

DAIRY COW RESPONSE TO ELECTRICAL ENVIRONMENT
FINAL REPORT
PART III. IMMUNE FUNCTION RESPONSE TO LOW-LEVEL
ELECTRICAL CURRENT EXPOSURE

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by

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ABSTRACT

Twelve lactating Holstein cows, housed in a stanchion barn, were exposed to 1 mA of 60 Hz electrical current from front to rear hooves for two weeks. Twelve cows acted as controls. Immune function was assessed by analyzing blood samples taken twice a week for thirteen different response variables. The measures for lymphocyte blastogenesis (concanavalin A and phytohemagglutinin mitogens), and oxidative burst (PMA-induced chemiluminescence) were chosen *a priori* as the best indicators of immune function response. Immunoglobulin production and interleukin 1 and 2 were also assessed. There was no statistically significant difference between control and treatment cows for any of the main response variables. The difference between the control and treatment cows was statistically significant for one of the secondary response variables but did not appear to be consistent with other observations. Collectively, these results suggest that exposure to 1 mA of current for two weeks had no significant effect on the immune function of dairy cattle.

INTRODUCTION AND LITERATURE REVIEW

The Minnesota Legislature authorized the Minnesota Public Utilities Commission to establish a committee of science advisors in response to claims by some dairy farmers that electric currents in the earth from electric utility distribution systems are somehow responsible for problems with behavior, health and production of dairy cows. A multidisciplinary group with expertise in the fields of agricultural engineering, animal physiology, biochemistry, electrical engineering, electrochemistry, epidemiology,

physics, soil science, and veterinary science were assembled to serve as science advisors. The consensus of the science advisors was that currents in the earth can only interact with dairy cows through their associated electric fields, magnetic fields and voltages, and that these parameters should be the focus of analysis. Five possible mechanisms were identified by which the electrical distribution system could conceivably affect dairy cows. A field study was conducted to investigate the magnitude of these hypothesized electrical factors on 19 Minnesota dairy farms. The combined electrical data from the field study indicated that while none of the five electrical hypotheses could be ruled out, only one of them was a priority for research. This hypothesis is that *continuous or frequently repeated contact of confined cows to sources of low level stray voltage may result in electric fields inside the cow at levels high enough to produce biological effects without producing observable or measurable behavior modifications.* The front to rear hoof step potential measured in the field study resulted in the continuous and longer-term exposure required to satisfy this low level voltage hypothesis. If a physiological response is to occur in dairy cows, it is more likely to be produced by step potential exposures in the stalls rather than outside because: 1. step potentials in the stall are larger than outside, and, 2. step potentials in the stall last longer because of long periods of cow confinement.

A physiological response in dairy cows that are exposed to low level voltages (1-100 mV) has not been specified. Various types of physiological responses (e.g., circulating hormones or their metabolites) to electric and magnetic field exposures have been shown in the published literature to occur in various animals other than dairy cows. These are neither equivalent to, nor indicative of, pathological effects that cause poor health and production in dairy cows. Since it is not possible to extrapolate to dairy cows, further studies were recommended that specifically examine exposure of dairy cows to step potentials lower than those threshold levels already known to elicit behavioral responses.

There have been several studies that have investigated the physiological response of dairy cows exposed to electrical current. Endocrine response experiments are summarized in the previous sections of this report. Gorewit et al. (1992) reported that dairy cows exposed to up to 4 V of 60 Hz while drinking, during the entire lactation, showed no difference in milk yield, somatic cell count, cow health or reproductive performance. Reinemann et al. (1996) reported that cows exposed to transient currents for three weeks showed no significant treatment effect for the following parameters: sodium, albumin, potassium, enzymatic CO₂, chloride, calcium, phosphorus, glucose, creatinine, and creatine kinase. The absence of significant changes in these laboratory data in treatment cattle over time (each cow serving as her own control), as well as the lack of difference between treatment and control cows, indicate that there was no alteration in circulating volume or acid-base balance, nor was there significant stress (as measured by glucose concentration) or muscle injury inflicted by the treatment. In both studies (Gorewit et al. 1992; Reinemann et al. 1996) cows were exposed to electrical current only while drinking, not continuously.

Physiological responses of farm animals to electrical environment have also been studied. Burchard et al. (1998) reported that nocturnal melatonin concentrations in dairy cows did not show any variation that could be attributed to exposure to a vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 μ T. Thompson et al. (1995) reported that cortisol concentrations, weight gain, and wool fiber length and diameter did

not differ between the controls and ewes exposed to a mean electric field of 6 kV and mean magnetic field of 40 mG.

Physiological responses of farm animals to stresses other than electrical exposure have been studied. Cummins and Brunner (1991) reported that housing in metal pens decreased cortisol, plasma ascorbate, IgG and specific antibody titres in dairy calves relative to calves housed in hutches. Elvinger et al. (1992) reported that the major effect of heat stress on immune function of dairy cows was decreased migration of leukocytes to the mammary gland after chemotactic challenge. In a study by Minton et al. (1995), reduced lymphocyte proliferative responses (PHA, Con A, PWM) were reported for lambs subjected to restraint and isolation stress for 6 h on three consecutive days. Treatment did not affect IL2 or MHCII.

