations of cortisol in human serum are normally above 100 ng/mL and the commonly used assays have been designed for accurate measurement of these values. The normal values in cattle are 2 - 20 ng/mL (Munksgaard and Simonsen, 1996; Ley et al., 1996).

Cortisol assays have been used previously on cows at times of high stress, such as near calving when circulating cortisol concentrations can be as much as 100-fold greater than normal (Peter and Bosu, 1987). We were interested in accurately evaluating potentially small changes in serum cortisol and, therefore, decided that we needed to design an assay with the ability to detect values near the normal serum concentration of cortisol in cattle.

Five different antibodies were evaluated. Antibodies were chosen based on sensitivity for cortisol and low cross-reactivity with other steroids (a monoclonal antibody, P01-92-92M, from Biostride Inc., Redwood City, CA). Like most antibodies, there was some cross-reactivity with other glucocorticoids (corticosterone = 22%; cortisone = 26%) but this was not considered to be a major problem in bovine serum. Unlike other antibodies, the one chosen 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 used in our studies would potentially have substantial concentrations of progesterone (4 ng/mL) and estrogens (10-100 pg/mL).

This antibody was used in an enzyme-linked immunosorbent assay (ELISA). Cortisol conjugated to horseradish peroxidase was obtained from Biostride Inc. We performed preliminary assays to establish an optimal antibody concentration (1:20,000 dilution) and an optimal amount of enzyme conjugated-cortisol (1:500 dilution).

In order to obtain sufficient precision and sensitivity, cortisol was extracted from the serum prior to analysis using a double extraction procedure with diethyl ether. The diethyl ether was then allowed to evaporate and the cortisol was re-suspended in assay buffer for analysis. This procedure results in over 90% recovery of cortisol from bovine serum. A 500 :L sample of serum was extracted and extracted cortisol was resuspended in 250 :L of assay buffer. This increased the sensitivity of the assay about 2-fold and produced a detection point of 50 pg/mL. The levels of sensitivity and specificity of this assay were considered optimal for accurate analysis of changes in cortisol concentrations during stress.

Stall Movement Pilot Study

A pilot study was conducted to determine the effects of moving cows from their normal stalls to the specially constructed test stalls. Four cows were placed in control stalls in the research barn. The normal amount of straw bedding was used in the control stalls. A light application of sawdust bedding was applied to the rear of the experimental stalls. The variability of current passing through the cow depends on the variability of resistance of the cow when standing and lying in the stall. Bedding increases the resistance and variability of resistance throughout the day. Complete elimination of bedding is problematic as cow discomfort and the risk of mastitis infection are increased. Application of sawdust bedding to the rear of the stalls was investigated to determine cow reaction to this amount and type of bedding and to determine the ability to control electrical current application. The experimental schedule and blood sample times are given below.

		Treatment Group	Control Group
Day	Time	Cows 4056 and 3993	Cows 4170 and 4291
1	10:00 - 13:00	Cows cannulated and blood sampled	
2	8, 9, 10, 11	Blood sample	Blood sample
2	11:45 to 12:00	Move from barn stalls to test stalls	Move from barn stalls to yard and back to barn stalls
2	12, 13, 14, 15, 16,	Blood sample	Blood sample
2	Aprox. 17:00	Blood sample when cows enter parlor	Blood sample when cows enter parlor
2	Aprox. 17:30	Cows returned to stall without going into yard	Cows returned to stall without going into yard
3	8, 9, 10, 11, 12	Blood sample	Blood sample

All four cows were cannulated on the afternoon of first day of the study. The cannulas remained in place and continued to function satisfactorily for the two-day period. Techniques were developed to draw blood both in the barn and parlor stalls with minimal disruption to the cow.

At approximately noon on the second day of the study the cows were moved from their stalls to an exercise yard. The feed bunks in all stalls were filled with feed. The control group was then placed back in their original stalls. The treatment group was placed in the stalls designed for electrical exposure.

These results are summarized in Figure 1. The cortisol concentrations in this study fluctuated between 1 to 20 ng/mL and showed an average cycle time of several hours between relative maxima. The range of cortisol concentrations and patterns of fluctuation agreed well with studies by several other researchers (Munksgaard and Simonsen, 1996; Ley et al., 1996).

Test Stall Design

The stalls used for these experiments consisted of two concrete pads with embedded steel reinforcing bars suspended by a wooden framework (Figure 2). 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 2 load cells on the rear corners. One of these load cells 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 weight measured by the load cell over time. The measurements from this activity monitoring system were compared to human observations of hoof lifting and cow movement as described below.

A schematic of the circuit to deliver and monitor the current applied to cows is shown in Figure 3. 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. Stalls were routinely checked for any current leakage paths using a standard cow-contact measurement device (copper plates placed 1-m apart and connected with shunt resistors ranging from 500 to 10,000 ohms).

For muzzle-to-hoof current application, current was applied to a ball-end, non-piercing nose ring used in previous experiments (Reinemann et al., 1999). The 4-hooves contact point was created by bonding the metal reinforcing bars in the front and rear concrete pads (Figure 2).

The current path was modified for the hoof-hoof pathway. Current was applied to the front concrete pad and returned through the rear concrete pad for the 2-front to 2-rear hooves pathway. In later experiments a 1-front to 2-rear hooves pathway was created in an attempt to amplify stepping behaviors. A wooden plate covered with two pieces of expanded metal mesh was used as the front hoof contact point. 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 that was wetted before tests to reduce the variability of contact resistance.

Activity Monitoring Pilot Studies

Several tests were done to calibrate the motion sensing system and develop an automated algorithm to detect changes in cow activity. The application of a steady current from 2-front to 2-rear hooves did not provide consistent results across cows. Human observers commonly noted a startle response (flinch) before the motion sensing system could detect a change in activity.

A pulsed current was compared to a steady current in an attempt to amplify activity changes in cows. Constant 60 Hz current, applied for 1-m was compared to pulsed 60 Hz current (0.5 s on and 2 s off, for 1 m). A 5-m observation period of each cow with no current applied was recorded at the beginning of each test. An ascending series of 0.7 mA rms increment was applied to 4 cows. Two cows received the pulsed and two cows received the constant current on day 1. The treatments were switched on day 2.

