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Effect of a nutritional immunomodulator in dry cows heat stressed with an electric blanket model

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ABSTRACT

Heat stress in the dry period reduces yield and health in the next lactation. Previous work indicates that feeding OmniGen AF (OMN; Phibro Animal Health) mitigates the detrimental effects of heat stress. Electric blankets (EB) can induce heat stress in lactating cows, but EB have not been used with dry cows. The objectives of this study were to explore efficacy of the EB on cows during the dry period, as well as to examine the effect of feeding OMN to heat-stressed cows. We hypothesized that EB would increase body temperature in dry cows and OMN would ameliorate the effects of heat stress. Fifty Holstein cows were housed individually in a tie-stall barn upon dry-off ~48 d before expected calving (223.7 ± 5 d carried calf) and cows were fitted with EB or no blanket (NB). Within EB and NB, cows were fed OMN (OMN; 56 g/d) or did not receive OMN (CON), which resulted in a 2 × 2 factorial of 4 treatments: NB-CON, EB-CON, NB-OMN, and EB-OMN. Throughout the dry period, DMI, water intake, and respiration rate (breaths/min) were measured daily, and rectal temperature was measured twice daily. After calving, all the cows were cooled and managed identically, and milk yield and composition were measured at each milking. Use of EB increased rectal temperature and respiration rate relative to NB regardless of diet; OMN treatment did not affect rectal temperature or respiration rate. Dry matter intake was reduced by over 1 kg/d with EB, and OMN feeding reversed this effect. Water intake increased with EB relative to NB, but OMN was without effect. Treatment did not affect gestation length. In early lactation, EB cows produced 6 kg/d less ECM relative to NB, and OMN reversed the effect on milk yield in EB cows. These data support the hypotheses that EB induce heat stress in dry cows and that OMN effectively mitigates the detrimental effects of heat stress in the dry period.

Key words: prenatal, hyperthermia, immunity, productivity

INTRODUCTION

Heat stress negatively affects production and health of dairy cows throughout the lactation cycle (Tao et al., 2018). Heat stress in the dry period reduces mammary development and limits milk yield capacity for the next lactation (Tao et al., 2012; Fabris et al., 2020), and adversely affects immune status from the dry period into lactation (do Amaral et al., 2011). The most effective way to avoid the negative outcomes associated with heat stress is to supply active cooling with fans and soakers (Collier et al., 2006); however, some nutritional supplements can also be used to overcome heat stress effects. In particular, there is evidence that an immunomodulator, OmniGen AF (Phibro Animal Health), reversed the effects of dry period heat stress on milk yield for the initial 60 d of the next lactation (Fabris et al., 2017) when treatment was initiated about 6 wk before cows were dried off and exposed to heat stress. However, the effects of providing OmniGen at dry-off concurrent with heat stress are unknown.

A common approach to initiate heat stress in hot humid climates is to simply remove access to active cooling under environmental conditions of excessive heat and humidity; usually characterized by a temperature-humidity index (**THI**) over 68 (Zimbelman et al., 2009). Recently, Al-Qaisi et al. (2019) reported a model of heat stress induced by the application of electric blankets during periods of low THI. Although the electric blanket model induced heat stress in lactating cows for relatively brief periods (i.e., 7–14 d), the effectiveness in dry cows that would require blanket treatment for 40 to 50 consecutive days is unknown. Therefore, the objective of the present study was to test the electric blanket model in dry cows and provide OmniGen AF supplementation at the time of heat stress initiation, to test the hypotheses that the electric blanket model would induce heat stress chronically and that OmniGen AF supplementation from the

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

beginning of the dry period would reverse the negative effect on milk yield in the next lactation.

MATERIALS AND METHODS

All procedures within the following study were approved by the University of Florida Institutional Animal Care and Use Committee. This study was conducted from January 2021 to May 2022. No treatments were imposed during the summer months (i.e., May through November), such that all cooled cows were under the relatively mild conditions of the fall, winter, and spring months in Florida.