OBJECTIVES

The specific objective of these experiments was to test the hypothesis proposed by the Science Advisors to the Minnesota Public utility Commission by measuring immune function response of dairy cows to continuously applied hoof-hoof voltage exposure below the level that would produce a behavioral response. Assays were chosen as rapid, routine measures to provide important initial information on immune system function.

MATERIALS AND METHODS

Test facilities were constructed for groups of 8 cows. Treatment animals were exposed to 1 mA of current flow for a period of 2 weeks. Each replicate used 4 control and 4 treatment animals. Blood samples were taken from all 8 cows twice a week for one week before electrical exposure and for the 2 weeks of electrical exposure. The change in immune function measures was compared between treatment and control groups. Three replicates of 8 cows each were performed using a total of 24 cows. Treatment and control cows had identical stall conditions except for the current treatment. The treatment and control stalls were selected in the systematic pattern shown in Figure 1. Cows were randomized to the stalls and hence the treatment conditions. The cows for this trial were selected on the following criteria:

Lactation number no less than 2 and no greater than 4 (multiparous).

Days in milk (DIM) greater than 150 (mid lactation).

Somatic Cell Count (SSC) less than 150,000 (no mastitis infection).

Days Carrying Calf (DCC) greater than 40 (confirmed pregnant).

The cows in this research herd normally receive BGH injections every 2 weeks. BGH was not administered during this trial so all cows would have missed one scheduled injection during these experiments. The information for the cows used in this study is given in the appendix.

The cows were released from their stalls for milking at approximately 5:30 a.m. and 5:30 p.m. After each milking, the cows were let out into an exercise yard. Cows were returned to the test stalls within 1 hour of being released.

Twelve cows were exposed to 1 mA of current for two weeks (treatment group) and 12 cows were not (control group). The statistical analysis method defined a priori was to take the difference between response variables measured on day 21 (at the end of the treatment period) minus the average of days 3 and 7 (during the pre-treatment period) for each cow. The response is, therefore, the difference from baseline for each cow with the experimental unit defined as an individual cow. The differences of the treatment cows were compared to the differences of the control cows using an independent t-test.

Test Stalls

The test stalls were constructed to allow precise control and measurement of electrical stimuli to individual cows and to eliminate interference from other electrical stimuli occurring in the cow environment. The test stalls consisted of a wooden framework filled with two 120x76 cm (48x30 in.) concrete pads (Figure 2). A 15x15 cm (6x6 in.) welded grid of 9.5 mm (3/8 in.) reinforcing steel was embedded in each pad. There is a 9 cm air gap between the front and rear pads. Cows were secured with head-locking stanchions supported on a wooden framework. When a cow stood in the stall, the front hooves were on the front concrete pad and the rear hooves were on the rear pad.

The front of the test stall was supported by a single 7.3 cm diameter PVC pipe section 5 cm high, located at the center of the stall front end. The rear of the stalls were suspended about 3 cm off the barn floor by two hangers attached at the back corners of the wooden stall frame and metal posts anchored in the concrete. This arrangement provided electrical insulation for all current other than the cow.

Several experiments were carried out to determine the best stall surface for maintaining current exposure levels over extended periods. Single day trials with bare concrete and several different types of organic bedding proved unsatisfactory. The back of the stall surface was periodically wetted with urine and then drained dry. This variable level of moisture in combination with accumulation of organic bedding on the animal hooves changed the animal resistance by a factor of 1000 times or more. It was not possible to maintain current exposure within +/- 10% unless very high source voltages were used.

The concrete surface of the pads were then covered with electrically conductive rubber mats 1.4 cm (9/16 in.) thick (American Health and Safety Inc., item number 1-786.3X5S). These resilient mats allowed the cows to be kept comfortably in the stalls without the use of organic bedding and reduced the risk of injuring feet and legs. The conductive mats with no bedding provided much better control of current exposure with cows both standing and lying than the bare concrete surface either with or without organic bedding.

The stalls were maintained twice a day when the cows were let out of the test stalls for milking. At each of these times the cow contact current level was checked as described below and recorded. Following this current check the stalls were cleaned by removing manure and other foreign material from the stall surface as well as areas surrounding the stalls. The rubber stall surfaces were then washed with a disinfectant (Muliquat, No. 455, Hydrite Chemical Co). The cow contact currents were then rechecked. If the current deviated by more than 10% of the treatment current (1 mA), the current level was adjusted by changing the source resistance. The water cups were also checked to make sure they were dispensing water properly.

Current Application

The intended treatment current was 1 mA through the cow's body. Current was applied continuously for two weeks in a 20-min cycle (10 min on, 10 min off). This cycled pattern was used because previous research suggests that the effects of electric fields may be more pronounced for changing electric fields than for steady fields.

A source voltage of 240 V was created using 120 V output from an uninterruptible power supply (UPS) with power conditioning capabilities and stepped up to 240 volts with an isolated transformer (Figure 3). Power was switched on and off in 10-min intervals using a repeat cycle timer/relay (Syrelec #ODRU, Dallas, Texas).

