An evaluation of an immunomodulatory feed ingredient in heat-stressed lactating Holstein cows: Effects on hormonal, physiological, and production responses

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ABSTRACT

Holstein cows ($n = 30$) were balanced by days in milk, milk production, and parity (91 ± 5.9 d in milk, 36.2 ± 2.5 kg/d, and 3.1 ± 1.4 , respectively) and fed OmniGen-AF (OG; Phibro Animal Health, Teaneck, NJ), an immune stimulant, at 0 g/cow per d for control (CON) or 56 g/cow per d for OG for 52 d on a commercial dairy. At 52 d of the study cows were randomly selected ($n = 12$) from both groups (6 OG and 6 CON) and housed in environmentally controlled rooms at the Agricultural Research Complex for 21 d at the University of Arizona. Cows were subjected to 7 d of thermoneutral (TN) conditions, 10 d of heat stress (HS), and 4 d of recovery (REC) under TN conditions. Feed intake, milk production, and milk composition were measured daily. Rectal temperatures (RT) and respiration rates (RR) were recorded 3 times per day (600, 1400, and 1800 h). Blood samples were taken on d 7 (TN), 8 (HS), 10 (HS), 17 (HS), and 18 (TN) during the Agricultural Research Complex segment. Cows in HS had higher RR and RT and water intake and lower dry matter intake and milk yield than these measures in TN. There was a treatment $\times$ environment interaction with cows fed OG having lower RR and RT and higher dry matter intake during peak thermal loads than CON. However, milk yield did not differ between groups. Cows fed OG had lower milk fat percent than CON (3.7 vs 4.3%) during HS. The SCC content of milk did not differ between treatment groups but rose in both groups during the REC phase following HS. Plasma insulin and plasma glucose levels were not different between groups. However, plasma insulin in both groups was lower during

acute HS, then rose across the HS period, and was highest during the REC phase. Plasma cortisol levels were highest in all cows on the first day of HS (d 8) but were lower in cows fed OG compared with CON. However, plasma ACTH concentrations were elevated in OG-fed animals at all times samples were collected. Plasma ACTH was also elevated in cows fed both OG and CON during HS. Feeding OG reduced plasma cortisol during acute but not chronic HS and increased basal plasma ACTH, suggesting that OG treatment may alter the hypothalamic pituitary adrenal axis.

Key words: cortisol, heat stress, immune stimulant

INTRODUCTION

A report by Kadzere et al. (2002) estimated that 48% or 4.2 million dairy cows in the United States are subjected to heat stress (HS) on an annual basis, negatively affecting milk yield, reproduction, and cow health. The economic impact of HS on the US dairy industry is estimated at \$879 million by St. Pierre et al. (2003). Dairy cows begin to physiologically adjust to the detrimental effects of HS when the ambient temperature exceeds 32.2°C or the temperature-humidity index (THI) reaches 68 (Kadzere et al., 2002; Ortiz et al., 2013; Allen et al., 2015). The physiological responses of dairy cows to HS include increased body temperature and elevated respiration rates (RR; Igono et al., 1992). These physiological adaptive responses are followed by decreased feed intake, milk yield, milk components, and reproductive efficiency (Johnson and Vanjonack, 1976; Jordan, 2003). In addition to the negative effect on production, immune function and cow health are also negatively affected by HS associated with elevated cortisol associated with exposure to heat (Christison and Johnson, 1972; Sordillo, 2013).

The feed additive Omnigen-AF (OG; Phibro Animal Health, Teaneck, NJ) is a nutritional supplement for ruminants that has been shown to bolster immune function in replacement dairy heifers (Ryman et al., 2013), lactating dairy cows (Wang et al., 2009; Brandão

Received November 28, 2017.

Accepted April 21, 2018.

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et al., 2016), and sheep (Wang et al., 2007). Multiparous dairy cows fed OG from dry-off through 60 d into lactation (Fabris et al., 2017) and exposed to HS were observed to have higher milk responses than the control (CON) cows. Leiva et al. (2017) reported that feeding OG to lactating dairy cows reduced HS responses. These studies prompted a more detailed investigation to evaluate the physiological, immunological, and production effects of prefeeding OG to lactating dairy cows under controlled thermoneutral (TN) and HS conditions. We postulate that feeding OG to lactating dairy cows before and during HS will also improve HS physiological responses.

