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Preliminary Evaluation of the Effect of Pulsed Alternating Wavelengths System on Hormonal Profiling and Peripartal Health of Transition Dairy Cows

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Abstract

The objective was to evaluate the effect of a recently developed technology, Pulsed Alternating Wavelengths System (PAWS; Xiant Technologies, Inc., Greely CO), on hormonal (melatonin [MEL] and somatotropin [bST]) and neurotransmitter (serotonin [SER]) profiles of peripartal Holstein cows. In addition, dystocia incidence, postpartum metabolic status (non-esterified fatty acids [NEFA], β-hydroxybutyrate [BHB], and calcium serum concentrations), and early lactation health were assessed. A total of 82 pregnant cows were blocked by parity and randomly assigned into 1 of 2 treatments: i) Exposed to a specific combination of pulses and light waves delivered

PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS. Blood samples for hormonal and metabolic assessment were collected at -1, -2, -3, 1, and 3 days relative to calving. Treatment effects were evaluated using multivariable logistic regression and repeated measures ANOVA. No significant effects were established on hormonal or neurotransmitter profiles, but treatment affected the incidence risk of dystocia that was lower in PAWS (P = 0.031). Although the repeated measures analysis indicated no significant effect for the interaction between treatment and time on serum concentrations of NEFA, values were higher in PAWS than in CON at d 1 and 3 postpartum (P = 0.014 and P = 0.013). No significant treatment effect was established on BHB and calcium serum concentrations or in other peripartal health conditions. In conclusion, exposure to this specific combination of pulses and light waves delivered by PAWS may reduce the presentation of dystocia and modify NEFA serum concentrations around calving.

INTRODUCTION

Metabolic changes occurring at the time of calving represent a significant challenge for dairy cows and result in nutritional and physiological imbalances leading to suboptimal immunity and early lactation disease (Esposito et al, 2014; Aleri et al, 2016). Strategies to reduce the impact of the stress associated with calving and the onset of a new lactation include pre and postpartum nutritional management, close monitoring of the approaching calving, and surveillance of peripartal diseases (Aleri et al, 2016; Sordillo, 2016).

The interrelationships among multiple hormones and minerals affecting the metabolic balance and the immunocompetence of the cow around parturition are well documented (Weaver et al., 2016; Hernandez, 2018; Hernandez-Castellano et al., 2019). Consequently, modulating

hormonal profiles in the periparturient cow as a mean to reduce stress and metabolic disruptions has also been considered as a potential option (Garcia-Ispieto et al., 2013; Hernandez-Castellano et al., 2019).

Melatonin (MEL), mainly synthesized in the pineal gland, modulates the circadian rhythm (Gillette and Tischkau 1999) and has been associated with reproductive (Reiter 1991; Tamura et al. 2008; Carlomagno et al, 2018), neuroendocrine (Cardinali and Pevet, 1998) and immunological processes (Reiter 1991; Cardinali and Pevet, 1998; Hardeland et al, 2006; Reiter et al. 2009). In addition, MEL influences availability of serum calcium, dry matter intake, and milk yield by modifying prolactin levels (Özçelik et al, 2017). Somatotropin (bST), produced in the anterior pituitary, acts in lactogenesis (Bauman, 1992) influencing glucose metabolism by increasing hepatic gluconeogenesis, reducing glucose oxidation, and increasing glycogen mobilization (Peel and Bauman,1987; Bauman et al., 1989). In addition, high concentrations of bST during the transition period have been associated with greater intensity of phagocytosis and oxidative burst by polymorphonuclear leukocyte, greater concentrations of antiovalbumin IgG, and lower incidence of retained fetal membranes and metritis (Burvenich et al., 1999, Silva et al., 2015, 2017). Serotonin (SER), known for its effect on immunity, behavior, and reproduction, also regulates calcium concentrations by promoting a biological activity on bone calcium resorption (Hernandez, 2018; Hernández-Castellano et al, 2019). In addition, SER has a role on glucose regulation and higher levels of SER can significantly reduce the impact of clinical and subclinical metabolic disorders (Laporta et al, 2013; Weaver et al, 2016).