Current to each of the four treatment stalls was controlled by an adjustable source resistance (decade box power resistor) for each stall. Each current application wire also had a 1k ohm resistor in series to measure the total current flow in that line by measuring the voltage drop across this resistor. The return wire from the rear pad of each test stall was grounded using a separately derived ground located just outside of the barn near the test stalls.

The treatment current level was measured in each treatment stall just before and after the twice-daily stall maintenance. The current exposure was measured using standard methodology used in field investigations of stray voltage. Copper plates (9x9 cm) were placed over wetted paper cloth at the center of the front and rear stall pads. A 3.6 kg weight was placed on the copper plates and the voltage across the plates was measured with 1k ohm shunt resistor and a Fluke 87 true rms multimeter. Leakage current was estimated by comparing the current measured at the 1k ohm resistor in the control box with the "cow contact" current measured at the 1k ohm resistor between the front and rear pads. The amount of leakage current is a function of the resistance of the intended path (pad-cow-pad) the resistance of alternate paths (debris bridging pads or from front pad to ground and wood rails connecting pads).

Periodic measurements of the step potential in control stalls were also made during the second replicate of this study using this method. The range of the measured values was 1.4 mV to 1.7 mV rms. The step potential values were much less than 5% of the 400 mV range specified as the lowest limit of accuracy by the manufacturer. As specified by the manufacturer, the offset of the Fluke 87 meter was checked with the test leads shorted and found to be 1.4 mV. This is a result of internal amplifier noise in the meter. Within the accuracy of this meter, the step potential was not different from zero.

Magnetic Field measurement

Background magnetic field levels were measured using an EmdexC magnetic field meter. This meter is designed to measure the resultant 3-axis 50-60 Hz magnetic field. Field readings were taken directly in front of each stall, in the center of the stall, and directly behind each stall at a height of 1 m from the floor. The average magnetic field at all test stall locations with all electrical devices in the barn running (lights and fans) was 0.3 mG. The magnetic field levels were between 0.14 and 0.4 mG at all locations except at the front of stall 1, which had readings of up to 0.54 mG.

Immune Function Assays

Blood samples were collected by tail bleeding twice weekly for assessment of immune function. Samples were collected for one week before exposure and for the two weeks of exposure. A sample was allowed to clot and resulting serum analyzed for immunoglobulin content by ELISA and IL1 and IL2 by bioassay (Wudhwa et al., 1991). Remaining blood was used to collect leukocytes, as previously described (Lohuis et al., 1990). Hypotonic lysis was used to remove red blood cells, and percoll gradient centrifugation was used to enrich target leukocyte populations. Leukocytes were used immediately for lymphocyte blastogenesis, antibody production and oxidative burst assays.

For lymphocyte blastogenesis (Lane et al., 1979), cells were diluted in Fisher's medium and 50 μ L containing 10^5 cells plated onto 96-well culture dishes. Responses to standard mitogens, including *S. aureus*, phytohemagglutinin, pokeweed mitogen and concanavalin A were determined. Phytohemagglutinin and concanavalin A activate largely T lymphocytes, pokeweed mitogen T and B lymphocytes and *S. aureus* cells B lymphocytes. After 72 hours, 1 μ Ci 3 H-thymidine was added, cells incubated an additional 4 hours and cells harvested using a 96-well plate harvester. Incorporation of 3 H-thymidine into DNA was used as an index of mitogenesis.

To assess immunoglobulin production (Lane et al., 1979), 3×10^6 cells were suspended in 300 μ L media. Cells were treated with or without pokeweed mitogen for 5-10 days and immunoglobulin production assessed by ELISA, using antibodies against specific bovine immunoglobulins.

To assess oxidative burst (Trush et al., 1978), chemiluminescence in response to standard activators of macrophage and neutrophil function was used. Leukocytes (10^6) were placed in 0.5 mL phenol red free Dulbecco's Modified Eagle's Medium (DMEM) containing 100 mg/mL luminol. Baseline luminescence was assessed after 10 minutes incubation. Next, 0 or 10 ng/mL phorbol myristate acetate (PMA) was added, cells incubated 1 minute and light emission determined again. The difference was used to estimate PMA-induced chemiluminescence.

The measures for lymphocyte blastogenesis using concanavalin A, and phytohemagglutinin mitogens and oxidative burst as measured by PMA-induced chemiluminescence were chosen *a priori* as the best indicators of immune function response. These questions were selected from the response variables to control the Type I error for the experiment's most important questions.

Other Responses

In addition to the blood measures, daily water volume and feed consumed, cow temperature and daily milk production were monitored. Each test stall was equipped with a water meter that was read once daily during the morning milking. Feed intake was monitored for each cow by measuring daily feed supplied minus leftover feed found in the feed bins. The amount of feed supplied was intended to keep some feed in the bins 24 hours a day. The milk meters in the milking parlor (BouMatic - Perfection), recorded milk yields.

The time and pattern of standing and lying were recorded on one of the control days and again near the end of the treatment period during the third replicate. The time for cows to reenter stalls after milking was measured. If the voltage/current exposure were perceived, the time and pattern of lying or time to enter stalls could be changed.