The activity measures did not produce consistent criteria for a behavioral response indicator. Human observers could clearly see changes in animal behavior, while the motion sensing system indicated no change, an increase or a decrease in activity. The most consistent behavioral change noted by human observers was a startle response (flinch), occurring immediately after the threshold current level was applied. This initial flinch may or may not have been followed by increased activity. There appeared to be little difference between the constant and pulsed exposure methods.

It was also clear during the pulsed exposure experiments that cows were being penalized for lifting their hooves. The current would be approximately divided between 2 hooves when all hooves were in contact with the platform. When one hoof was lifted (either front or rear), the current flow through the other would be approximately doubled.

The test stall was modified so that the front pad was divided into right and left quadrants as described above. Current was applied alternately to the right and left side of the pad so that current would flow through only one front hoof at a time. Previous results had shown that front hoof activity was a better indicator of response than rear hooves.

Experimental Designs and Results

Muzzle-Hoof Compared to hoof-hoof sensitivity

An experiment was performed to determine the relationship between muzzle-hoof and hoof-hoof exposure pathways. A total of 8 Holstein cows, 2nd to 4th lactation, 51 to 192 days in milk, and producing 65 to 103 pounds of milk per day were used for this experiment. The exposure path was from one-front to two-rear hooves. Exposure to the front hooves was alternated between front right and front left hooves every 2 seconds. Each series began with a 5-m observation, during which no current was applied, followed by 1-m exposure periods separated by 1-m periods with no exposure. An ascending series of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 rms mA of current was used.

Reaction levels were defined as the current level at which two humans observed a flinch. One or two additional current increments were recorded to gather more motion data and to confirm the flinch observation. The results of this experiment are summarized in Figure 4 and the table below.

Reaction Level (mA, rms) for 1-front to 2-rear-hooves compared to muzzle to 4-hooves.

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

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 to the 1-front to 2-rear-hooves pathway. These results for the 1-front to 2-rear-hooves pathway are in good agreement with the study by Currence et al., (1990), who used a similar exposure pathway.

Although on average there was a small increase in activity at and above the human observer identified point of reaction, some cows appeared to respond negatively (e.g., remained still). This justified the use of the flinch as the primary indicator of response to avoid subjecting cows to undue pain.

Cortisol and Behavioral Studies

A second experiment was done to examine the relationship between behavioral response thresholds to current exposure and cortisol response. The same group of 8 cows used for the experiment described above were used for this experiment. Blood samples were taken in 5-m intervals. Cortisol is excreted in pulses and has a half-life of about 20 m, consequently sampling every 5 ms will detect any release of cortisol. Each series began with blood samples beginning 20-m before the first current exposure. Each current exposure lasted for 5-m using the same alternating front hoof to 2-rear hooves method described above. The time between current exposures was 10 m. The current exposure levels used were 50, 75, 100 and 150 % of the human observer defined reaction level for each cow as determined in the first experiment (e.g. for cow 4106 the 50% level is 1.5 mA, the 75% level is 2.25 mA, the 100% level is 3 mA, and the 150% level is 4.5 mA). The 50% and 75% reaction levels were chosen to determine if a cortisol response would occur at levels below which a behavioral reaction could be observed. The 100% reaction level was chosen to determine if the level of stimulus required to produce an observable behavioral response would produce a cortisol response. The 150% level was chosen as a level of annoyance which was shown in previously studies (Reinemann et al., 1995) to cause avoidance of water bowls.

The cortisol data are summarized in Figures 5 and 6. The range of cortisol concentrations was similar to those recorded in the stall movement study. 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 showed an increasing trend toward the end of the experiment. This is probably due to the normal periodic fluctuation of cortisol concentration in the blood.

In Figure 6 the 15-m average cortisol immediately before each exposure interval are compared to the 15-m average immediately after that exposure. A positive value indicates that cortisol concentration is increasing after exposure, while a negative valued indicated a decreasing trend in cortisol concentration. None of the averages was significantly different from zero.

The average change in activity from the 5-m preceding the current exposure to the 5-m of current exposure is shown in Figure 7. The threshold used to count events was a change in load of 9 kg/s. This value was slightly more sensitive than steps as counted by human observers. On average there was a small but significant ($p<0.05$) increase in activity associated with the 100 % reaction level exposure. This was not consistent across cows, however. None of the other exposure levels had a significant ($p<0.05$) change in activity. Human observers noted that a flinch at the beginning of the exposure period was the most consistent behavioral change

It appears for these results that behavioral changes are more sensitive indicator of response to voltage than blood cortisol levels. This is in agreement with previous results (Henke et al., 1982; Lefcourt et al., 1986).

Hoof Trimming Positive Control Study

As a positive control for measuring stress induced cortisol increases, blood samples were taken from 8 cows before and after hoof trimming. The eight cows were scheduled for routine hoof trimming at the UW Arlington experiment station. The same assays as used previously were used to measure cortisol concentrations. 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 while the cow was still in the trimming stall. Cow hoof trimming takes between 10 to 30 m. The cows are severely restrained in the trimming stall to avoid injury to the hoof trimmer or cow. Straps are run under the cow to hold it up while one leg is forcibly lifted and held in place during trimming. This is common practice on dairy farms. Information on the cows used for this study is given in the Appendix. The plasma cortisol concentrations measured before and after trimming are presented in Figure 8 and below.

Cortisol concentrations before and after hoof trimming.

Cow number	Before Trimming ng/mL	After 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

The results of a paired T-test of the before and after hoof trimming data showed that the mean increase in cortisol concentration of 32 ng/mL (standard deviation of differences = 12 ng/mL) was significant ($p < 0.0001$). 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 8.

Conclusions

Dairy cows were more sensitive (reacted at lower current) to current applied from 1-front to 2-rear hooves than current applied from muzzle to 4-hooves. No increase in cortisol level was observed for cow subjected to 5-m of 1.5 times the current required to produce a behavioral response. A cortisol increase was observed in response to hoof trimming. Behavior responses are a more sensitive indicator of perception or annoyance than cortisol levels in dairy cows.