In a study with human subjects, light intensity, time of exposure, and the spectrum of light affected hormone concentrations and certain behaviors (Bonmati-Carrion et al, 2014). Blue light was reported as the most chronodisruptive of the wavelengths, while red light increased

levels of melatonin around sleep time, potentially adjusting the circadian clock and improving the quality of sleep (Bonmati-Carrion et al, 2014; Ho Mien et al, 2014).

In domestic species, variable exposure to light has been used to enhance growth and production and most of the research has focused on photoperiod, which is essentially a change in the time of daily light exposure (Dahl, 2000; Dahl et al., 2012). In dairy cows, alterations in photoperiod have resulted in changes in milk yield, estrous cyclicity, timing of puberty, and mammary growth (Dahl et al., 2004). However, as dairy cattle display circadian rhythms for behavior, concentrations of hormones, (Dahl et al., 2012), and for metabolites (Bitman et al., 1990; Lefcourt et al., 1999), light management including other light features, such as variable pulses and light waves, may result in alterations of these cycles.

Studies on the effect of exposure to different light wavelengths at specific pulsing rates are scarce. A study in broilers indicated a preference for blue and green light, as compared to white or red light and these colors resulted in calmer behaviors (Prayitno et al., 1997). Subsequent studies in broiler chickens indicated that controlled artificial light had positive biological responses manifested by better production and welfare (Yang et al, 2018) and similar effects were presented in dairy cows (Penev et al, 2014).

A new developed methodology based on a Pulsed Alternating Wavelengths System (PAWS; Xiant Technologies, Inc., Greely CO) has been aimed at modifying endogenous hormone production via light. The application of this technology provides opportunity to manipulate multiple features of light exposure at strategic times within production systems and PAWS exposure in laying hens and broiler chickens resulted in increments in numbers of eggs and weight gain, respectively (non-published data).

It is plausible to hypothesize that exposure to PAWS could modify hormonal profiles in periparturient cows. Moreover, this changes could affect the impact of stressful events, such as calving, as well as hormone concentrations around parturition, reducing the incidence of dystocia and improving the transition to the milking stage. Hence, our objective was to evaluate the effect of specific combination of pulses and light waves delivered by PAWS on hormonal (melatonin [MEL] and somatotropin [bST]) and neurotransmitter (serotonin [SER]) profiles of peripartal Holstein cows. In addition, calving behavior, dystocia incidence, metabolic status (non-esterified fatty acids [NEFA], β-hydroxybutyrate [BHB], and calcium serum concentrations), and early lactation health were assessed.

MATERIALS AND METHODS

The procedures completed on this research were approved by the Institutional Animal Care and Use Committee (IACUC) at Colorado State University (Fort Collins, CO) in the protocols 18-7862A (newborn calves) and 18-7885A (adult cows).

Study Population and Pre-trial PAWS Assessment

The study included a pilot analysis in newborn calves (experiment 1), followed by the main trial in periparturient cows (experiment 2). The initial small-scale experiment was completed in newborn Holstein heifer calves to explore any potential effect of PAWS on hormonal (MEL and cortisol) and neurotransmitter (SER) concentrations. Calves (3 d old) born in June 2018 were housed individually in polyethylene hutches with a front yard of 2.25 m^2 with sand bedding and assigned into 1 of 2 treatments: (1) PAWS exposed (PAWS; n = 4); and (2) control (CON; n = 4). Hutches in the PAWS group had interior lights affixed to the hutch roof that were constantly on. All the study calves had free access to the enclosed front yard. Calves were fed and managed according to the farm management program. Blood samples were

collected for determination of serum MEL and SER concentrations at 0600 h (pre-PAWS exposure), 1200 h, 1800 h, and 2400 h on d 1 (enrollment), d 3, d 5, and d 15. Hair samples were collected for cortisol determination on d 1 (pre-PAWS exposure), d 14, d 40, and d 60.

As a follow-up of experiment 1, the second experiment was conducted in a dairy farm under certified organic management located in northern Colorado, USA. Pregnant Holstein cows were enrolled between December 2018 and February 2019 at the maternity facility. Cows that were 14 days to the expected due date were housed in pens with sand packed floors with *ad libitum* access to clean water. Cows received a total mixed ration (TMR) twice a day according to the National Research Council recommendations for transition cows (NRC, 2001) and anionic salts were included into the ration (dietary cation anion difference = −100 mEq/kg).