MATERIALS AND METHODS

All aspects of this protocol were approved by the Animal Care and Use Committee of the University of Arizona. Cattle were selected and sorted by DIM, production (previous lactation and current lactation), and parity. The 2 phases of the study were the on-dairy phase and the Agriculture Research Complex (ARC) phase. The on-dairy phase was conducted at a commercial dairy in Eloy, Arizona. The ARC phase took place at the University of Arizona, Tucson.

The on-dairy part of the study was needed to elicit an immune response before arrival at the ARC. Previous studies have established that a 52-d feeding period of OG supplementation is required to demonstrate differences in markers of immune function (e.g., IL-8 receptor, beta) between OG-fed cows and CON (Wang et al., 2004; Wu et al., 2017). The dairy used for the on-dairy phase (Caballero Dairy, Eloy, AZ) is a dry lot dairy with Saudi barns, which were cooled by Advanced Dairy Systems-Shade Tracker (ADS-ST, Chandler, AZ), an oscillating evaporative cooling system. Cows were milked 3 times daily in a rotary style parlor. A total of 504 cows were placed in 2 pens of 252 cows each. Both pens were in the same barn on opposite sides of the feed alley. All cows in each pen were fed CON or OG diets. All cows in the barn were fed and milked at the same time each day. The cooling systems (oscillating fans) for each side of the barn were operated by the same control system, which was set to begin cooling at a THI of 72. The CON and OG cows were offered fresh feed twice daily. The OG fed group received the same

base TMR as the CON, and OG was mixed in at 56 g/cow per d. At the beginning of the on-farm phase, a total of 30 cows (15 CON and 15 OG) balanced by DIM, milk production, and parity were identified to obtain a pool of animals to be used in the ARC phase of the study. Animals in the on-farm phase were pen fed, and pen was confounded with treatment. Therefore, on-farm milk yields and metabolites were not tested.

Of the original 30 cows in the on-farm phase, 6 cows were selected from each group to provide 12 cows to be used for the ARC phase of the study. Cows in each group were balanced for milk yield, parity, and DIM. After arrival at the William Parker Agricultural Research Complex in Tucson, Arizona, the cows were weighed and fitted with halters to accommodate their tiestalls. The 12 cows were then randomly assigned by coin flip to 1 of 2 environmentally controlled rooms with 3 CON and 3 OG cows per room for a total of 6 cows per treatment. The stalls in each room were of identical size, and the room dimensions of both rooms were identical. Stalls faced north in one room and south in the second room. A single heating, cooling, and humidifying system was used to control the environment in both rooms. Cows were continuously monitored for the first 48 h following arrival to prevent injury during acclimation to the rooms and the tiestalls. During the night, cows were observed through remote access cameras.

The ARC phase of the trial lasted 21 d and was composed of 3 periods. There were 7 d of acclimation at TN to allow the cows to settle and adjust to their surroundings. After the acclimation period, the cows were subjected to 10 d of HS. Cows were then given a recovery period at TN for 4 d before returning to the commercial dairy (Figure 1).

Feeding and milking in the ARC phase occurred twice daily at 0500 and 1700 h. Cows were individually fed and milked in their own tiestalls. The CON cows were fed the base TMR (Table 1) plus ~25 g of molasses (as-fed) mixed into the top one-third of each meal, and the OG cows received the TMR + molasses + 28 g of OG mixed into the top third of each meal. Orts were removed daily at 1645 h and weighed. Water consumption was metered and recorded daily before the AM feeding. Milk bucket weights were taken, and a daily milk sample was taken from the AM milk. Samples

Figure 1. Thermal conditions in the controlled environment rooms during the 21-d Agriculture Research Complex phase of the study.

Table 1. Ingredients and chemical composition of the diet¹

Item	% of DM
Alfalfa hay	65.02
Corn (steam flaked)	22.12
Whole cottonseed	7.28
Distillers grains (dry)	2.58
Supplement RS-1299 ²	2.04
Maxxer ³	0.96
Chemical analysis	
CP (%)	19.75
NDF (%)	25.39
ADF (%)	19.75
Fat (%)	4.74
DM (%)	53
NE _L (Mcal/kg)	1.76

¹Diet DM averaged 53% by weight of DM and moisture including added water.

²The supplement contained 1.14% fat, 10.42% Ca, 4.49% P, 3.80% Mg, 0.49% S, 0.19% K, 15.83% Na, 7.52% Cl, 2,029.06 mg/kg of Zn, 1,991.82 mg/kg of Mn, 974.24 mg/kg of Fe, 583.45 mg/kg of Cu, 67.86 mg/kg of Co, 12.28 mg/kg of Se, 6.81 mg/kg of Mo, 43.68 mg/kg of I, 304.9 IU/g of vitamin A, 30.2 IU/g of vitamin D, and 1.0 IU/g of vitamin E (Dairy Nutrition Services, Chandler, AZ).