RESULTS

Current Application

The results of the twice-daily measurements of cow contact current are summarized in Table I. The average cow current was within the +/- 10% target value for all cows except 4262 in replicate II. This cow was fistulated and leaking rumen fluid caused the stall surface to remain wet and created a leakage path. The average value of 0.6 mA is probably an under-estimate of the true average as these measurements were taken at the end of each 12-hour observation period, when the stall condition was likely at its worst. Immediately after these measurements were taken, the stalls were cleaned and the current levels readjusted to 1 mA.

Table I. results of the twice-daily measurements of cow contact current.

Cow Number	Replicate	Average Current (mA)	Standard Deviation (mA)
3910	I	1.00	0.15
4066	I	1.04	0.06
4161	I	0.93	0.34
4192	I	1.10	0.21
3861	II	0.94	0.20
4243	II	1.00	0.16
4262	II	0.60	0.28
4084	II	0.97	0.23
3987	III	0.98	0.03
4057	III	1.03	0.02
4157	III	0.98	0.05
4279	III	0.99	0.02

Further measurements were done to estimate the stability of the cow current in the time between the twice-daily cow current measurements. Tests were done periodically using shunt resistor values of 0.5k, 1k, 5, and 10k ohms. The cow contact current was within +/- 0.1 mA for all resistance values except the 10k resistor, which fell just outside the

10% deviation with an average cow contact current of 0.89 mA. The test stalls were thus able to maintain a cow contact current within +/- 10% for the practical range of cow and contact resistances.

The average source resistance, recorded twice daily was 196k ohms. Values were between 170k and 230k ohms for all tests except for cow 4262 in replicate II (leaky fistula) in which case the source resistance was typically 100k to 150k ohms. The resistance of the rest of the circuit (pads, mats, cow and contact resistance) was between 10k and 70 k ohms with a standard deviation of individual stalls between 1k to 3k ohms (or less than 2 % of the total circuit resistance). The only exception to this was the cow with a leaking fistula in which case the standard deviation increased to 32k ohms or 13 % of the total circuit resistance.

The current measured at the 1k ohm resistor in the control box was monitored for 24 hours on all test stalls during the third replicate. The 12 hour average current was compared to the last 10 minute interval (corresponding to the twice-daily cow-current checks). The ratio of the 12 hour average current to the last 10 minutes was between 94 and 99 % with standard deviations between 8 and 10 %. The voltage between the wires connected to front and rear pads was also monitored for 24 hours for each stall during replicate III. The expected range of voltages for this measurement is 10 to 70 V corresponding to the source voltage and 10 to 70k ohm resistance measured for this part of the circuit. The 24 hour average measured pad to pad voltage was 28 V with a standard deviation of 18 V. Less than 1% of the data points were in excess of 76 V. These values are within the expected range and indicate that the current exposure was stable during the treatment periods.

The combination of these measurements show that the average cow contact current was within the design range of 1 mA +/- 0.1 mA except for the cow with a leaking fistula in which case the average current exposure was probably about 0.8 mA +/- 0.3 mA.

Immune Function Responses

The summary statistics for the 3 replicates of current exposure experiment are given in Table II. Box plots of the main response variables are given in Figures 4-7. Statistical analysis was done after taking the natural log of all immune response data. This log transform yielded a more normal distribution of the data. The difference from baseline level for each measure was used as the response variable for each cow. The difference values for the treatment animals were then compared to the difference values for the control cows using an independent, two-tailed t-test. The questions for this work have been divided into two groups--the main questions and other questions. The comparison-wise Type I error for the main questions was $p=0.05$.

Table II. Summary statistics for immune function measures. The main questions are indicated in Bold. *Data analyzed as difference of natural logs, n of controls = 12, n of treatments = 12, DPM = Disintegration per minute, RLU = Relative Light Units*

Main Response Variables	Mean Change of Controls	Mean Difference (Treatment–Control)	P-value Two Tailed Independent Test
	Mean Change of Treatments		
Conconavalin A ln(DPM)	1.267	-0.247	0.724
	1.020		
Phytohemagglutinin ln(DPM)	0.799	-0.128	0.647
	0.671		
Chemiluminescence PMA, ln(RLU)	0.483	-0.414	0.280
	0.069		
Secondary Response Variables			
<i>S. aureus</i> , ln(DPM)	0.632	-0.637	0.038
	-0.005		
Pokeweed, ln(DPM)	0.668	-0.286	0.272
	0.382		
IgG Serum, ln(mg/mL)	-0.034	0.017	0.771
	-0.017		
IgG in vitro, ln(mg/mL)	-0.154	-0.035	0.862
	-0.189		
IgA Serum, ln(mg/mL)	-0.005	0.017	0.796
	0.012		
IL1 Serum, ln(pg/mL)	-0.085	0.535	0.071
	0.450		
IL1 in vitro, ln(pg/mL)	-0.063	0.041	0.410
	-0.022		
IL2 Serum, ln(pg/mL)	-0.041	-0.098	0.218
	-0.139		
IL2 in vitro, ln(pg/mL)	-0.060	-0.203	0.351
	-0.263		
Cortisol, ln(ng/mL)	-0.427	0.044	0.900
	-0.383		

Positive Control

An experiment was done to validate the immune assays using the well-know immune response of cows to dexamethasone as a positive control. Four non-pregnant cows were injected with dexamethasone for 4 days. Each of the treatment cows received two injections of 15 mg of Dexamethasone (Dexamethasone, Sodium phosphate, Steris Laboratories Inc. Phoenix, Arizona 85043 USA) per day at 12-hour intervals for four days (Monday, Tuesday, Wednesday, and Thursday, approximately 7 a.m. and 7 p.m.). Blood samples were taken prior to the injection on Monday and at 7 a.m. Friday.