Heat Stress and Dietary Treatment

Fifty multiparous Holstein cows were blocked by genomic predicted transmitting ability and assigned randomly at dry-off (~48 d before expected calving) in a 2 × 2 factorial design. Treatments included being fed a top-dress base of dried distillers grain and ground corn including 56 g/d OmniGen AF (**OMN**) or fed a top dress with no OmniGen AF (**CON**), and either exposed to heat stress conditions using electric blankets (**EB**) or no heat stress (no blankets, **NB**), resulting in 4 treatments (NB-CON, n = 13; EB-CON, n = 13; NB-OMN, n = 12; EB-OMN, n = 12). OmniGen AF contains a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, brewer's dehydrated yeast, mineral oil, calcium carbonate, rice hulls, niacin supplement, biotin, calcium D-pantothenate, vitamin B_{12} supplement, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate, and folic acid, but the full formulation is proprietary. Cows were housed in a tie-stall barn with mechanical ventilation during the dry period, and then a freestall barn during lactation. Heat stress was induced by applying a Thermotex Equine FAR Heating Blanket (Thermotex Therapy Systems, Calgary, AB, Canada) to the cow, whereas no blanket was applied to the control cows. The blankets were set at "high" during the day (0700 to 2000 h) and "low" at night (2000 to 0700 h) to simulate a diurnal pattern of exposure. Ambient temperature and relative humidity were recorded every 15 min and averaged hourly from October to May using 2 Hobo Pro Series Temp Probes (Onset Computer Corp., Pocasset, MA) placed in the barn. The THI was calculated using the equation established by the NRC and suggested for use in subtropical environments (Dikmen and Hansen, 2009). The THI was calculated based on the equation reported by Dikmen et al. (2008): $\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$, where T = air temperature (°C) and RH = relative humidity (%). The THI values were used within each block as a covariate, and thus individual values are not shown.

Cows were fed far-off and close-up TMR (Table 1) ad libitum at 0800 h for the first 3 wk and the following 4 wk before expected calving, respectively, according to the standard operating procedures of the University of Florida dairy. During lactation, all cows received the same TMR formulated for lactating cows (Table 1). Samples of the TMR were collected once weekly at 1000 h for DM determination and chemical analysis (Cumberland Valley Analytical Services Inc., Waynesboro, PA). Upon birth, calf and dam were separated, and the dam was milked to collect colostrum.

Treatments, Experimental Design, and Animals

During the dry period, cows were housed in a tie-stall barn with either deep sand (block 1 and 2) or air mattresses with dry shavings as bedding (block 3–5). Cows were housed in a sand-bedded freestall barn during lactation and were kept under the same cooling system and management. Active cooling systems under shade including soakers (Rain Bird Manufacturing, Glendale, CA) and fans (J&D Manufacturing, Eau Claire, WI) were available for all cows in lactation. When the ambient temperature exceeded 21.1°C, fans automatically turned on and the soakers were activated for 1 min at 5 min intervals. Photoperiod (14 h light/10 h dark) of the barn for dry cows on all treatments was controlled using fluorescent lights. The lights provided ~50 lx intensity at eye level of cows and were kept on from 0600 to 2000 h.

Data Measures and Sample Collection

Daily DMI of individual cows was measured from dry-off to calving by weighing distributed TMR and orts. Total daily water intake for each cow was recorded during the dry period using water meters on individual water bowls. Three times each week (Monday, Wednesday, and Friday), rectal temperature (**RT**) was measured twice daily (0730 and 1430 h) by Genuine Pavia Rectal Veterinary Thermometer (Pavia Sales Group, Plymouth, MN), and respiration rates (**RR**, breaths/min) were measured twice daily before RT (0725 and 1425 h) by counting the flank movements for 1 min for all cows to confirm the heat stress outcomes.