³Calcium salts of palm oil (Tarome Inc., Eloy, AZ).

were individually stored with a preservative (bronopol tablet, D&F CON Systems, San Ramon, CA) at 4°C. Aliquots were analyzed by Arizona DHIA (Tempe, AZ) by infrared for butter fat, protein, SCC, lactose, and SNF.

Measurements for rectal temperatures (**RT**) and RR were taken 3 times per d at 0600, 1400, and 1800 h. Rectal temperatures were taken using a GLA M700 (San Luis Obispo, CA) high-performance digital thermometer. Respiration rates were visually counted as breaths per minute. Temperatures and RR were recorded at each time for all cows.

During the ARC phase, blood was collected on d 1, 7, 8, 14, 17, and 18. The blood was collected 6 times per day (0400, 0800, 1200, 1600, 2000, and 2400 h) on d 7, 8, 17, and 18 at the time of arrival at the ARC on d 1, and at 2000 h on d 14. Blood samples were collected by venipuncture from the coccygeal vein. The area was wiped with sterile gauze, sprayed with 70% ethanol, and wiped clean with sterile gauze. Samples for metabolites, insulin, ACTH, and cortisol assays were collected in vacuum tubes containing sodium heparin. All blood samples were immediately placed on ice after collection. Blood samples for plasma were centrifuged at $1,500 \times g$ for 15 min at 4°C within 15 min of collection. Plasma was collected and transferred into aliquots and stored at -20°C until analyzed.

Plasma nonesterified fatty acids levels were determined enzymatically through a commercial kit [Wako NEFA-HR(2), Wako Chemicals USA, Richmond, VA]; inter- and intraassay coefficients of variation (**CV**) were 8 and 4% respectively. Plasma insulin levels were

determined using a Siemens Medical Solutions Diagnostics (Los Angeles, CA) RIA, and plasma glucose was quantified using a colorimetric assay (Pointe Scientific Inc., Canton, MI), both described and validated by Long and Schafer (2013). The intra- and interassay CV were less than 10 and 5% for insulin and 5 and 7% for glucose, respectively. Plasma ACTH levels were determined using a bovine adrenocorticotrophic hormone ELISA kit (MyBioSource, San Diego, CA) previously published by Ponchon et al. (2017). The intra- and interassay CV were 15.8 and 10.4%, respectively; Ponchon et al. (2017) reported intra- and interassay CV of 7.4 and 18.4% for the same assay. Concentrations of cortisol were determined as described previously by Dong et al. (2008) using Coat-A-Count Cortisol RIA with a sensitivity of 0.5 µg/dL (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with an intra- and interassay CV <5%.

Data were analyzed with PROC MIXED procedures of SAS (ver. 9.4, SAS Institute, Cary, NC). Dry matter intake, water intake, milk yield, and milk composition were analyzed according to the following model:

$$Y_{ij} = \mu + T_i + C_j + A_k + H_{l(j)} + T_i C_j + T_i H_{l(j)} + e_{ij},$$

where Y_{ij} = individual data point, μ = overall mean, T_i = fixed effect of treatment (i = CON and OG), C_j = fixed environment effect (j = TN, HS, and REC), A_k = random effect of cow (k = 1 to 12), $H_{l(j)}$ = fixed effect of day (l = 1 to 22) nested within environment, and e_{ij} = residual error. The Kenward-Roger option was used for determining degrees of freedom. Day nested within environment was considered as repeated measures and cow nested within treatment as the subject. A covariance structure with the smallest Akaike's information criterion value was chosen from compound symmetry, Toeplitz, variance components, and autoregressive. Least squares means were calculated and compared under a PDIFF option. Data are presented as least squares means with significance declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

The environmental conditions in the barns during the on-dairy phase of the study were not recorded. However, all cows were housed in the same barn, and oscillating fans were set to keep cows below a THI exposure of 72. Environmental conditions for the TN and HS periods during the ARC phase of the study are shown in Figure 2a and 2b. The dashed line in each figure identifies the THI value of 68, which is the threshold for HS in lactating dairy cows (Zimbelman et al., 2009; Anderson et al., 2013). Cows exposed to THI above this threshold

display a RR greater than 60 (Berman, 2005) and RT above 38.5°C (Allen et al., 2015). During TN the average hourly THI values remained below 68 for the entire 24-h period of each day of the TN period. During HS, average THI values remained above 68 for 17 h of the day. Thus, the environment in HS should have produced measurable changes in physiological measures of HS as well as feed intake and milk yield, whereas these measures would not be affected in TN.