The 3 control cows received a placebo shot of the saline solution only. These shots were given at the same time that the treatment cows receive their shots. The cows had identical stall conditions. Blood samples were taken prior to the injection on Monday and on Friday for the control cows as well. Cows were handled in the same way as in the current exposure experiments except that no current was applied during this study. Seven cows were available for this trial, 4 were randomly selected as treatments and 3 as controls. Cows were selected on the following criteria:

Lactating and no less than 2 and no greater than 4 if possible.

DIM greater than 40.

SSC less than 150 If possible.

Non-Pregnant.

Good feet and legs.

Information on the cows used for this trial is given in the appendix. Summary statistics of the positive control experiment are given in Table III and raw data in Figure 8.

One of the control cows (2336) injured her right front teat on the morning of 5/9/99 and subsequently developed a mastitis infection. She was treated and stayed in the experiment. This cow showed a reduction in all 3 of the main immune function responses.

Table III. Summary statistics for immune function measures for positive control experiment, dexamethazone injection. The main questions are indicated in Bold. *Difference of natural logs, n of controls = 4, n of treatments = 3, DPM = Disintegration per minute, RLU = Relative Light Units*

Main Response Variables	Mean Change of Controls	Mean Difference (Treatment–Control)	P-value Two Tailed Independent Test
	Mean Change of Treatments		
Conconavalin A ln(DPM)	-1.858	-2.291	0.044
	-4.149		
Phytohemagglutinin ln(DPM)	-0.898	0.368	0.767
	-0.530		
Chemiluminescence PMA ln(RLU)	-0.418	-1.036	0.278
	-1.454		
Secondary Response Variables			
<i>S. aureus</i> , ln(DPM)	-1.060	0.250	0.799
	-0.810		
Pokeweed, ln(DPM)	-0.739	0.845	0.336
	0.106		
IgG serum, ln(mg/mL)	-0.089	0.001	0.997
	-0.088		
IgG in vitro, ln(mg/mL)	0.0563	-0.843	0.010
	-0.787		
IgA serum, ln(mg/mL)	0.248	-0.032	0.958
	0.216		
IL1 serum, ln(pg/mL)	0.347	-0.331	0.580
	0.016		
IL1 in vitro, ln(pg/mL)	-0.025	-0.380	0.005
	-0.405		
IL2 serum, ln(pg/mL)	-0.173	0.207	0.362
	0.034		
IL2 in vitro, ln(pg/mL)	0.031	-0.556	0.163
	-0.525		
Cortisol, ln(ng/mL)	2.055	-5.017	0.003
	-2.961		

Other Responses

The standing and lying behavior of cows was analyzed in two ways. First the percentage of time spent standing was calculated for each of the control and treatment cows during the pre-exposure period. The change in this value for each cow was compared for control and treatment cows measured again at the end of the current exposure period. The same analysis was done using the percentage of periods in which cows changed status (from standing to lying or from lying to standing). The results of these tests are summarized below.

Percent of time standing	Pre-Exposure	End of Exposure
Control Cows	56%	48%
Treatment Cows	44%	37%

A two tailed t-test indicated that the difference between control and treatment cows was not significant ($p= 0.95$)

Percent of time Change in Status	Pre-Exposure	End of Exposure
Control Cows	11%	9%
Treatment Cows	17%	7%

A two-tailed t-test indicated that the difference between control and treatment cows was not significant ($p= 0.35$)

The time required for the cows to move from the center alley into the stalls was measured on 3 consecutive days near the end of the exposure period of the third replicate. None of the cows showed any hesitation to enter the stalls. The mean of the treatment cows was 3.5 s with a standard deviation of 1.0 s. The mean of the control cows was 4.2 s with a standard deviation of 1.6 s. The difference between the control and treatment animals was not significant ($p=0.46$).

Data for cow temperature, daily milk weights, water consumption, and twice-daily current measurements are given in the appendix.

DISCUSSION

Lymphocyte mitogenesis (blastogenesis) is a well-documented response to lectins and is generally recognized as a useful measure of systemic immune function (Lohuis et al., 1990). Chemiluminescence is widely used as a measure of respiratory burst in phagocytic cells, a key event in phagocytosis and intracellular killing of bacteria (Thrush et al., 1978). These two measures together provide important measures of lymphocyte and phagocyte function in response to various treatments. These measures represent several of the major immunological processes and are the most likely to be altered if systemic immune function is suppressed by the treatments. The two-week exposure period appears justified as previous work on housing stress in farm animals has shown significant immune system response within 3 days (Minton et al., 1995) and one of the

control cows in this experiment showed a change in immune function within 4 days in response to a mastitis infection.