Experimental Design and Treatment Allocation

Prepartum Holstein cows were blocked by their parity number (1; ≥2) in the approaching calving, aiming at approximately 35% primiparous cows to represent the study farm demographics. Cows were randomly assigned within parity block into 1 of 2 treatment groups: (1) exposed to Pulsed Alternating Wavelengths System (PAWS, n = 40) within 7±4 days before calving; or (2) non-exposed control group during the same period (CON, n = 42) (Figure 3.1). Study cows were maintained in two sand packed pens with an area of 48 m^2 in the opposite extremes of the maternity barn in an East-West orientation (Figure 1). The pen for PAWS group had lights affixed to wires at about 3 m height that were constantly on (Figure 1). Regular barn lights were constantly on for both groups. Study cows had no access to the adjacent yard, and as cows were calved, new cows were sequentially enrolled to maintain 10 cows in each pen.

Trained personnel and farm veterinarians performed calving monitoring and daily general health checking from enrollment to d 28 postpartum.

Hormonal and Metabolic Assessment

A subsample of 30 cows (PAWS = 17; CON = 13) was randomly selected to determine MEL, bST, and SER serum concentrations at enrollment (7 ± 4 days before calving) and at -1 d, and 1 d relative to calving for hormonal assessment. A group of 46 cows (PAWS = 26; CON = 20) was also selected for determination of NEFA, BHB, and calcium serum concentrations at 1d and 3 d post calving.

Blood samples were collected every morning (0600 to 0700 h) from enrollment to calving in the maternity, and from 0800 to 0900 h at 1 d and 3 d post calving after milking, using 10 ml serum Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were stored in a transportation cooler and the serum was separated within 12 hours of collection (centrifuged at 3,500 rpm for 15 minutes at room temperature). Serum was stored frozen at -80˚C from until the analyses were completed at Colorado State University (Fort Collins, CO) in the Proteomics & Metabolomics Laboratory (MEL and SER), the Animal Reproduction & Biotechnology Laboratory (bST), and the Clinical Pathology Laboratory of the Veterinary Teaching Hospital (NEFA, BHB, and calcium).

Activity monitoring and Definitions of Dystocia and Health Events

All cows had IceQube accelerometers (IceQube®, IceRobotics, Edinburgh, Scotland) previously attached in the rear leg for activity assessment. Activity measurements included lying time (min), number of steps, and number of lying bouts summarized in 15 minutes intervals. Records for behavior analyses were summarized to include 12 hours before the time of calving, determined using video records from cameras in the study pens.

Health related events included dystocia, stillbirth, clinical hypocalcemia, clinical ketosis, puerperal metritis, clinical endometritis, clinical mastitis, and other (retention of fetal

membranes, digestive problems, respiratory disease, and lameness). Farm workers performed supervisory rounds every 30 minutes through the maternity pens to detect cows in labor and the progression of calvings. Cows unable to expel the calf within one-hour post amniotic sac presentation and assisted by 2 people were considered as a dystocia case (Schuenemann et al., 2011; Funnell and Hilton, 2016). A stillbirth was defined as a calf that was either born dead or died within the first 24 hours after birth and a case of retention of fetal membranes was a cow unable to expel the placenta within 24 hours after the expulsion of the offspring (Kelton et al., 1998).

Cows with clinical hypocalcemia were identified as downer-cows (in head-down recumbency with paresis of the four limbs) 24 hours after parturition responding to intravenous calcium administration (Mahjoubi et al, 2018). Clinical ketosis was defined as presence of acetoacetate in urine that resulted in any color change in the urine test strip, combined with staggering, excessive object chewing, and unusual behavior (Ketostix, Bayer, Leverkusen, Germany).

Diagnosis of uterine diseases was done by transrectal massage of the uterus combined with vaginal discharge collection with a Metricheck device (Metricheck, SimcroTech, Hamilton, New Zealand). Puerperal metritis (PMET) was defined as an abnormally enlarged uterus and a fetid, watery, reddish/brownish uterine discharge, associated with signs of systemic illness (e.g. reduced milk yield and appetite, dullness) and fever ($\geq$ 39.5˚C) within 21 days after calving. Clinical endometritis was assessed at 21 DIM and defined as vaginal discharge with a content of pus greater than the content of mucus (more than 50% pus) noticeable in the vagina (Sheldon et al, 2006).