Part II. Comparison of Treatments Applied during Milking

Objectives

The specific objective of this part of the study was to compare commonly encountered milking machine problems to exposure to electrical current. The current exposure was 1 mA of current applied from front to rear hooves during milking. The milking machine problems applied were either a pulsator failure producing no massage (D phase), or the use of excessively aged liners.

Materials and Methods

The experimental design was a completely randomized two-level factorial ($CRF_{2,2}$). One factor was current applied from front to rear hooves. The other factor was one of two commonly occurring milking machine problems (either pulsation failure or aged liners). These experiments were conducted in one stall of the four-stall milking parlor in the UW-Madison Dairy Cattle Research and Instruction Center. Tests took place during three consecutive evening milkings. All cows were milked using normal procedures and equipment on the first and third milkings (low level milking line, BouMatic Flow star Claws, BouMatic Detachers, milking vacuum level approximately 36 kPa). The 2x2 factorial was administered on the second milking. Four groups of four cows each received no treatment, milking machine problem, 1 mA of current exposure, and a combination of milking machine problem and current exposure. The milking machine was allowed to automatically detach without any human interference for all tests.

The test cows were systematically sampled from the groups of four from the available study cows in the barn. Characteristics of the cows used in these studies are given in the Appendix. The cows were let out of their housing stalls and brought to the milking parlor in groups of four with the experimental cows being directed into the instrumented stall. The same operator milked all the cows used in this study for the three nights of testing.

A schematic of the current exposure apparatus is shown in Figure 9. Two aluminum plates were placed in the milking stall. These plates were supported by rubber strips around the edges and one support down the center. The rear plate was fitted with a load cell on one of its edges (Figure 10). When current exposure was called for, an operator would apply a voltage from the front to rear plates when the milking unit was attached to the cow. The operator adjusted the source resistance while monitoring the current flow so that 1 mA rms current was passing through the cow for the duration of the milking. The current was removed when the milking unit detached.

Pulsation failure

For the treatments requiring pulsation failure, a one-way valve was placed in each of the two long pulse tubes. This one-way valve would allow the pulsation chamber to be evacuated (opening the liner) in its normal fashion. When the pulsation chamber was opened to atmospheric pressure the valve would shut and prevent the liner from closing completely. This resulted in the absence of a D (massage) phase of pulsation. A malfunctioning pulsator is a problem commonly encountered in the field and was expected to produce mild discomfort to the cows. A 2x2 factorial using 16 cows with treatments of pulsation failure and current exposure was replicated twice with a total of 32 cows.

Aged liners

The liners used for this study (BouMatic R-2CV) were artificially aged by soaking them in clarified butter oil at 100/C for 72 hours. This artificial aging process reduced the tension that the liners were mounted under from 74 N to 38 N. This reduction in tension was expected to reduce the massage applied to the cows' teats during milking, thus causing mild discomfort to the cows. A 2x2 factorial using 16 cows with treatments of aged liners and current exposure was performed.

Response Measures

The response variables measured in these studies were milk yield, maximum milk flow rate, average milk flow rate, liner slips, cow activity, and strip yield. All responses were taken as the value of the variable on the p.m. milking of the treatment day minus the average of that variable for the same cow for p.m. milkings on the two control days (before and after treatment).

Milk yield was recorded using the milk meters installed in the UW parlor (BouMatic - Perfection). A computer-based data acquisition system was used interfaced with the milk meter to record milk flow rate every 5 s. The maximum milk flow rate was taken as the maximum 30-s rolling average of these milk flow rates. Average milk flow rate was taken as the milk yield divided by the time of milking. Cows release the hormone oxytocin during milking to contract the alveoli in the udder and eject milk. The maximum and average milk flow rates may be affected by changes in the milking machine and could also be affected by changes in the oxytocin release of cows during milking. Changes in these parameters, therefore, could indicate changes in the endocrine response of cows due to current exposure.

Milking vacuum was measured in the short milk tube as recommended by Rasmussen et al (1999). The time of milking was taken as the interval during which the 5 second average milking vacuum was greater than 5 kPa. A liner slip occurs when the seal between the cow's teat and the liner of the milking machine is broken. This results in an inrush of air into the milking unit and is considered to increase the risk of mastitis infections. Increased liner slips may be caused by changes in milking machine parameters or by increased activity of cows during milking. Liner slip events were recorded when the milking vacuum dropped by more than 8 kPa with a rate of change exceeding 500 kPa/s based on the work of Rasmussen et al (1999).

Cow activity was quantified by monitoring the load cell placed under one edge of the rear aluminum plate in the parlor stall (Figure 10). Load was measured at a frequency of 100 Hz.. A weight shift event was defined as the derivative of the change in load over time in excess of 25 kg/s. This rate of change in load corresponded approximately to a cow lifting its hoof, as confirmed by human observers.

Strip yield is a measure of the completeness of milk removal by the milking machine. Strip yield may be affected by changes in the milking machine and is also another measure of changes in endocrine response during milking. Strip yield was measured by hand milking immediately after the automatic detacher removed the milking unit. The number of quarters that yielded more than 10 mL of milk were recorded for each cow.

Results and Discussion

The mean difference measures and standard deviation of differences for each response variable were as follows. Response measures that were statistically significant (p<0.05) are indicated in bold.

	Experiment I (n = 32)		Experiment II (n = 16)	
	Pulsation Failure	1 mA Current	Aged Liners	1 mA Current
Milk Yield (kg)	1.2^{+} (2.1)	0.1 (2.1)	**2.2* (1.3)**	-0.6 (1.8)
Average Flow Rate (kg/min)	0.54^{+} (0.78)	0.32 (0.83)	**-0.77* (0.56)**	-0.13 (0.68)
Maximum Flow Rate (kg/min)	0.29^{+} (0.42)	0.04 (0.45)	**-1.2** (0.54)**	-0.26 (0.75)
Activity (weight shifts / milking)	**-5.8** (4.5)**	-1.3 (5.3)	-8.9 (11)	-0.31 (13)
Strip Yield (% of quarters > 10 mL)	-10 (19)	8.6 (19)	-3.0 (27)	-16 (27)
Liner Slips / milking	-0.38 (1.5)	-0.88 (1.6)	**21** (4.0)**	0.1 (12)

*Note: values given are mean effect size and (standard deviation) Statistical treatment effects are indicated by + = p<0.10, * = p<0.05, ** = p < 0.01.*

There was no statistically significant main effect for current exposure for any of the response variables for either experiment. Pulsation failure produced a significant decrease in cow activity (5.8 fewer weight shifts). Aged liners produced a significant effect on milk yield (2.2 kg increase), average milk flow rate (0.77 kg/min decrease), maximum milk flow rate (1.2 kg/min decrease), and liner slips (21 more per milking).