The assays used in the present study are standard methods of assessing immunological function in mammals. Lymphocyte blastogenesis in response to concanavalin A and Phytohemagglutinin measures activation of T lymphocytes, while *S. aureus* measures B lymphocyte activation and pokeweed mitogen measures both T and B lymphocyte activation (Lane et al., 1979). Of these measures, only *S. aureus*-induced blastogenesis was significantly affected by 2 weeks of voltage exposure. This response would suggest a change in responsiveness of B cells (cells that eventually differentiate to produce immunoglobulins). However, no other measures, including pokeweed mitogen-induced blastogenesis, pokeweed mitogen-induced immunoglobulin production or in vivo antibody concentrations were affected. In addition, the difference in *S. aureus* was caused by an increase in the control cows while the treatment cows showed no change. Thus, it is possible that this response was a type I error. Concanavalin A-induced blastogenesis was inhibited in positive controls cows (dexamethasone treated).

Chemiluminescence is a widely used measure of respiratory burst, a key event in intracellular killing of bacteria (Thrush et al., 1978). The present study found no effect of voltage exposure on chemiluminescence, suggesting that bactericidal activity of circulating phagocytic cells was unaffected by treatment. Treatment with dexamethasone (a glucocorticoid used as a positive control) significantly inhibited chemiluminescence.

In addition, immunoglobulin levels in vivo and in vitro in response to pokeweed mitogen were measured as indices of immune function. As indicated earlier, these responses were unaffected by voltage exposure of cattle. However, dexamethasone significantly inhibited pokeweed mitogen-induced antibody production in vitro.

Two major cytokines regulating immune function, interleukin 1 and 2, were measured. Measurements included both serum concentrations and pokeweed mitogen-induced interleukin production in vitro. Interleukin 1 concentrations in serum were slightly elevated upon voltage exposure for 2 weeks ($P < 0.07$), but serum interleukin 2 concentration and interleukin 1 and 2 production in vitro were unaffected. IL1 change in the combined data (all 3 replicates) appeared to be strongly influenced by one replicate (replicate 2, $p < 0.06$), with minimal change in the other two replicates ($p = 0.96$ and $p = 0.46$). The bioassays used do not differentiate between a and b forms of interleukin 1 (Wudhwa et al., 1991), so the possibility that one isoform was selectively affected cannot be excluded. In positive controls, dexamethasone decreased interleukin 1 production.

CONCLUSION

Collectively, these results suggest that exposure to 1 mA of 60 Hz electrical current for two weeks had no significant effect on immune function of dairy cattle. One of 13 response variables was statistically significant but did not appear to be entirely consistent with other observations.

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Figure 1. Location of treatment and control stalls in the barn. .

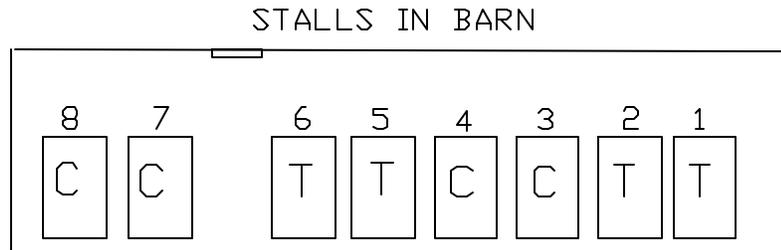


Figure 2. Diagram of test stalls.

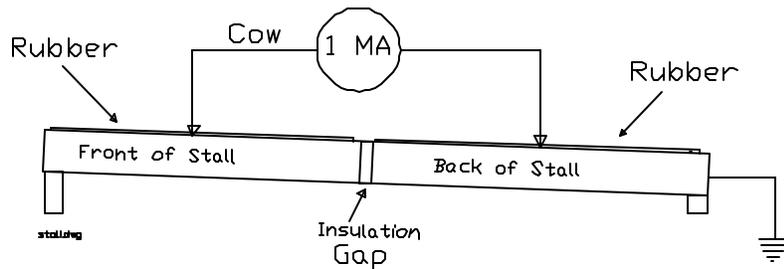


Figure 3. Schematic of electrical exposure circuit.

