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Exposure of Pregnant Dairy Heifer to Magnetic Fields at 60 Hz and 30 μT

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Thirty-two pregnant Holstein heifers weighing 499 ± 45 kg, at 3.1 ± .7 months of gestation and 21 ± 2.0 months of age were confined and exposed to 30 μT magnetic fields (MFs) and a 12 h light/12 h dark light cycle. The heifers were divided into two replicates of 16 animals. Each replicate was divided into two groups of eight animals each, one group the non-exposed and the second, the exposed group. The animals were subjected to the different treatments for 4 weeks. After 4 weeks, the animals switched treatment, the exposed group becoming the non-exposed group and vice versa. Then the treatment continued for 4 more weeks. Catheters were inserted into the jugular vein, and blood samples were collected twice a week to estimate the concentration of progesterone (P4), melatonin (MLT), prolactin (PRL), and insulin-like growth factor 1 (IGF-1). Feed consumption was measured daily. The results indicated that exposure of pregnant heifers to MF similar to those encountered underneath a 735 kV high tension electrical power line for 20 h/day during a period of 4 weeks produces slight effects. This is evidenced by statistically significant higher body weight (1.2%), higher weekly body weight gain (30%), and decreases in the concentration of PRL (15%) and IGF-1 (4%) in blood serum. The absence of abnormal clinical signs and the absolute magnitude of the significant changes detected during MF exposure, make it plausible to preclude any major animal health hazard. Bioelectromagnetics 28:471–476, 2007. © 2007 Wiley-Liss, Inc.

Key words: magnetic field; ELF; dairy cattle; prolactin; melatonin; progesterone; IGF-1

INTRODUCTION

Previous studies have shown that dairy cattle exposed to worst case scenario electric and magnetic fields (EMFs), as found directly under 735 kV AC power lines carrying around 2000 A, increased their feed consumption, milk yield, progesterone (P4) concentrations in blood plasma [Burchard et al., 1996] and estrous cycle length [Burchard et al., 1998b]. These EMFs have also been associated with changes in the concentrations of macro and trace elements in blood plasma [Burchard et al., 1999] and neurotransmitter metabolites in cerebrospinal fluid [Burchard et al., 1998c] and slight variations in plasma thyroxine [Burchard et al., 2006] in dairy cattle. Lactating pregnant dairy cows exposed to EMF (60 Hz, 10 kV/m, and 30 μT) had increased blood prolactin (PRL) and decreased diurnal melatonin (MLT) concentrations [Rodriguez et al., 2004]. Non-pregnant dairy cattle exposed to EMF (60 Hz, 10 kV/m, and 30 μT) during estrous cycle did not show changes in PRL and a decreasing tendency in diurnal MLT [Rodriguez et al., 2004]. Exposure of pregnant dairy cattle to a 60 Hz, 10 k/V electric field (EF) did not have any effect on MLT, P4, PRL or insulin-like growth factor I (IGF-I) [Burchard et al., 2004].

The above-mentioned research established mild effects of EMF on some physiological variables in dairy cattle. Exposure of dairy cattle to EF did not replicate these effects. The experiment reported herein is an attempt to discern if the effects observed with EMF exposure may be attributable to the magnetic field (MF) component.

MATERIALS AND METHODS

Animal Care and Exposure

All the experimental procedures comply with the good laboratory practices (GLP) recommended by the

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guide to the care and use of experimental animals of the Canadian Council on Animal Care [CCAC, 1984].

Thirty-two pregnant Holstein heifers weighing 499 ± 45 kg, at 3.1 ± .7 months of gestation and 21 ± 2.0 months of age (Table 1), obtained from commercial herds in the province of Québec, were confined to wooden metabolism cages in exposure chambers for the duration of the experiment. These chambers were designed and constructed to resemble commercial tie stall barns prevailing in the province of Québec. Further description of the MF exposure chamber can be found elsewhere [Burchard et al., 1999; Nguyen et al., 2005]. The animals were exposed to artificial light with a cycle of 12 h of light with 178 lx (PANLUX Gossen # 3C10075, Nürnberg, Germany) followed by 12 h of darkness. Every day during the study, lights in the chambers were turned on and off at 04:00 and 16:00 h, respectively.

The animals were fed twice daily, to meet NRC requirements [NRC, 2001]. Water and feed were available ad libitum. Test animals were fed a total mixed ration consisting of a mix of forages, and a commercial supplement containing 12% crude protein and required minerals and vitamins.