Clinical mastitis was defined as a mammary quarter with signs of inflammation (heat, pain, redness, or swelling) and/or changes in the appearance in the milk (e.g. flakes, clots, pus, etc.) (International Dairy Federation. 1971). Trained workers and veterinarians diagnosed respiratory disease by the auscultation of lung sounds and the presence of cough, polypnea, and nasal discharge. Records of clinical diseases from calving to 28 DIM were exported from PCDART® (Dairy Records Management Systems, Raleigh, NC) into Microsoft® Office Excel (Microsoft Corp., Redmond, WA) spreadsheets for subsequent organization and analysis.

Data Management and Statistical Analyses

Sample size calculations were performed using the data analysis application SAS Power and Sample Size (release 9.4; SAS, Inst. Inc., Cary, NC). Based on the results from experiment 1, the sample size was calculated to detect a difference of 2.5 pg/mL in MEL serum concentration in favor of PAWS group. The anticipated average MEL serum concentration in the CON group was 20 pg/mL (Buchard et al., 1998). Considering power = 80% and confidence = 95%, the number of cows required to show a significant difference between the 2 treatment groups was estimated to be 42 cows per group (PROC POWER, SAS; release 9.4; SAS, Inst. Inc., Cary, NC).

Randomization of cows into treatment groups was performed using the RAND function of Excel, based on a list of cows expected to calve within 2 weeks at the time of enrollment start. All data were exported to Microsoft Excel (Microsoft Corp., Redmond, WA), where data were organized for further analysis using SAS.

Due to lack of normality, hormone and metabolite concentrations were Log10-transformed to perform the analyses (PROC MIXED). After the analyses, the Log10 LSM and CI were back-transformed for reporting in the original units.

Disease frequency analyses considered incidence risk, as the time at risk of all the health-related outcomes was well delimited and short in comparison to the duration of the whole lactation (Dohoo et al., 2009). Multivariable models included treatment, parity category (primiparous; multiparous), and the interaction treatment by parity. Binary variables were analyzed using chi-square test (PROC FREQ, SAS 9.4) and logistic regression (PROC GLIMMIX). Continuous variables with multiple values across time were analyzed using repeated measures ANOVA (PROC MIXED) followed by Tukey test (PROC GLM) and least squared means calculation (PROC LSMEANS). Models included treatment, parity category (primiparous; multiparous), sampling time, and the interactions treatment by time and treatment by parity. Significance and tendency levels were declared at $P < 0.05$ and $P < 0.1$, respectively.

RESULTS

Experiment 1: Calf Pre-trial

No differences for hair cortisol concentrations were determined between treatment groups. Average ± SE hair cortisol values for CON vs. PAWS were 592 ± 201 pg/mg vs. 663 ± 201 pg/mg at d 1, 365 ± 99 pg/mg vs. 569 ± 98 pg/mg at d 15, and 309 ± 93 pg/mg vs. 211 ± 93 pg/mg at d 40 of the study. Repeated measures analyses established no significance for the interaction between treatment and time ($P = 0.59$). Average MEL and SER serum concentrations for the overall monitoring period, by day, and by day at 2400 h, which was the sampling time of maximum exposure to the treatment, are presented in Table 1. Overall, PAWS calves had higher MEL concentrations, as indicated by a significant interaction between treatment and time ($P = 0.02$).

Experiment 2: Peripartum Trial

Descriptive Statistics

A total of 82 Holstein cows (23 primiparous, 59 multiparous) were enrolled (PAWS = 40; CON = 42). One cow (primiparous, PAWS) left the study at 11 DIM due to death caused by puerperal metritis combined with respiratory disease. Average ± SD lactation numbers were 2.80 ± 1.83 and 2.52 ± 1.55 for PAWS and CON, respectively. Average ± SD number of days from enrollment to calving were 4.00 ± 2.74 and 5.00 ± 2.75 for PAWS and CON, respectively. None of these descriptive variables were significantly different between treatment groups.