Some interaction effects were significant, but none of these were repeatable across experiments. The interaction between pulsation failure and current exposure was significant for milk weight in experiment I (p=0.03), with current exposure increasing milk yield 1.4 kg with pulsation failure but not without. The interaction between pulsation failure and current exposure was also significant (p=0.003) for activity in experiment I, with current reducing the effects of activity observed when pulsation failure was applied alone. Neither of these interactive effects was repeated in the second experiment. The interaction between aged liners and current exposure was significant (p=0.006) for strip yield in experiment II, with the combination of aged liners and current exposure tending to reduce strip yield (cows milked out better). One cow, which had lower strip yield on the treatment day, was a major contributor to this effect.

Conclusions

Several significant effects were measured when commonly encountered milking machine problems were applied to cows. No adverse effects were observed for cows exposed to 1 mA of current applied from front to rear hooves. Exposure to 1 mA rms of 60 Hz electrical current produced no significant change in milk yield, milk flow rate, strip yield, cow activity or liner slip. Some interactions between milking machine problems and current exposure were significant in some experiments, but the magnitudes were small and they were not repeatable across experiments.

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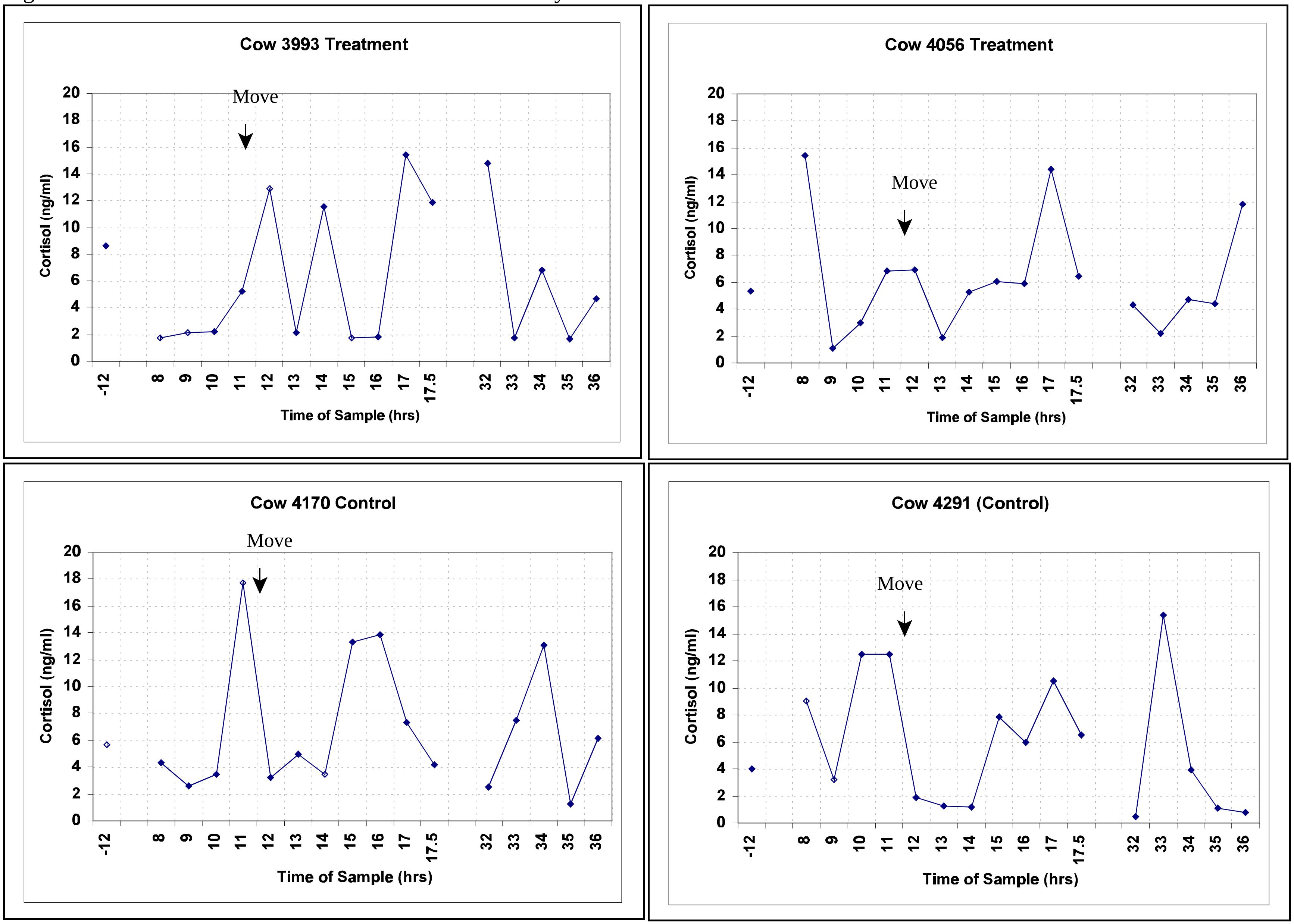
Figure 1. Cortisol concentrations for stall movement study.