This study was carried out in two replicates; the first and second replicate were executed between October and December, 2001 (16 animals) and between February and March, 2004 (16 animals), respectively. The MF exposure chamber was 15 m long, 10 m wide, and 3 m high; contained eight wooden metabolism cages, each housing one animal. Measurements of the MF were performed with probes installed at three locations in the chamber and were recorded with a data acquisition system every 5 min. The field intensities obtained by the probes were relative values and were calibrated before the experiments with a commercial probe, the Holaday model 3604 (Holaday Industries, Inc., Eden, MN). The software controlling the input voltage and current for MF generation in the exposure chamber, also acquired field values every 5 min to assess the stability and uniformity of the field intensities in the exposure and control rooms. The intensity of the MF chosen for this experiment resembles the exposure encountered by animals standing continuously under a 60 Hz 735-kV power line carrying 2000 A. The control chamber had the same design as the MF exposure chamber without the coil to generate MF. A detailed description of the exposure chamber has been published elsewhere [Nguyen et al., 2005].

The animals were housed in the MF and control chambers for a 6-day-adaptation phase immediately before the initiation of treatment. Treatment exposure was conducted using a cross-over design. A total of thirty-two heifers were divided into two replicates of 16 animals each. Each replicate was divided into two groups of eight animals, one group becoming the non-exposed and the second group, the exposed group. The animals were subjected continuously to the different treatments for 4 weeks, except during the time required for cleaning, feeding and sampling. After 4 weeks of treatment, the animals switched rooms; the non-exposed group was moved to the MF exposure chamber and the exposed group was moved to the control chamber, the MF were deactivated and a period of 1 week without any treatment was allowed. Subsequently, treatments were applied for 4 additional weeks.

Blood and Feed Sampling

Catheters were inserted into the jugular vein 3 days before blood sampling. After sedation, (Rompun, Bayvet, Etobicoke, Ont., Canada), an angiocatheter (intracath IV catheter, Vialon, Becton & Dickinson cat # 3831621 Oakville, Ont., Canada) was inserted into the jugular vein and fixed to the skin and left in place for the duration of the experiment. The procedure for the installation and maintenance of the jugular catheters has been described elsewhere [Burchard et al., 2004].

Blood samples were collected on Tuesdays at 09:00 and on Thursdays at 09:00, 10:00, 11:00, and 12:00 AM for the duration of experiment. Blood samples were collected into plain vacutainer tubes, stored in the refrigerator for 12 h to allow coagulation, then centrifuged at 1000*g* for 15 min. The serum was

TABLE 1. Body Weight, Age, and Gestation Length (Mean ± Standard Deviation) of the Animals at the Beginning of the Study for Each Replicate and Treatment Group

Replicate	Group	Weight (kg)	Age (months)	Gestation (months)
1	Off–on	500.63 ± 43.30	20.25 ± .71	2.86 ± .78
	On–off	502.50 ± 44.70	20.13 ± .42	2.78 ± .82
2	Off–on	482.75 ± 49.80	20.88 ± 1.89	3.36 ± .66
	On–off	511.75 ± 49.90	20.38 ± 1.30	3.29 ± .42
Both	Off–on	491.69 ± 46.00	20.56 ± 1.41	3.11 ± .75
	On–off	507.13 ± 46.00	20.25 ± 1.88	3.03 ± .68

stored at −20 °C pending analysis. Dry matter intake, a measure of feed consumption, was measured daily and the animals were weighed weekly. Feed samples were collected on Tuesday and Thursday mornings, composited into a weekly sample and then analyzed to estimate their nutritional components.

Blood Analyses

Serum P4 concentrations were estimated using a solid phase radioimmunoassay (RIA) (Coat Count, Diagnostic Product Corporation, Los Angeles, CA). Serum MLT concentrations were estimated with a direct RIA previously validated [Fraser et al., 1983], using the Guilford antiserum from Stockgrand Ltd. (Guilford, Surrey, UK; batch g/s/704-6483) and tracer from Amersham Biosciences (Buckinghamshire, UK; TRK 798). Serum PRL was estimated with a double antibody RIA using iodinated PRL and a primary antibody (bPRL AFP-4835B; kindly donated by Dr. Parlow, NHPP, and NIDDK, Torrance, CA), validated previously [Miller et al., 1999]. Quantification of serum IGF-I was done with an immunoradiometric assay (IRMA) (DSL-2800 kit, Diagnostic Systems Laboratories, Inc., Houston, TX) previously validated [León et al., 2004].