Hormonal Profiles, Peripartal Conditions, Metabolic Status, and Activity

No differences were established between treatments for the overall serum concentrations of MEL, bST, or SER for any of the sampling points (enrollment; d -1 and d 1 relative to calving; Table 2). Treatment had a significant effect on the incidence risk of dystocia (0% in PAWS vs. 5% in CON; $P = 0.031$). However, there was no significant effect of PAWS on the presentation of other health conditions from enrollment to 28 DIM (Table 3).

Although no significance was determined for the effect of the interaction treatment by time on serum concentration of NEFA, this metabolite was higher in PAWS than in CON at d 1 and d 3 postpartum (P = 0.014 and P = 0.013, respectively). Exposure to PAWS did not have a significant effect on BHB or calcium (Table 4).

There was a tendency of PAWS to reduce the number of lying bouts (5.5 ± 0.8 in PAWS vs 8.4 ± 1.3 in CON; $P = 0.065$); however, no significant effect was found in any of the other activity parameters. Average values for number of steps, lying time and number of bouts (number of times the cows went down to lie on the ground) within -12 h relative to calving are shown in Table 5.

DISCUSSION

This preliminary investigation included an initial experiment consisting of a small-scale trial completed in newborn Holstein calves. The aim was to test potential differences in hormonal and neurotransmitter concentrations in PAWS exposed vs. unexposed control calves. A significant finding for this pre-trial study was the difference in MEL concentrations in favor of PAWS. Although, likely due to the limited sample size, differences were not significant when evaluated in specific time points, MEL concentrations at midnight were more than doble in PAWS vs. CON calves. This is an interesting finding, as during the night calves were inside the hutch and exposure to treatment was maximized. Based on these results and considering the multiple activities described for MEL(Cardinali and Pevet, 1988; Bonmati-Carrion et al., 1999; Hardeland et al., 2006), the objective of the second experiment of the study was to expand the analyses on the effects of PAWS in periparturient Holstein cows.

Although the peripartum period offers unique opportunities for testing the potential effect of PAWS on stress reduction, this is also a challenging time for evidencing differences in hormonal profiles, as hormone concentrations are changing drastically around parturition (Hernández et al., 2018; Hernández-Castellano et al., 2019). This issue was evident in our study, as indicated by significant P-values for the effect of time in the concentrations of the hormones in analysis (Table 2). Remarkably, the effect of PAWS on MEL serum concentrations established in calves was not replicated in mature cows. Moreover, no differences were established for SER and bST at any of the sampling points. In addition to the challenges presented by calving, it is plausible to hypothesize that the time of exposure to treatment was not long enough to evidence effects on the outcomes in analysis. Moreover, one sample per day, especially for MEL, may not capture a treatment effect due to the significant variation in blood concentrations at different hours of the day (Suarez-Trujillo et al., 2020).

Our main finding was a lower incidence risk of dystocia in PAWS (0%) compared with CON (11.9%) cows. Incidence of dystocia in the US (USDA, 2010) are 19% and 11% in primiparous multiparous cows, respectively. In the current study, the overall incidence was lower (6.1%) and values were higher in multiparous (4.3%) than primiparous (6.8%) cows; a possible explanation could be the use of different criteria in the definition of dystocia.

Calving easy is associated to multiple factors, such as calf size, parity, and calf position (Funnell and Hilton, 2016, Zaborski et al, 2009) but stress also plays a significant role on the adequate progression of calving. It is likely that excessive levels of environmental stress and hormonal asynchrony may result in incomplete dilation of cervix, causing dystocia (Funnell and Hilton, 2016). Although not evident in the serum concentrations of the hormones analyzed in this study, PAWS may have reduced environmental stress in the exposed group by providing a more favorable synchronization in the hormonal response around calving with an improvement in calving ease (Funnell and Hilton, 2016).

Multiple behaviors, especially lying activity, can be considered for the assessment of comfort and welfare in lactating cows (Hendricks et al, 2019). In our study, supporting the possibility of lower stress in the PAWS exposed group, a statistical tendency to lower number of lying bouts was established for these cows. Although, this difference did not affect the time spent lying the number of times that the cow shifted positions could be indicative of uneasiness.