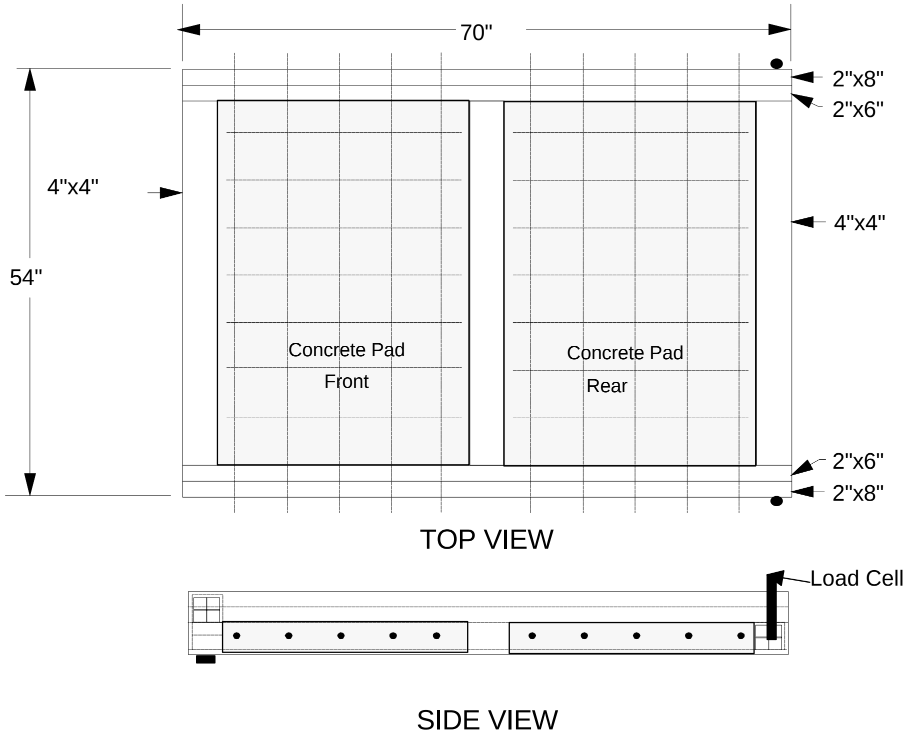


Figure 2. Diagram of experimental stall.

Figure 3. Schematic of current circuit for behavioral and cortisol studies.

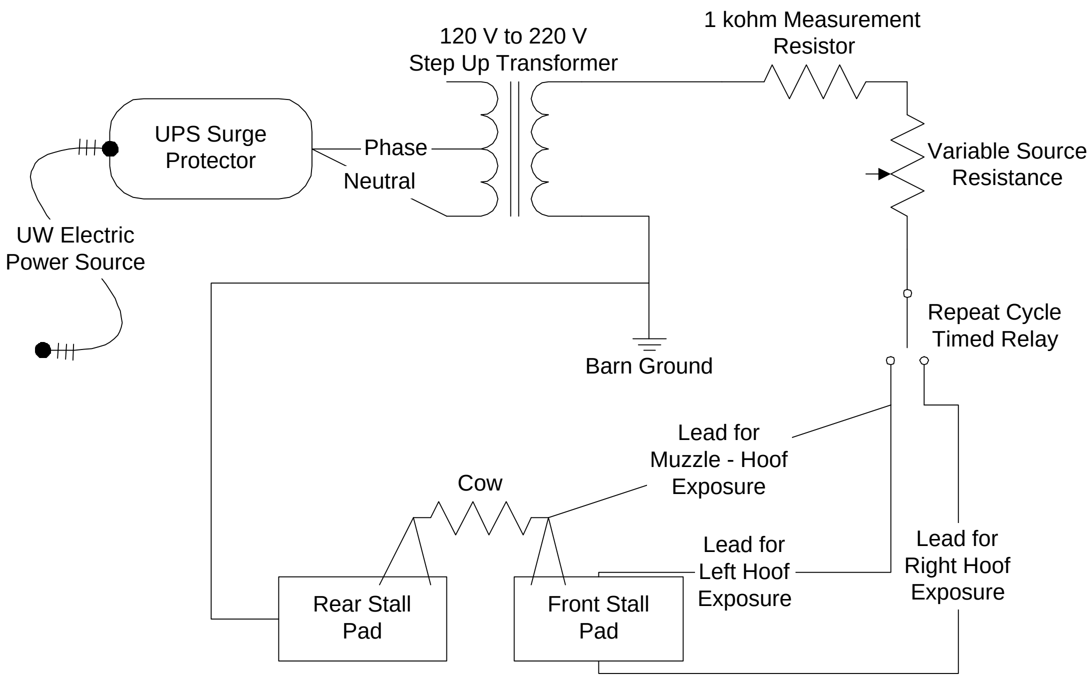


Figure 4. Box plot of muzzle to 4-hooves behavioral reaction threshold compared to 1-front to 2-rear hooves behavioral reaction threshold. The horizontal white line is the mean of the data. The box includes +/- 25% of the data from the median. The horizontal black lines are the maximum and minimum values.

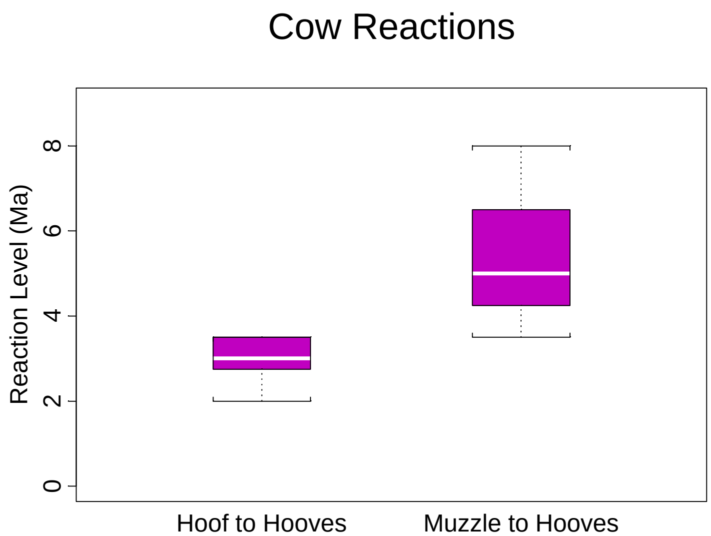


Figure 5. Cortisol concentrations for increasing current exposure.