Intra-assay and inter-assay variations for P4, MLT, PRL, and IGF-1 were 8.60 and 10.27%, 4.96 and 13.70%, 8.46 and 9.74%, and 2.97 and 12.91%, respectively.

Statistical Analysis

This experiment was carried out as a cross-over trial to remove the between-cow variation from the experimental error; hence, each treatment is compared on each cow [Ratkowsky et al., 1993]. Briefly, each cow was initially on one treatment, then half way through the experiment was changed over to the other treatment. The statistical analysis took into consideration the treatment cross-over design structure with repeated measurements. The design had two replicates of 16 animals each. For each replicate the animals were randomly split into two groups of eight animals each, initially assigned to one of the two treatments, and after 4 weeks the animals switched treatments. The statistical methodology is described in detail elsewhere [Burchard et al., 2004]. In order to normalize the estimations of P4, MLT, PRL, and IGF-1 concentrations, a log-transformation was applied.

RESULTS

Initial comparison of the animals revealed no differences regarding weight and age. There was a minor difference in gestation length between replicates (Tables 1 and 2). The analysis of the power of the test for variables in the study revealed that, overall, the power of the test was adequate (Table 3). For example, in the case of MLT the difference between treatments is 6% and the power of the test is 96% with 32 animals. This means that the test would yield a significant treatment effect about 96 times out of 100 on average, when the difference between treatments is 6%. The results obtained for DMI, protein and energy intake, are in agreement with the expected intake according to the nutritional requirements for dairy cattle of the National Research Council [NRC, 2001].

The control and treated animals were exposed to a horizontal MF of .58 ± .01 μT or 30.5 ± .04 μT for an average of 20.2 ± .43 h/day. During the experiment, temperature and humidity in the control and exposure rooms were 17.5 ± .04 °C, 42 ± .1%; 17.8 ± .02 °C and 44 ± .1%, respectively. Globally, exposure to MF did not affect feed consumption; however, a treatment by week interaction was detected ($P = .0013$) (Table 4, Fig. 1). On week 3 DMI was 7.5% higher ($P = .025$) during exposure; however, when applying the Bonferroni correction, to avoid type I error in a multiple testing problem, this difference became a trend ($P = .097$). Body weight increased 1.2% ($P = .01$) during exposure and there was a treatment by week interaction ($P = .0001$) (Fig. 2). The average weekly body weight gain was 30% higher ($P = .012$) during exposure (Table 4, Fig. 3). Results for MLT, P4, PRL, and IGF-1 are presented in Table 5 and are in agreement with those obtained elsewhere [Mäntysaari et al., 1999; Burchard et al., 2004; León et al., 2004; Muthurama-

TABLE 2. Two-Way ANOVA With Replicate and Group Effects on Body Weight, Age, and Gestation Length

		Weight			Age			Gestation length		
Source	df	MS	F-value	Pr > F	MS	F-value	Pr > F	MS	F-value	Pr > F
Replicate	1	148.78	.07	.7972	1.5313	.53	.4733	2.0503	4.32	.0470
Group	1	1906.53	.86	.3610	.7813	.27	.6077	.0528	.11	.7413
Replicate × Group	1	1471.53	.67	.4215	.2813	.10	.7577	.0003	.00	.9797
Error	28	2211.03			2.8973			.4750		
Corrected total	31									

TABLE 3. Power of the Test for the Treatment Effect With an Expected Detectable Difference between Treatments

Variable	Detectable difference (%)	Power of the test (%)
Dry matter intake	10	98
Crude protein intake	10	98
Energy intake	10	96
Body weight	2	99
Weekly weight gain	30	72
Melatonin	6	96
Progesterone	7	81
Prolactin	10	78
IGFI-1	2	86

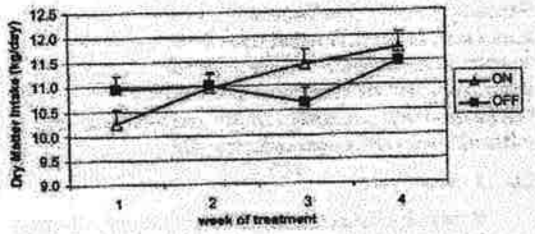

Fig. 1. Dry matter intake in pregnant dairy heifers not exposed (OFF) or exposed (ON) to 60-Hz magnetic fields (MFs).

lingam et al., 2006]. Serum concentrations of MLT and P4 did not differ between heifers exposed and not exposed to MF. Heifers exposed to MF had a 15% decrease in serum PRL (Table 5, Fig. 4). The IGF-1 ANOVA detected a MF exposure residual effect on measurements taken during the fifth experimental week (no-treatment period). Consequently, the test for treatment effect was carried out using the first 5 weeks of the experiment, where it is not possible to encounter a carry-over effect. This revealed a 4% decrease in serum IGF-1 during exposure (Table 5, Fig. 5).