Mean RR and RT in CON and OG fed cows during the ARC phase of the study are shown in Table 2. Diet had no overall effect on these 2 variables. However, there was an effect of environment ($P < 0.001$) as cows exposed to a higher THI resulted in increased RT and RR in all cows. There was also a treatment (TRT) $\times$ environment (ENV) interaction for RT at 1400 h (Table 2, $P < 0.008$) and for RR at 1400 and 1800 h, respectively (Table 2, $P < 0.03$ and $P < 0.05$).

Figure 2. Dry bulb and temperature-humidity index (THI) values in the rooms during the Agriculture Research Complex phase of the study in cows fed OmniGen-AF (Phibro Animal Health, Teaneck, NJ) and control. The dashed line represents nonstressed THI value.

Table 2. Least squares means and tests of significance for respiration rates (RR) and rectal temperatures (RT) of cows fed OmniGen-AF (Phibro Animal Health, Teaneck, NJ) or control diets and exposed to thermoneutral (TN) or heat stress (HS) conditions during the Agriculture Research Center phase of the study

Measure	Time (h)	Control		OmniGen-AF		Mean SEM	P-value ¹				
		TN	HS	TN	HS		ENV	Day (ENV)	TRT	ENV × TRT	Day × TRT (ENV)
RR (breaths/min)	0600	26.83	29.75	26.61	31.08	1.50	0.0031	0.5254	0.7897	0.485	0.7065
	1400	34.33	57.16	30.66	53.00	2.31	<0.0001	<0.0001	0.2102	0.03	0.9844
	1800	32.80	59.26	29.53	50.33	1.86	<0.0001	0.0025	0.213	0.05	0.9571
RT (°C)	0600	38.15	38.00	38.09	38.08	0.05	0.0308	<0.0001	0.8977	0.0591	0.6285
	1400	38.02	38.57	38.11	38.35	0.08	<0.0001	0.1928	0.5234	0.0085	0.1157
	1800	38.22	39.08	38.30	38.87	0.08	<0.0001	0.4394	0.4726	0.44	0.7809

¹ENV = environment; TRT = treatment.

Others have reported that feeding OG reduced RT during thermal stress (Fabris et al., 2017) or following an endotoxin challenge (Brandão et al., 2016).

Feed intake, water intake, milk yield, and components during the ARC phase are shown in Table 3. Dry matter intake increased during TN as the cows adjusted to tiestalls and the 2 rooms in both CON and OG groups (Table 3, Figure 3). Feed intakes also fell in CON during HS ($P < 0.0001$, Table 4, Figure 3), then rose again during REC. Feed intakes did not differ during TN, but were higher in HS for cows fed OG compared with the CON (24.8 vs 22.8 kg of DM/d, TRT × ENV, $P < 0.05$, Table 3, Figure 3). Water intake was increased in all animals during HS ($P < 0.001$, Table 3, Figure 4). There was also TRT × ENV interaction in water intake ($P < 0.01$, Table 3). Testing of intakes within periods indicated water intake was higher in CON during TN ($P < 0.007$, Figure 4). However, water intake did not differ between groups during HS or REC ($P < 0.12$ and $P < 0.77$, Figure 4, tests between groups within periods not shown). The cause of the higher water intake in CON during TN is unknown, but the variance for water intake was normally distributed across the study. Uri-

nary output was not measured during this study so the route of water loss cannot be identified, but the volume of intake for both groups was in the normal range for lactating dairy cows (Meyer et al., 2004).

We did not detect a difference in milk and FCM yields recorded during the ARC phase between the cows fed CON and OG (Table 3). Milk yield in both groups declined during HS ($P < 0.001$, Table 3, Figure 5), then rose during REC. We did not detect an effect of dietary treatment or environment on any milk components, and no TRT × ENV interactions were detected (Table 3). Milk SCC did not differ between TRT groups but a large increase occurred in SCC in both groups during REC ($P < 0.03$, Table 3, Figure 6); however, no TRT × ENV interaction was present. Changes in SCC during the recovery period were surprisingly high. The milk from all cows was tested for microbial content and all milk samples were negative for mastitis pathogens, so no evidence indicated that the increase in SCC in the recovery period was associated with microbial infections in the animals. The estimation of somatic cells may be skewed by loss of epithelial cells from alveoli that were damaged by HS and were sloughed off during