After parturition, all cows were managed identically as a single group and all cows were cooled during lactation. Cows were milked twice a day, and daily milk yield was recorded for the first 9 wk of lactation. Milk components (protein, fat, and lactose) were measured using the AfiLab milk analyzer (Kibbutz Afikim, Israel) at each milking and SCS was collected and analyzed monthly from DHIA records. The AfiLab milk analyzer is based on the optical characteristics of light scattering of matter such as milk fat, protein, and lactose; and the values

Table 1. Ration formulation and nutrient content of far-off, close-up, and lactation TMR fed to EB heat-stressed and OmniGen (Phibro Animal Health)-supplemented dry cows, and the ration fed to those cows during lactation after treatments ended

	Dry cow diet[1]		
Item	Far-off	Close-up	Lactation
Ingredient, % of DM			
Corn silage	50.53	68.18	37.71
Corn grain	—	—	18.18
Citrus pulp pellets	—	—	10.88
Grass hay	34.84	8.42	—
Soybean meal 44	10.86	14.73	9.77
Amino plus[2]	—	—	5.86
UF dry cow premix[3]	3.77	—	—
UF close-up premix[4]	—	8.67	—
UF lactation premix[5]	—	—	5.45
Oat/grass silage	—	—	4.46
Molasses	—	—	4.19
Nurisol[6]	—	—	1.29
Brewers grains	—	—	1.19
Wheat straw	—	—	0.70
Cottonseed	—	—	0.33
Nutrient content (DM basis)			
DM, %	49.10	45.66	
CP, %	13.29	14.14	
Fat, %	3.04	3.28	
ADF, %	26.24	22.94	
aNDFom,[7] %	42.93	35.55	
NFC,[8] %	29.53	32.33	
Lignin, %	6.51	6.06	
Ethanol-soluble carbohydrates, %	3.32	3.29	
Starch, %	19.77	23.26	
Ash, %	7.51	8.19	
Ca, %	0.77	1.68	
P, %	0.30	0.31	
Mg, %	0.41	0.48	
K, %	1.48	1.17	
Na, %	0.42	0.12	
Cl, %	1.08	0.90	
S, %	0.19	0.40	
NE_L,[9] Mcal/kg	0.75	0.75	
DCAD, mEq/100 g of DM	13.91	15.40	

[1] The far-off diet was fed from 222 to 243 d of gestation (~47 d prepartum), and the close-up diet was fed from 244 d of gestation to calving (~277 d of gestation).

[2] High bypass protein supplement (Ag Processing Inc.), 44% CP of which 72% was RUP, 1.06% fat.

[3] Ingredients and percentage of mix (%) for far-off premix: calcium carbonate (22.80), magnesium oxide (10.10), NaCl (25.40), Rumensin (0.48; Elanco), wheat mids (33.65), Progressive Dairy Solutions L65 selenium (7.56), and vitamin A (0.01). UF = University of Florida.

[4] Ingredients and percentage of mix (%) for close-up premix: calcium carbonate (38.86), magnesium oxide (3.40), NaCl (2.74), Rumensin (0.20), calcium sulfate (1.94), magnesium sulfate (3.89), ReaShure (5.92; Balchem), wheat mids (5.61), PDS L65 selenium (3.43), vitamin A (0.01), and Animate (34.0; Phibro Animal Health).

[5] Ingredients and percentage of mix (%) for lactation diet premix: calcium carbonate (11.65), magnesium oxide (4.12), NaCl (5.16), Rumensin (0.16), calcium diphosphate (7.77), sodium bicarbonate (23.81), PDS *Aspergillus oryzae* and *Yucca schidigera* extracts (0.86), corn grain (19.76), PDS L65 selenium (6.36), PDS yeast (0.84), and PDS protein supplement (19.53).

[6] Calcium salt of long-chain fatty acids (GlobalAgri Trade Corp.).

[7] NDFa = ash-free NDF.

[8] NFC calculated as NFC = DM − (ash + CP + ether extract + NDF − NDF insoluble CP).

[9] Calculated using the NRC (2001) according to the chemical composition of the diet and adjusted to lactation stage.

obtained using AfiLab are well correlated with DHIA measures (Kaniyamattam and De Vries, 2014).