DISCUSSION

The similarity of design, temperatures, humidity, and light intensity of the exposure and control rooms contributed to minimize the room factor as a confounding effect. The serum concentrations of P4 and MLT were not affected by MF exposure. Progesterone results do not agree with those obtained in similar experiments where P4 was elevated in pregnant lactating dairy cows [Burchard et al., 1996] but are in agreement with studies using non-pregnant lactating [Burchard et al., 1998b], non-pregnant non-lactating mature dairy cattle [Rodriguez et al., 2003] exposed to 10 kV/m, 30 μT EMF, and dairy heifers exposed to 10 kV/m MF [Burchard et al., 2004]. MLT has been hypothesized as the mediator of the effects caused by EMF exposure in different species [Wood et al., 1998; Takebe et al., 1999]. Mature dairy cattle exposure to EMF decreased diurnal MLT in blood plasma but did not affect nocturnal MLT. Exposure of pregnant dairy heifers to EF did not affect diurnal MLT. Simultaneous exposure to EMF resulted in increases in PRL and IGF-1 in mature cattle but not in pregnant heifers exposed only to EF [Burchard et al., 1998a, 2004; Rodriguez et al., 2004].

PRL and IGF-1 have been associated with DMI and milk production increases in dairy cattle [Petitclerc et al., 1983; Dominique et al., 1992; Dahl et al., 2000] and PRL and IGF-1 are positively correlated in dairy cattle [Nosbush et al., 1996]. Even though exposure to MF did not globally affect DMI, there was an increase of 1.2% of body weight and 30% weekly body weight gain in exposed animals. The fact that the effect of MF was not uniform during different weeks, evidenced by the treatment by week interaction, might contribute to explain this MF effect on body weight and weekly body weight gain. Simultaneous application of EF and MF increased DMI [Burchard et al., 1996; Rodriguez et al., 2002; Burchard et al., 2003] and weight gain [Rodriguez et al., 2002] in dairy cattle. Conversely, exposure to 10 kV/m EFs does not affect DMI in dairy heifers [Burchard et al., 2004]. Increases in bovine body

TABLE 4. Least-Squares Means ± Standard Error (SE) for Dry Matter Intake (DMI), Crude Protein (CP), and Energy (NeG) Consumption; Body Weight (BW) and Weekly Body Weight Gain (WBWG) in pregnant Dairy Heifers not Exposed (OFF) or Exposed (ON) to 60-Hz Magnetic Fields (MFs), and the Associated Probability Value (*P*)

Variable	MF on	MF off	*P*
DMI (kg/day)	11.11 ± .25	11.02 ± .25	.7458
CP (kg/day)	1.83 ± .04	1.81 ± .04	.7070
NeG (Mcal/day)	15.72 ± .34	15.58 ± .34	.8560
BW (kg)	561.37 ± 7.93	554.73 ± 7.93	.0102
WBWG (kg)	11.95 ± .73	9.18 ± .74	.0122

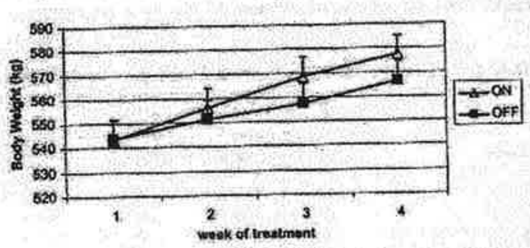

Fig. 2. Body weight in pregnant dairy heifers not exposed (OFF) or exposed (ON) to 60-Hz magnetic fields (MFs).

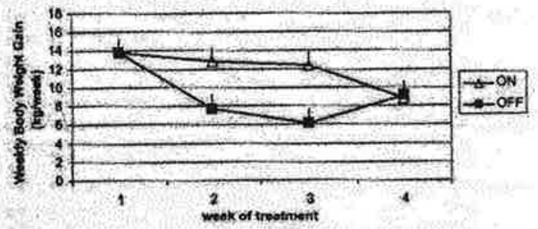

Fig. 3. Weekly body weight gain in pregnant dairy heifers not exposed (OFF) or exposed (ON) to 60-Hz magnetic fields (MFs).