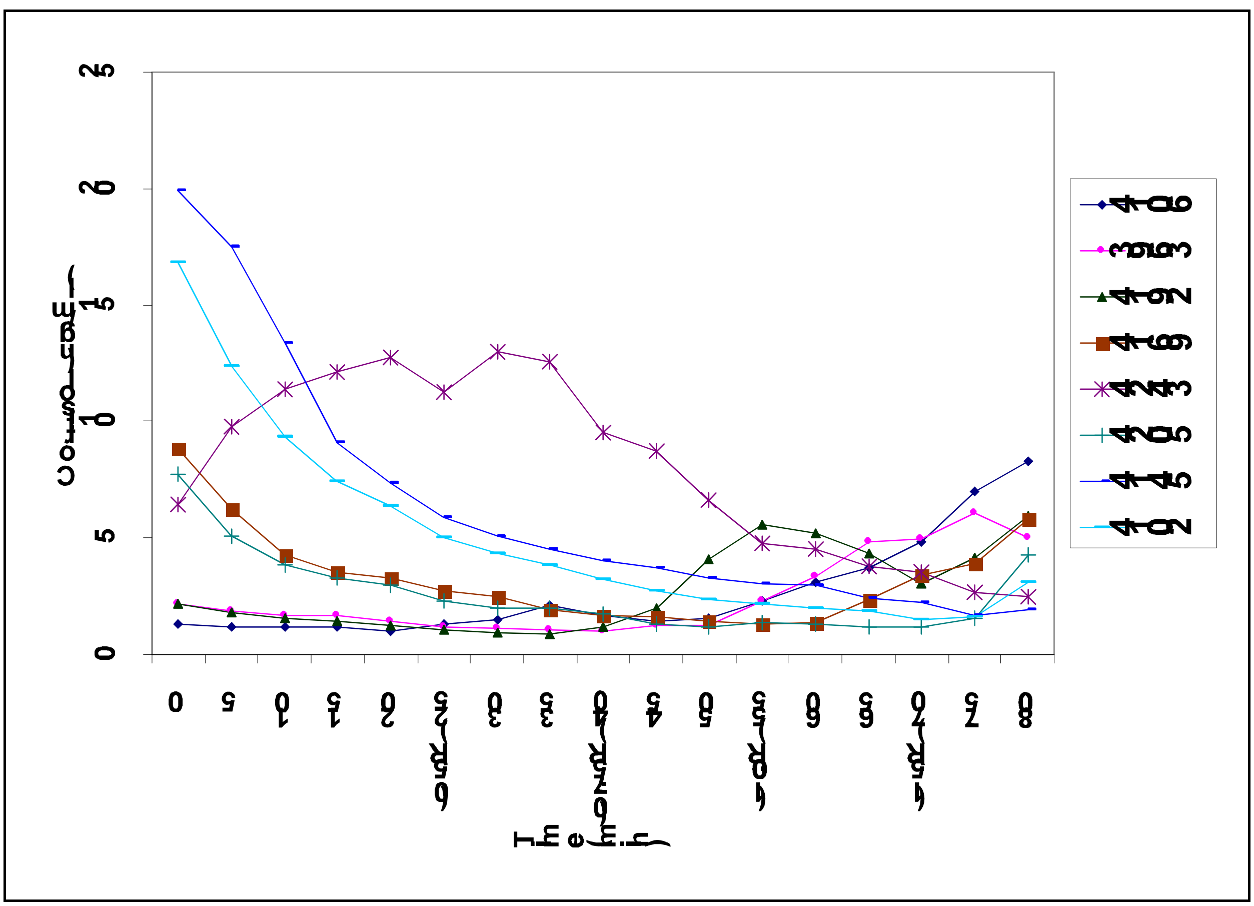


Figure 6. Box plot of the change in 15-m average cortisol concentration for cows exposed to 0.5, 0.75, 1.0 and 1.5 times the current required to produce a behavioral response (R).

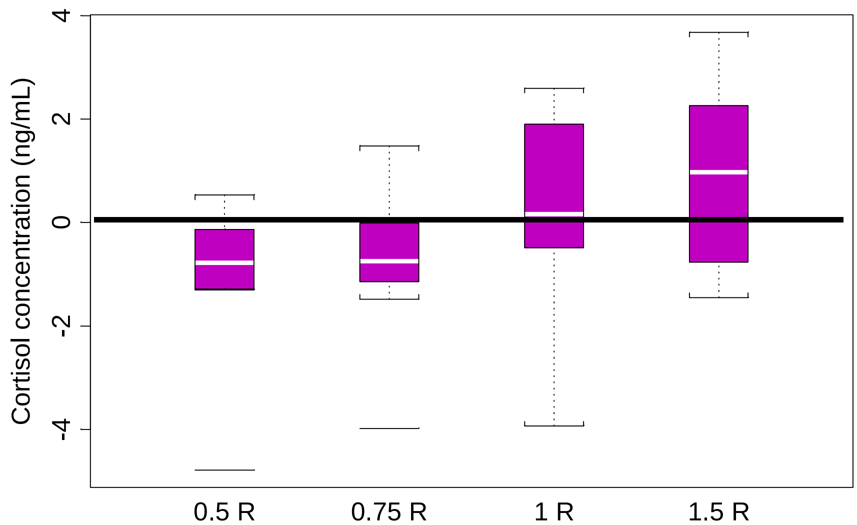


Figure 7. Box plot of 5-m average change in activity of cows exposed to 0.5, 0.75, 1.0 and 1.5 times the current required to produce a behavioral response (R).

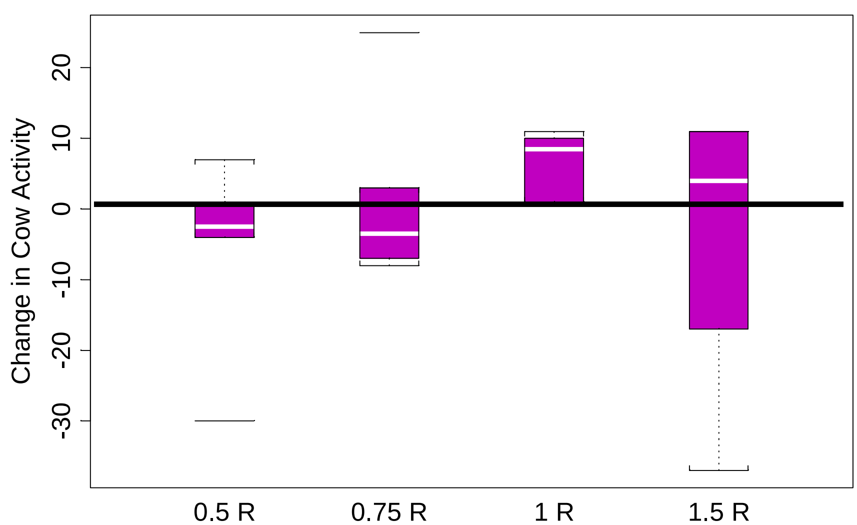


Figure 8. Box plot of cortisol concentrations of 8 cows before and after hoof trimming, and 8 cows before and after exposure to 1.5 times the current required to produce a behavioral response (1.5R).