Statistical Analysis

For the dry period, the average daily RT and RR and daily DMI were analyzed using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC), after condensing daily values into weekly means. The statistical models included the fixed effects of prepartum heat stress (NB vs. EB), diet (CON vs. OMN), week prepartum (−1, −2, −3, −4, −5, −6, −7), the interactions between week prepartum, heat stress, diet, and the random effect of cow nested within treatment. During the dry period, the average daily THI that each cow was exposed to was used as covariate for analyses of RT, RR and DMI.

For the postpartum period, the responses milk yield, milk components, and SCS were analyzed using the MIXED procedure of SAS. The statistical models included the fixed effects of prepartum heat stress (NB vs. EB), prepartum OMN, and week postpartum (1, 2, 3, 4, 5, 6, 7, 8, 9), and the random effect of cow nested within dietary treatment and heat stress.

Models were fit to the data and distribution of residuals and homogeneity of variance were evaluated. Data that did not fit the assumptions of normality were transformed before analyses and LSM and respective SEM were back-transformed for data presentation. The Kenward-Roger method was used to obtain the approximate degrees of freedom. The covariance structure that resulted in the best fit model based on the smallest Akaike's criterion was selected for the analysis of data with repeated measurements. The LSM ± SEM are reported. Differences with $P \leq 0.05$ were considered statistically significant, and $0.05 \leq P \leq 0.10$ was referenced as a tendency.

RESULTS

Application of EB to cows increased the RT ~0.2°C on average, and RR at least 12 breaths/min compared with those that had no blankets, indicating that heat stress was greater with EB treatment (Table 2). In contrast, OMN supplementation did not affect RT or RR independent of the temperature effects. Heat stress induced with EB did not affect gestation length compared with NB (Table 2). Similarly, neither EB nor OMN altered the duration of the dry period, thus all cows had similar dry period lengths (Table 2). Calf birthweight was also not influenced by EB or OMN (Table 2).

Electric blanket treatment reduced DMI by over 1 kg/d during the dry period relative to cows that did not have blankets (Figure 1). Supplementation of OMN reversed this effect of heat stress on intake, resulting in

Table 2. Rectal temperatures, respiration rates, gestation and dry period lengths, and calf birth weights of cows exposed to heat stress via electric blankets (EB vs. NB) and supplemented with OmniGen (Phibro Animal Health) or not (OMN vs. CON) during the dry period[1]

	Treatment[2]					*P*-value		
Item	NB-CON	NB-OMN	EB-CON	EB-OMN	SEM	Heat	Diet	Heat × diet
Rectal temperature, °C	38.17	38.07	38.39	38.36	0.08	<0.01	0.60	0.25
Respiration rate, breaths/min	37.47	37.24	49.51	51.84	1.82	<0.01	0.89	0.18
Gestation length, d	275.1	272.5	273.6	273.0	2.17	0.63	0.35	0.40
Dry period length, d	48.7	48.5	45.4	48.6	2.37	0.30	0.35	0.26
Calf birth weight, kg	38.7	37.5	37.1	36.7	2.38	0.43	0.62	0.77

[1] Data are presented as LSM ± SE of each treatment.

[2] NB-CON and EB-CON: n = 13 per treatment; NB-OMN and EB-OMN: n = 12 per treatment.

a significant interaction of the heat treatment with diet supplementation. Heat (i.e., EB) also increased water consumption relative to no heat, but there was no effect of OMN to alter that response on water intake (Figure 2).

During the initial 9 wk of lactation, cows that were fitted with EB during the entire dry period produced 6 kg/d less ECM compared with control cows that did not have blankets when dry (Figure 3). Supplementation with OMN to EB dry cows reversed the effect on early lactation milk yield, which is consistent with the observed tendency for an interaction of OMN and EB treatment effects. Patterns of actual milk yield were similar to ECM, as there were no differences in milk composition among treatments (data not shown).