All the study cows, regardless of the treatment group, spent around 75% of the time standing. As presented in previous studies (Piñeiro et al., 2019) this was expected, as activity was measured in the 12 hours before calving, a period characterized by restlessness and anxiety.

Contrary to our hypothesis, none of the peripartal conditions was affected by treatment. Moreover, unexpectedly, PAWS exposed cows presented higher NEFA serum concentrations at

d1 and d3 postpartum compared with CON cows. No significant difference between groups was determined on serum BHB or calcium concentrations at d1 and d3 postcalving. The main effect of daylight on calcium homeostasis relies on the synthesis of calcitriol, an essential component for calcium intestinal absorption and renal reabsorption (Özçelik et al, 2017). In this experiment, both groups had relatively the same amount of exposure to natural day light, which may have masked a treatment effect.

In conclusion, exposure to this specific combination of pulses and light waves delivered by PAWS resulted in lower incidence of dystocia and greater NEFA serum concentrations within 3 days after calving. No significant effects were found in hormonal profiles and other health and metabolic outcomes. Future approaches including assessment at multiple stages of lactation for prolongated periods are necessary to properly support an inference about the effect of PAWS on the results obtained.

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TABLE 1 Least square means (SE) for melatonin and serotonin serum concentrations in newborn calves for the overall monitoring period, by day, and by day at 2400 h, which was the time of maximum exposure to the treatment.

	Melatonin by treatment[1]			Serotonin by treatment		
Time	CON	PAWS	P-value	CON	PAWS	P-value
Overall	5.47 (1.86)	11.6 (1.85)	0.02	1,644 (91.5)	1,462 (91.6)	0.16
Daily average						
day 1	2.66 (2.08)	9.98 (2.08)	0.02	1,406 (118)	1,268 (119)	0.42
day 3	4.58 (2.27)	10.3 (2.27)	0.08	1,926 (196)	1,850 (196)	0.78
day 5	4.99 (2.36)	9.8 (2.36)	0.16	1,369 (192)	1,279 (192)	0.74
day 15	9.10 (6.8)	17.0 (6.8)	0.41	1,870 (172)	1,458 (172)	0.1
Sample at 2400 h						
day 1	4.56 (7.66)	22.6 (7.66)	0.14	1,781 (311)	1,458 (311)	0.49
day 3	5.13 (7.13)	23.7 (7.13)	0.11	2,700 (405)	2,543 (405)	0.79
day 5	10.1 (9.06)	20.8 (9.07)	0.43	2,169 (470)	1,758 (470)	0.55
day 15	4.45 (9.62)	15.4 (8.33)	0.43	1,803 (320)	1,925 (277)	0.78

[1]Treatment groups = Cows were assigned into 1 of 2 treatments: i) Exposed to PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS.

TABLE 2 Least square means (SE) from the repeated measure analyses for assessment of treatment differences in melatonin, serotonin, and bovine somatotropin serum concentrations at enrollment (-7 ± 4 days before calving) and at -1 and 1 day relative to calving. Models included treatment, parity category, time, and the interactions between treatment and time and treatment and parity category as fixed effects.

		LSM by time relative to calving (d)						P-value				
Variable	Treatment (T)[1]	-7		-1		1		Treatment	Parity (P)	Time (t)	T x P	T x t
Melatonin (pg/mL)	CON	9.28	(1.36)	9.99	(1.65)	10.4	(1.5)	0.77	0.18	<0.001	0.43	0.98
	PAWS	9.27	(1.41)	10.3	(1.5)	10.4	(1.82)					
Serotonin (ng/mL)	CON	462.5	(65)	468.6	(69)	659.4	(65)	0.44	0.28	0.008	0.20	0.38
	PAWS	546.0	(73)	465.1	(76)	618.9	(73)					
bST (ng/mL)	CON	12.7	(1.22)	10.8	(1.22)	13.1	(1.22)	0.147	0.41	0.022	0.018	0.58
	PAWS	9.72	(1.19)	7.27	(1.19)	8.55	(1.19)					

[1]Treatment groups = Cows were assigned into 1 of 2 treatments: i) Exposed to PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS.

TABLE 3 Incidence risk (%) of health-related events by treatment group and parity category. Models included treatment, parity category and the interaction treatment by parity. No significant effect for the interaction treatment by parity was established.