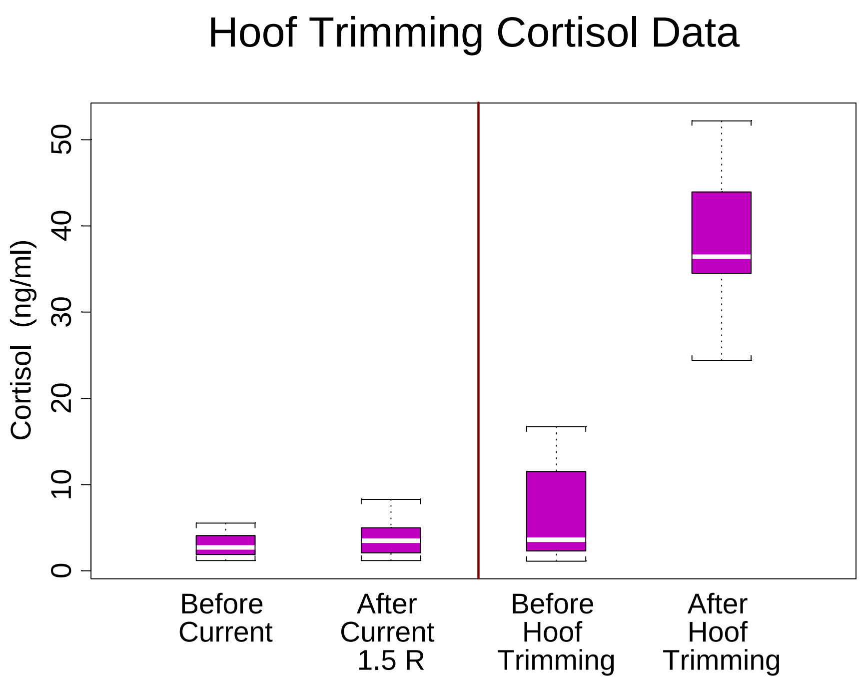


Figure 9. Schematic of electrical apparatus for milking time tests.

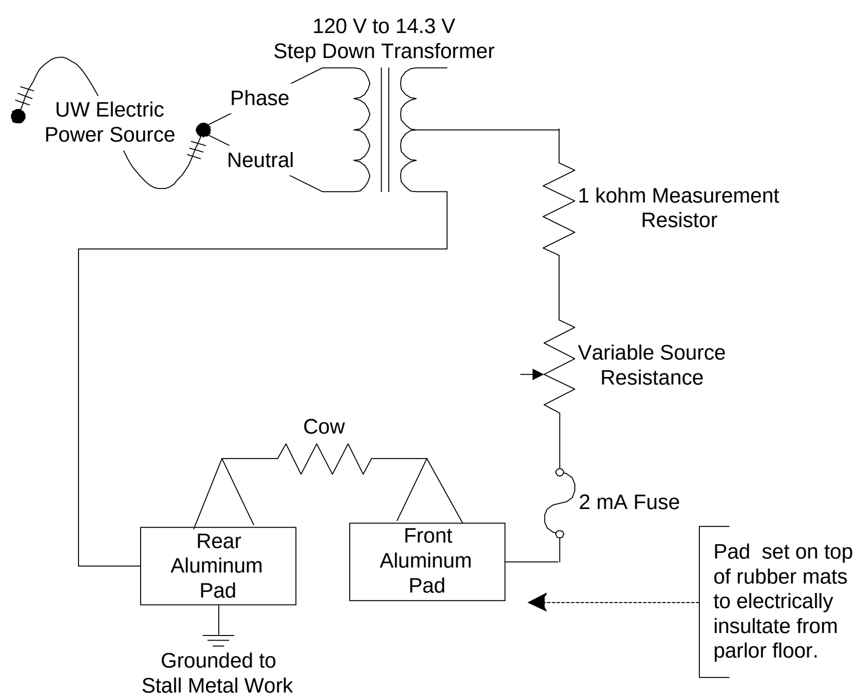
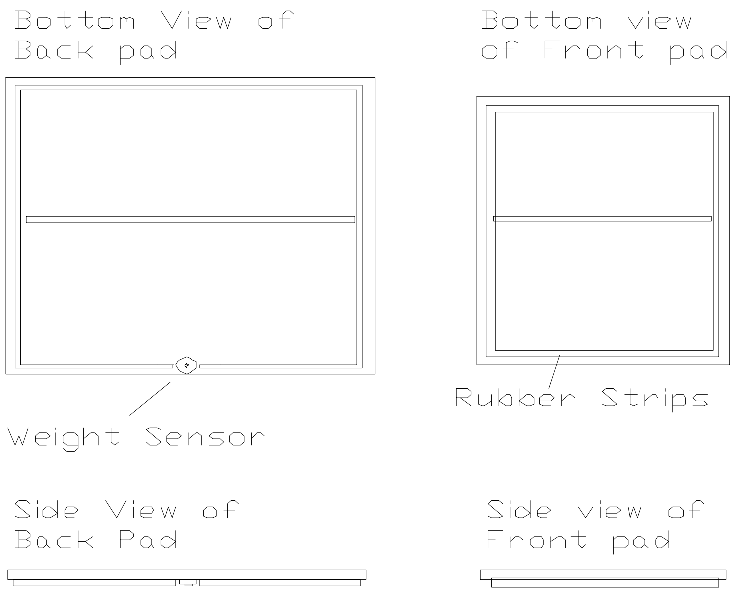


Figure 10. Activity monitoring device for milking time tests.