DISCUSSION

Heat stress during the dry period of dairy cows results in substantial reductions in health, productivity, and calf performance in late gestation and the subsequent lactation (reviewed in Ouellet et al., 2020; Cattaneo et al., 2023). The potential losses to the US dairy industry for the dam and calf have been estimated at $1.4 billion annually, which rivals estimated losses due to heat stress during lactation (Ferreira et al., 2016; Laporta et al., 2020). Therefore, continued research to understand the mechanisms of heat stress related effects on productivity and health, as well as identification of mitigation strategies, are critical to the dairy industry. Herein we used a model of heat stress developed for brief periods of time (i.e., 7–14 d; Al-Qaisi et al., 2019) and applied it for the entire dry period of mature cows. The increase in RR and RT of cows that were fitted with EB indicated that they experienced significant heat stress relative to the control cows, so the effect of OMN supplementation can be accurately assessed compared with a thermoneutral condition.

Absolute values of RT and RR were elevated with the blanket treatment, but they did not reach the levels ob-

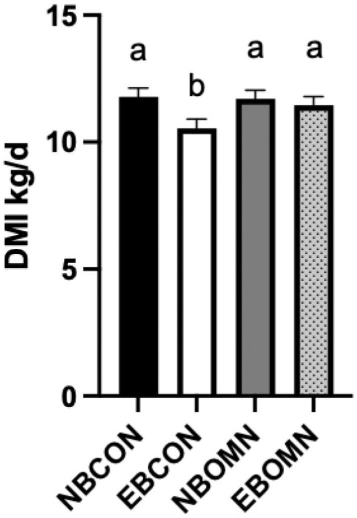

Figure 1. Dry matter intake of cows exposed to heat stress via electric blankets (EB vs. NB) and supplemented with OmniGen (Phibro Animal Health) or not (OMN vs. CON) during the dry period. Data are presented as LSM ± SE of each treatment. Different letters (a vs. b) indicate significance ($P \leq 0.05$).

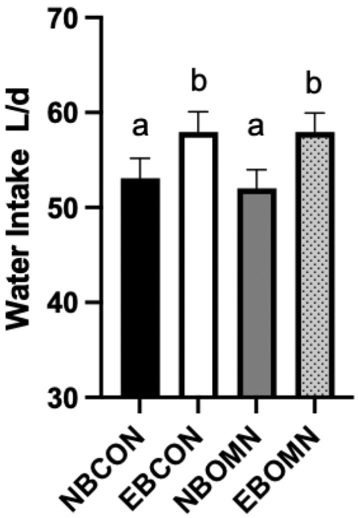

Figure 2. Water intake (L/d) of cows exposed to heat stress via electric blankets (EB vs. NB) and supplemented with OmniGen (Phibro Animal Health) or not (OMN vs. CON) during the dry period. Data are presented as LSM ± SE of each treatment. Different letters (a vs. b) indicate significance ($P \leq 0.05$).

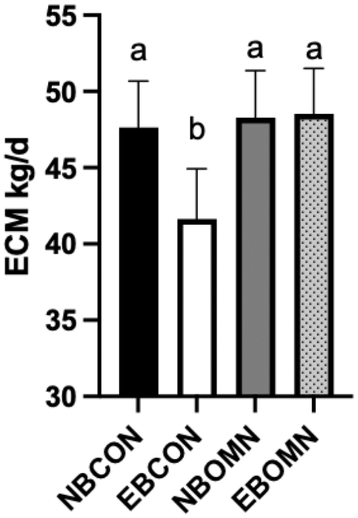

Figure 3. Energy-corrected-milk yield (kg/d) of cows exposed to heat stress via electric blankets (EB vs. NB) and supplemented with OmniGen (Phibro Animal Health) or not (OMN vs. CON) during the dry period. Data are presented as LSM ± SE of each treatment for the first 9 wk of lactation. Different letters (a vs. b) indicate significance ($P \leq 0.05$).

served with other heat stress models wherein cows are held under conditions of high ambient temperatures and humidity without heat abatement (Toledo et al., 2020). In cows under high heat and humidity such as the subtropical environment of Florida, there is essentially no capacity to escape the ambient heat load, especially at high humidity levels. Indeed, only evaporative processes can be employed under such conditions, which is why soakers and fans are prevalent in the Southeastern United States as a heat abatement strategy (Collier et al., 2006). In contrast, the EB treatment occurred during periods of relatively low humidity, and the entire body surface of the cows was not covered by the blanket, thus some capacity to eliminate heat remained available. Despite this limitation, the EB treatment was clearly associated with physiological responses consistent with an elevated heat load.