Variable	Parity (P)	Treatment (T)[1]		
		CON, n (%)	PAWS, n (%)	P-value
Dystocia	Overall	5 (11.9)	0 (0)	0.031
	Multiparous	4 (13.3)	0 (0)	0.060
	Primiparous	1 (8.30)	0 (0)	0.522
Stillbirth	Overall	1 (2.38)	3 (7.50)	0.237
	Multiparous	1 (3.33)	0 (0)	0.509
	Primiparous	0 (0)	3 (27.27)	0.093
Clinical hypocalcemia	Overall	3 (7.14)	2 (5)	0.329
	Multiparous	3 (10.00)	2 (6.90)	0.329
	Primiparous	0 (0)	0 (0)	.
Clinical ketosis	Overall	2 (4.76)	2 (5.00)	0.384
	Multiparous	2 (6.67)	1 (3.45)	0.388
	Primiparous	0 (0)	1 (9.09)	0.478
Puerperal Metritis	Overall	2 (4.76)	4 (10.00)	0.225
	Multiparous	1 (3.33)	3 (10.34)	0.241
	Primiparous	1 (8.33)	1 (9.09)	0.521
Clinical endometritis	Overall	3 (7.14)	3 (7.50)	0.324
	Multiparous	3 (10.00)	3 (10.34)	0.329
	Primiparous	0 (0)	0 (0)	.
Clinical mastitis	Overall	9 (21.43)	5 (12.50)	0.133
	Multiparous	4 (13.33)	3 (10.34)	0.294
	Primiparous	5 (41.67)	2 (18.18)	0.178
Other events[2]	Overall	6 (14.29)	2 (5.00)	0.115
	Multiparous	4 (13.33)	2 (6.90)	0.247
	Primiparous	2 (16.67)	0 (0)	0.261

[1]Treatment groups = Cows were assigned into 1 of 2 treatments: i) Exposed to PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS.
[2]Other events included retention of fetal membranes (n = 1), digestive (n = 1; constipation), respiratory (n = 3) and lameness cases (n = 3; sole ulcer, white line disease and a pinched nerve).

TABLE 4 Least square means (SE) from the repeated measure analyses for assessment of treatment differences in metabolic status (NEFA = non-esterified fatty acids, BHB = beta hydroxybutyrate, and calcium) at 1 DIM = 1 day in milk and 3 DIM = 3 days in milk.

Variable	Treatment[1] (T)	1 DIM		3 DIM		Treatment	Parity (P)	Time (t)	T x P	T x t
NEFA (meq/L)	CON	0.46	0.08	0.49	0.09	0.013	0.014	0.732	0.002	0.262
	PAWS	0.73	0.07	0.64	0.08					
BHB (mmol/L)	CON	0.69	0.1	0.78	0.10	0.906	0.014	<0.001	0.017	0.217
	PAWS	0.66	0.1	0.83	0.10					
Calcium (mg/dL)	CON	8.52	1.03	8.57	1.03	0.752	0.013	0.774	0.115	0.601
	PAWS	8.37	1.03	8.54	1.03					

[1]Treatment groups = Cows were assigned into 1 of 2 treatments: i) Exposed to PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS.

TABLE 5 Least square means (SE) from the repeated measure analyses for assessment of treatment differences in activity variables. Activity data were summarized in 15 minutes intervals for the period of 12 hours before calving, which was determined using video records from cameras in the study pens.

Variable		Values in 12 hours*		P-values				
	Treatment (T)[1]	Mean	SE	Treatment	Parity (P)	Time (t)	T x P	T x t
Number of steps	CON	301	92	0.642	0.933	0.125	0.770	0.919
	PAWS	251	51					
Lying time (minutes)	CON	233	62	0.892	0.181	0.222	0.378	0.909
	PAWS	224	35					
Number of bouts**	CON	8.4	1.3	0.065	0.138	0.140	0.083	0.203
	PAWS	5.5	0.8					

[1]Treatment groups = Cows were assigned into 1 of 2 treatments: i) Exposed to PAWS (PAWS; n=40) during -7 ± 4 days before calving; and ii) Control (CON; n=42) non-exposed to PAWS.