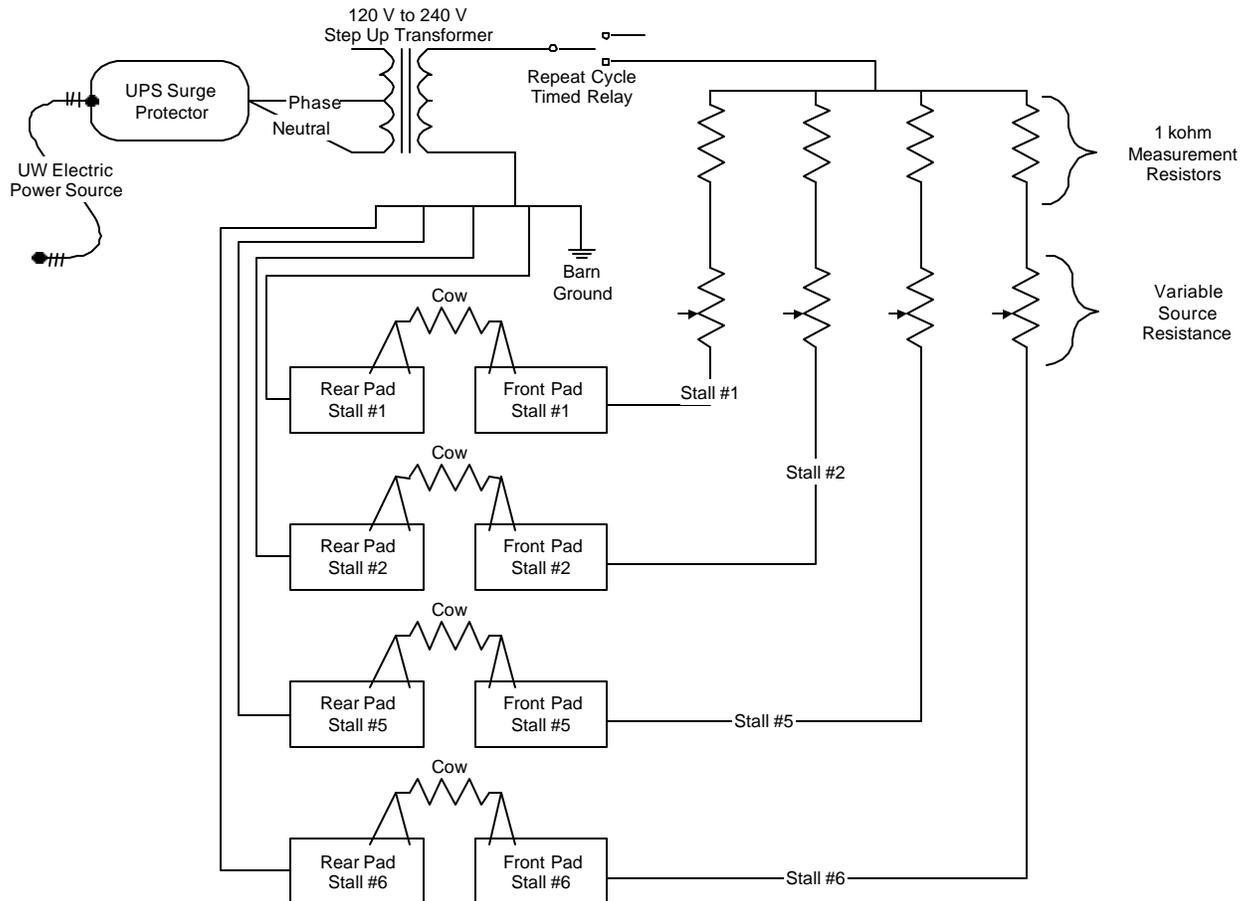


Figure 4. Box plots of the main response variables. The horizontal white line is the mean of the data. The box includes +/- 25% of the data from the median. The horizontal black lines are the maximum and minimum values. Current exposure started on day 8.