Consistent with numerous previous studies (reviewed in Ouellet et al., 2020), heat stress during the dry period resulted in reduced milk yield in the next lactation without affecting milk composition. Dry period heat stress appears to reduce the capacity for milk secretion by impairing mammary development in late gestation (Tao et al., 2011; Fabris et al., 2020). Consistent with previous studies (Brandão, et al., 2016; Fabris et al., 2017), OMN supplementation reversed the effect of dry period heat stress on productivity, as evidenced by the similar milk yields of the blanket cows that received OMN with the control and control OMN supplemented groups, all of which were greater than the nonsupplemented blanket cows. Of interest, OMN treatment began coincident with dry off and imposition of the heat stress, which differs from our previous study where cows began OMN in late lactation before dry-off and heat stress exposure. The reasoning was that OMN responses have been shown to improve with a priming period before stress challenge to fully observe the impacts of OMN on immune stimulation (Nace et al., 2014). The current study and that of Brandão et al. (2016) support the concept that OMN supplementation at the start of the dry period can produce positive results in cows experiencing heat stress, and there is no requirement of a stimulation period to observe effects on subsequent milk yield.

Gestation length and thus dry period length were not affected by heat stress, in contrast to previous studies (reviewed in Cattaneo et al., 2023). This may reflect the less severe heat load on the EB cows relative to ambient heat stress. The lack of effect on calf birth weight would also support the idea that heat load, and potential effects on placental function, had less effect with the EB model compared with the typical approach to induce heat stress by limiting active cooling in a high THI environment. Supplementation with OMN had no effect on gestation length and the duration of the dry period relative to control, also in contrast with earlier work (Fabris et al., 2017). Although the primary focus of placental function revolves around support of the developing fetus, it is also the source of hormonal outputs that support mammary gland development, which peak in late gestation (Collier et al., 1982). Thus, any extension of placental life, and its function, would be expected to increase stimulation of calf and mammary development simultaneously. Lacking evidence to support that concept of improved placental function as a mechanism explaining the effects of OMN under EB stress, it is possible that OMN increased blood flow and perfusion of the mammary gland when heat stress occurred with EB treatment, and thus supported greater mammary development in late gestation. Although blood flow was not directly measured in this study, some components of OMN, including niacin, have been shown to increase peripheral perfusion and alleviate heat stress (Chen et al., 2019).

Supplementation of OMN to EB cows also increased DMI relative to the cows under EB alone. A reduction in DMI with heat stress in dry cows is a hallmark of the response (Cattaneo et al., 2023). Indeed, Seyed Almoosavi et al. (2021) used a pair feeding model to investigate the potential effect of the lower intake on subsequent productivity and estimated that as much as 60% of the milk loss could be attributed to the reduced DMI in the dry period. Although the effect on DMI observed with pair feeding and heat stress was substantially more than that of the current study (1.9 vs. 1.2 kg/cow per day), it is reasonable to expect that lower DMI in the dry period may negatively affect later productivity. Therefore, maintaining higher levels of DMI with OMN supplementation would be consistent with greater yields relative to cows that were only exposed to heat stress with the EB application.

CONCLUSIONS

In summary, the EB model is a useful addition to the toolbox for heat stress studies with dry cows. OmniGen AF supplementation alters the trajectory of the dry period heat stress response even when supplementation begins at the time heat stress is initiated at dry off, extending previous observations where cows received OMN 4–5 weeks before heat stress was imposed. Further studies to assess the role of immune function associated with involution processes offer a target to investigate the mechanism of action of OMN with heat stress.

NOTES

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Nonstandard abbreviations used: CON = control, no OmniGen AF; EB = electric blankets; NB = no blanket; OMN = OmniGen AF; RR = respiration rates; RT = rectal temperature; THI = temperature-humidity index; UF = University of Florida.

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