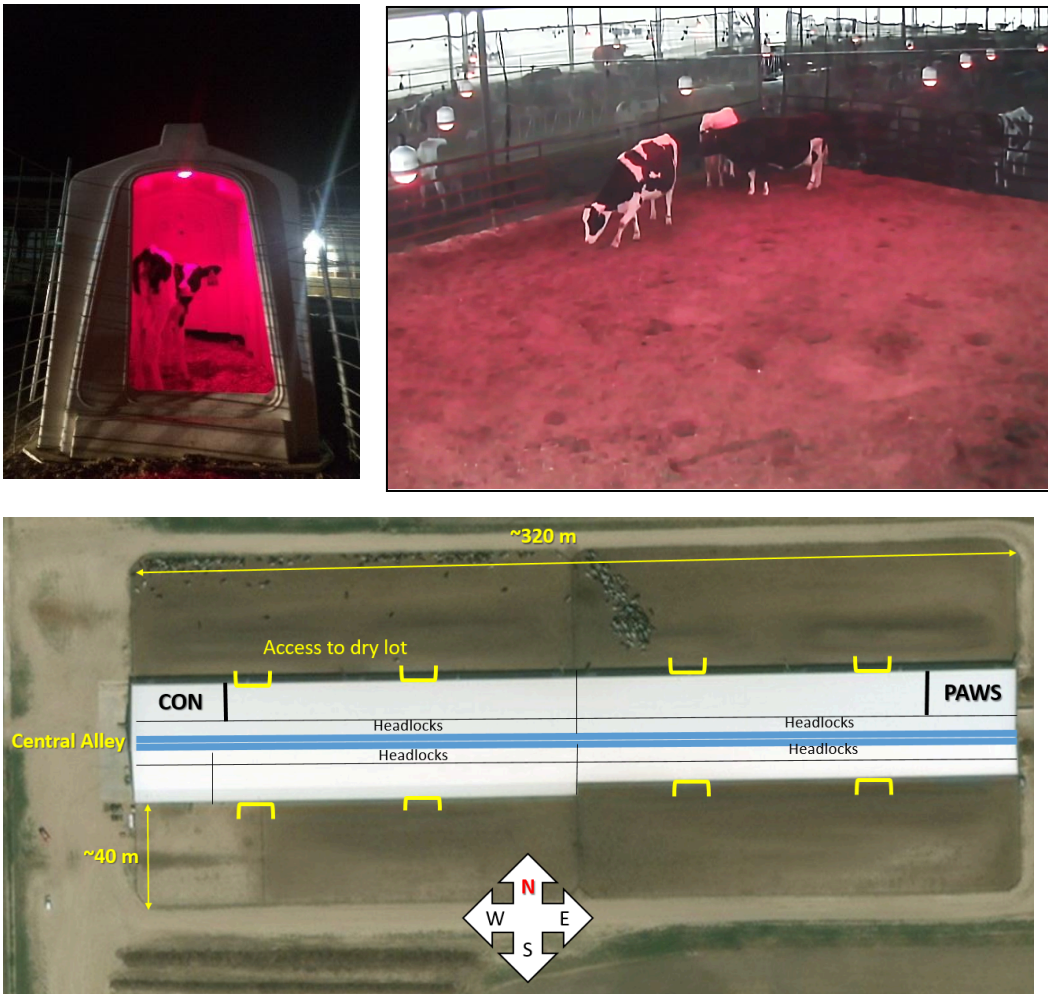

FIGURE 1: Lights were attached in the interior of the individual hutches in the calf pre-trial (top left panel). Lights affixed to wires at 3 m height on top the pen of cows exposed to PAWS (top right). Distribution of PAWS and CON groups within the maternity complex (top panel).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest. One of the authors (RP) worked at Xiant Technologies Inc. at the time of the study, but he did not participate in the data analyses. We thank the participant dairy farms that allowed this research.

Author Contributions

GS, DM, JV, LE-C, and PP had substantial contribution to the work through concept, design, analysis and/or interpretation. GS, DM, PP, AV, LH, and RP had significant contribution trough sample and data collection. Additionally, all authors revised and edited the draft and approved of publication.

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Data Availability Statement

All data sets generated for this study can be made available upon request of the authors.