Data Appendix

Cows used in milking time experiment, pulsation failure and current exposure.

Cow Number	Days in Milk	Lactation Number	Cow Number	Days in Milk	Lactation Number
2336	88	7	4128	168	3
3744	121	6	4029	164	4
3963	102	4	3744	230	6
3970	67	4	3990	138	3
3992	166	4	4252	172	2
3996	69	4	938	284	5
4005	49	4	4286	135	2
4066	223	3	4219	315	2
4131	64	3	4015	70	4
4134	96	3	4405	152	1
4212	254	2	4419	150	1
4226	152	2	4225	288	2
4237	175	2	4397	201	1
4278	68	2	4408	139	1
4284	68	2	4425	135	1
4425	26	1	4145	214	3

Results of milking time experiment, pulsation failure and current exposure. *Note these differences are the average of the variable on the control days minus the value of the variable n the treatment day (a positive value indicates a reduction in the value on the treatment day).*

Cow	Current	Pulse Failure	Strip Yield difference	Milking Time Difference (s)	Peak flow Diff (kg/min)	Activity Diff	Milk Yield Diff (lb)	Ave flow Diff (lb/min)
4212	yes	no	-0.125	27.5	-0.25	-2.5	4	0.14
4134	no	no	-0.125	12.5	0.05	-3.5	2.5	0.16
4278	yes	yes	0.00	-5	0.1	0.5	-8	-0.84
3963	no	yes	0.00	17.5	-0.65	2	1.5	-0.14
4005	no	yes	0.00	-22.5	-0.25	1.5	-4	-0.17
3970	yes	yes	0.125	7.5	-0.35	6	-0.5	-0.06
4131	yes	no	0.00	-5	0.4	-0.5	9	1.37
3996	no	no	0.125	10	-0.5	-6	-5	-0.89
3744	yes	yes	0.375	70	-0.4	6.5	3	-0.57
2336	no	no	-0.125	-45	0.4	-5.5	1.5	0.65
3992	yes	no	0.00	17.5	0.15	-8.5	5	0.36
4237	no	yes	0.00	32.5	-0.4	13	2.5	0.01
4066	no	yes	-0.125	7.5	-0.2	10	4	0.31
4284	yes	yes	0.00	82.5	-0.6	-4.5	-9.5	-2.70
4425	no	no	0.25	42.5	0.15	-3.5	3.5	-0.09
4226	yes	no	0.00	32.5	-0.05	4	2	-0.14
4128	yes	yes	0.125	-2.5	-0.475	3	-6.35	-0.97
4029	no	yes	0.125	-10	-0.64	2.5	-8.65	-1.07
3744	no	no	0.00	-115	0.15	-6	-3.3	0.82
3990	yes	no	0.00	-32.5	0.375	-1	-4.15	-0.07
4252	yes	yes	0.00	20	0.605	0	-1.25	-0.59
938	no	no	0.50	-130	0.37	-9	-4.75	0.60
4286	no	yes	0.50	-50	1.12	1	5.74	1.40
4219	yes	no	-0.25	37.5	-0.65	3.5	2.15	-0.52
4015	no	no	0.00	47.5	-0.03	-6	3.2	-0.19
4405	no	yes	0.50	-2.5	0.005	2.5	4.55	0.88
4419	yes	no	0.125	65	-0.315	8.5	7.85	-0.46
4225	yes	yes	0.375	7.5	-0.445	0.5	-0.85	-0.25
4397	yes	no	0.00	-65	0.42	-1.5	-1.75	0.80
4408	no	no	0.375	-10	0.405	-4.5	0.75	0.45
4425	yes	yes	0.125	-37.5	-0.145	1	-1.3	0.64
4145	no	yes	0.25	45	-0.91	6	-0.26	-1.51

Cows used for milking-time experiment, aged liners and current exposure.

Cows	DIM	LACT	SSC	AVE Milk (lb)
4128	196	3	87	78
4015	98	4	16	108
3744	258	6	76	85
4252	200	2	47	76
938	312	5	60	86
4279	191	2	58	101
4226	289	2	34	59
4264	293	2	198	73
4244	258	2	38	74
4082	309	3	136	62
4399	198	1	196	71
4412	176	1	19	76
4436	126	1	41	76
4405	180	1	39	71
3763	222	7	197	94
919	252	6	NA	86

Results of milking time experiment aged liners and current exposure. *Note these differences are the average of the variable on the control days minus the value of the variable n the treatment day (a positive value indicates a reduction in the value on the treatment day).*

Cow	Current	Aged Liners	Strip Yield Diff	Milking Time Diff (s)	Peak flow Diff (kg/min)	Activity Diff	Milk Yield Diff (lb)	Ave flow Diff (lb/min)	Slips Diff
4128	yes	yes	0.25	-85.0	1.32	10.5	-4.95	0.82	-19
4015	no	no	0.25	27.5	-0.11	3.5	3.15	-0.01	-2
3744	yes	no	-0.125	52.5	-1.28	5.5	-1.10	-0.86	0
4252	no	yes	-0.125	-85.0	0.40	15	-8.85	0.37	-27
938	yes	no	0.00	-25.0	-0.83	7	-4.25	-0.20	0
4279	no	no	-0.25	-30.0	-0.01	8	-0.40	-0.06	1
4226	yes	yes	0.625	-152.5	1.86	-0.5	-1.45	1.49	-11
4264	no	yes	-0.25	-65.0	0.65	14	-4.85	0.39	-26
4244	yes	yes	0.00	-2.5	0.84	7.5	-1.00	-0.16	-26
4082	yes	no	-0.25	17.5	0.35	-4.5	5.90	1.02	0
4399	no	no	0.00	25.0	-0.22	13	0.00	-0.59	0
4412	no	yes	-0.5	-67.5	0.53	-12.5	-5.40	0.21	-17
4436	yes	no	-0.25	7.5	0.53	0	0.35	-0.20	-3.5
4405	no	yes	-0.25	-112.5	0.91	26	-5.20	1.35	-18
3763	no	no	0.125	-27.5	-0.15	-6	-2.80	0.09	2.5
919	yes	yes	0.00	-147.5	1.25	38	-7.15	0.88	-28