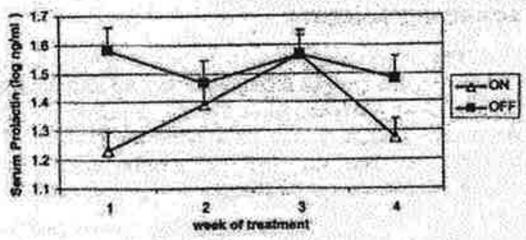

Fig. 4. Serum prolactin in pregnant dairy heifers not exposed (OFF) or exposed (ON) to 60-Hz magnetic fields (MFs).

weight are associated with increases in IGF-1 when compared to animals with restricted diets [Nosbush et al., 1996; León et al., 2004]. In the present experiment, the animals in both groups were fed normal diets and were growing normally during the whole experiment. Exposure to MF resulted in slight decreases in IGF-1 and PRL, and increases in body weight and weekly body weight gain when compared to control animals. In a similar experiment with lactating pregnant cows, EMF exposure resulted in a 4.2% greater body weight, and 9% and 31% increases in IGF-1 and PRL concentrations, respectively, compared to control animals [Rodriguez et al., 2002, 2004]. It is possible that the relatively small decreases in IGF-1 and PRL concentrations during MF exposure throughout the experimental period of time, are not enough to override the predominant effect of MF exposure on body weight observed in previous experiments. Body weight losses of 15–18% over a period of 60 days under restricted diets are associated with 50–70% reductions in IGF-1 blood concentrations [León et al., 2004]. Reductions of 17% of daily weight gain were associated with decreases of 16% and 13% in blood PRL and IGF-1, respectively in prepubertal dairy heifers [Nosbush et al., 1996]. The fact that the statistical analysis revealed a carry-over effect in the case of IGF-1 implies that the MF exposure produces some residual effects. A similar situation was suggested elsewhere in relation to PRL in EMF exposed animals [Rodriguez et al., 2004]. The duration of this residual effect cannot be determined from the data collected in this experiment. Exposure to 10 kV/m EF did not cause any change in body weight or weekly weight gain [Burchard et al., 2004]. It might be possible that the summation or interaction of the EF and MF components is necessary to elicit the responses observed in previous trials. Furthermore, the trials cited for comparison were slightly different with respect to the age, production status and reproduction condition of the cattle used, and light regime [Burchard et al., 1996, 2003; Rodriguez et al., 2002].

CONCLUSIONS

Exposure of pregnant Holstein heifers for 20 h/day to a 30 μT MF during a period of 4 weeks produced slight effects. This is evidenced by statistically significant increases in body weight, weekly body weight gain, and decreases in the concentration of PRL and IGF-1 in blood serum. The effect of exposure was found to be residual in the case of IGF-1. Under the experimental conditions described herein, the absence of abnormal clinical signs and the absolute magnitude of the changes detected during MF exposure, make it plausible to preclude any major animal health hazard.

TABLE 5. Least-Squares Mean ± Standard Error of the Log Transformation for Melatonin (MLT), Progesterone (P4), Insulin Growth Factor 1 (IGF-1), and Prolactin (PRL) in n Pregnant Dairy Heifers not Exposed (OFF) or Exposed (ON) to 60-Hz Magnetic Fields (MFs) and the Associated Probability Value (*P*)

Variable	MF on	MF off	*P*
Melatonin (pg/ml)	3.33 ± .06	3.35 ± .06	.6757
Progesterone (ng/ml)	1.42 ± .05	1.46 ± .05	.8773
IGF-1 (ng/ml)	5.61 ± .065	5.87 ± .065	.0001
Prolactin (ng/ml)	1.37 ± .06	1.52 ± .06	.0057

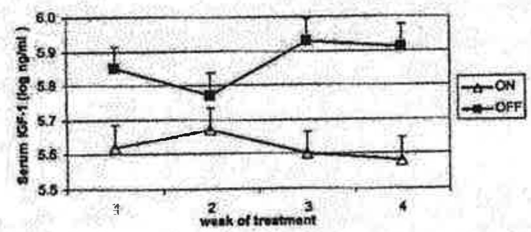

Fig. 5. Serum IGF-1 in pregnant dairy heifers not exposed (OFF) or exposed (ON) to 60-Hz magnetic fields (MFs). Due to carry-over effect only the first 5 weeks of treatment are considered.

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