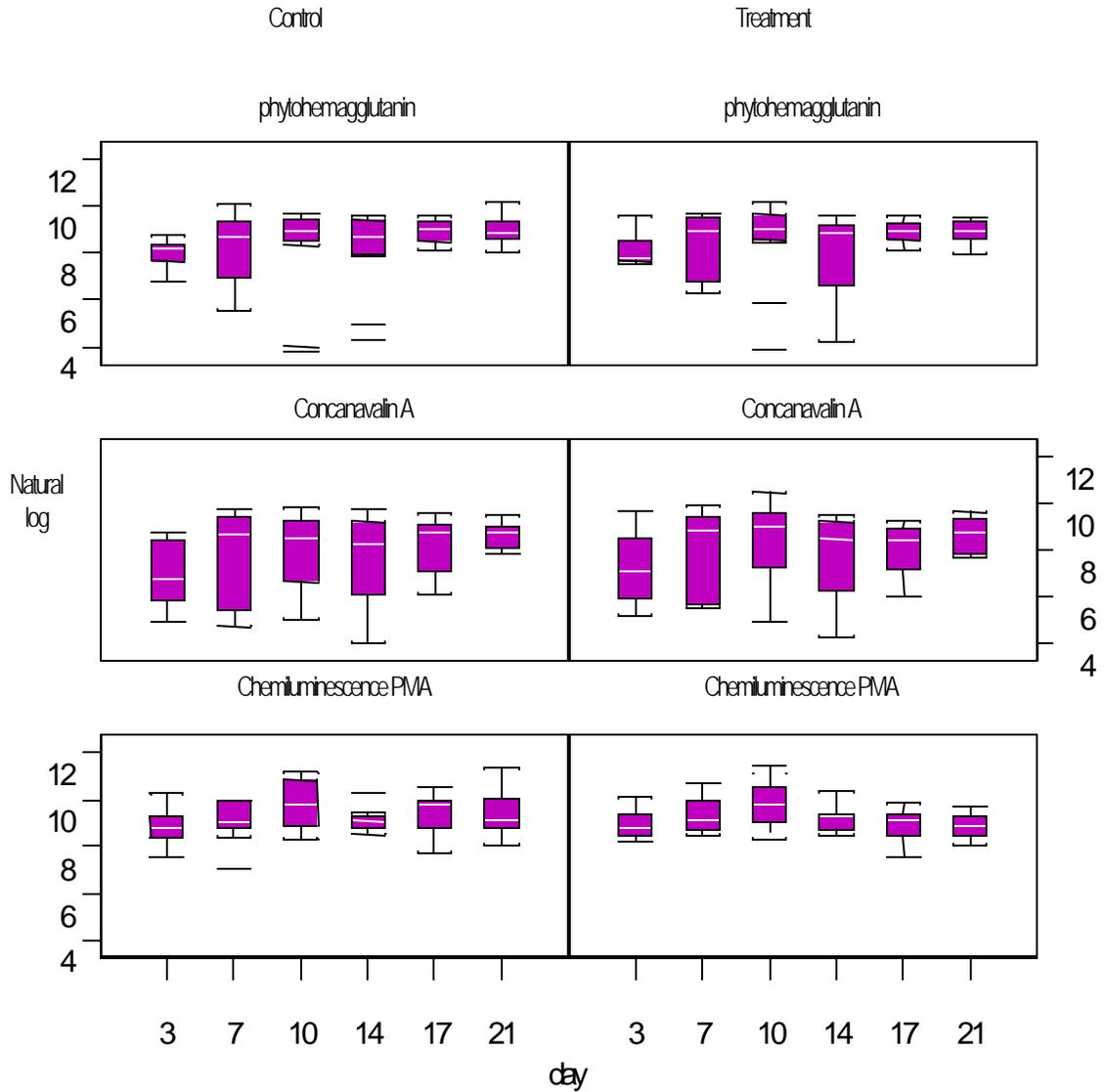


Figure 5. Natural Log of Chemiluminescence
Control and Treatment (Difference Values)

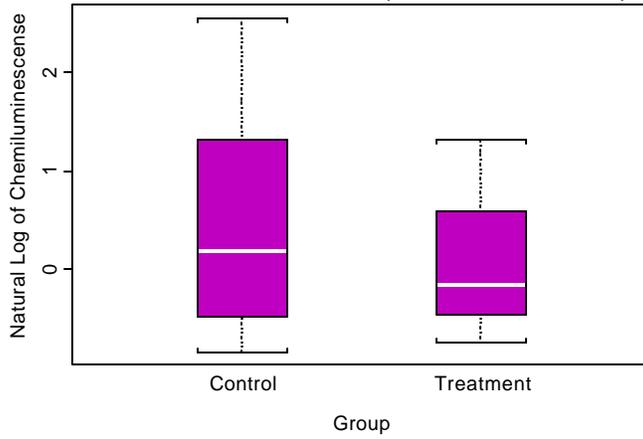


Figure 6. Natural Log of Concanavalin A
Control and Treatment (Difference Values)

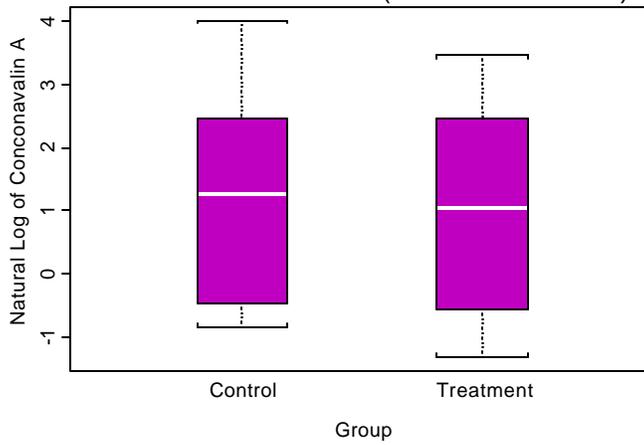


Figure 7. Natural Log of Phytohemagglutani
Control and Treatment (Difference Values)

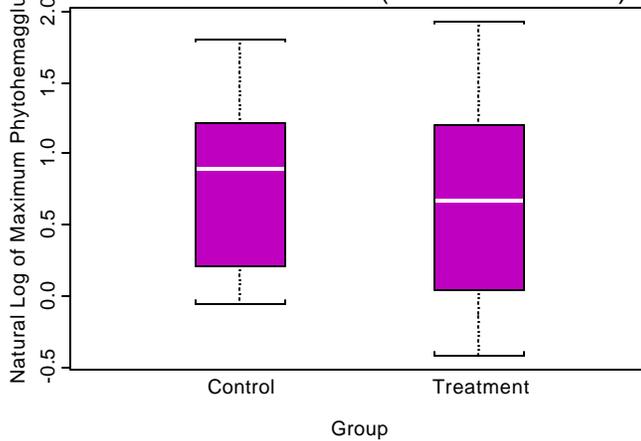


Figure 8. Main Response variables for positive control experiment.