Table 3. Dry matter and water intake, milk yield, and milk composition of animals fed control or OmniGen-AF (Phibro Animal Health, Teaneck, NJ) diets and housed under thermoneutral (TN) or heat stress (HS) conditions in environmentally controlled rooms

Item	Control			OmniGen-AF			SEM	P-value ¹				
	TN	HS	REC ²	TN	HS	REC		ENV	Day (ENV)	TRT	ENV × TRT	Day × TRT (ENV)
DMI (kg)	21.6	22.8	25.2	21.6	24.8	26.1	4.1	<0.0001	<0.001	0.4648	0.0571	0.2551
Water intake (L)	37.2	48.5	34.7	27.0	36.1	33.5	5.6	<0.0001	<0.001	0.304	0.0116	0.2782
Milk yield (kg)	31.9	30.4	30.4	32.6	31.4	31.3	2.2	<0.0001	<0.001	0.779	0.856	0.793
FCM (kg/d)	32.0	31.2	31.1	32.1	30.4	30.2	1.8	0.175	<0.001	0.825	0.7369	0.377
Fat (%)	4.0	4.3	4.2	3.8	3.7	3.9	0.2	0.811	0.014	0.1153	0.4712	0.6294
Protein (%)	3.0	3.0	2.9	2.9	2.8	2.8	0.1	0.193	0.038	0.3097	0.2549	0.6334
Lactose (%)	4.9	4.9	5.0	4.9	4.8	5.0	0.1	0.175	0.967	0.8613	0.7836	0.9994
SCC (×10,000)	19.8	24.8	61.0	19.6	24.5	26.3	13.1	0.081	0.0329	0.5052	0.166	0.8947

¹ENV = environment; TRT = treatment.²REC = recovery.

Figure 3. Mean daily DMI in cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during the Agriculture Research Complex phase of the study. Dry matter intake differed by environment ($P < 0.001$), and a treatment $\times$ environment interaction ($P < 0.05$) was present. TN = thermoneutral; HS = heat stress.

the recovery period. This deserves further study as a potential model for estimating effects of HS on milk SCC.

The data presented for plasma nonesterified fatty acids, glucose, and insulin shown in Table 4 and Figures 7 and 8 are the mean levels by group for the time points samples were collected for that day. The data included for d 14 in both figures and for that day in Table 4 only represent the 2000 h time point. No differences were detected in plasma nonesterified fatty acids due to TRT, ENV, or TRT $\times$ ENV (Table 4). However, there was an effect of time of day on plasma nonesterified fatty acids ($P < 0.01$). Glucose levels did not differ between CON and OG-fed animals (Table 4, $P = 0.32$ or ENV, $P = 0.26$, Figure 7) and no TRT $\times$ ENV

interaction was present. We did not detect differences in plasma insulin between TRT groups ($P = 0.80$) but an effect of ENV was observed (Table 4, $P = 0.001$) as insulin declined abruptly during acute HS (Figure 8), then steadily increased across the HS period (Figure 8). Insulin concentrations rose again during REC to their highest levels (Table 4, Figure 8). A decline in insulin during HS has not been previously reported. Plasma insulin has been reported to be elevated in HS heifers (Burdick Sanchez et al., 2013), lactating cows (Itoh et al., 1998; Baumgard and Rhoads, 2013), and sheep (Achmadi et al., 1993). Similarly, Tao et al. (2012) observed an increase in plasma insulin in HS lactating cows relative to cooled controls. It is possible that other studies did not evaluate the effect of acute heat shock

Table 4. Least squares means and tests of significance for hormones and substrates of cows fed OmniGen-AF (Phibro Animal Health, Teaneck, NJ) or control diets and exposed to thermoneutral (TN) or heat stress (HS) conditions during the Agriculture Research Center phase of the study

Measure	Control			OmniGen-AF			Mean SEM	P-value ¹							
	TN	HS	REC ²	TN	HS	REC		ENV	Time	Day (ENV)	TRT	ENV $\times$ TRT	ENV $\times$ Time	Day $\times$ TRT (ENV)	
NEFA ³ (mEq/L)	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.314	0.012	0.630	0.454	0.798	0.111	0.247	
Insulin (μ IU/mL)	6.2	4.3	8.1	5.7	4.8	7.2	0.8	<0.0001	0.548	0.208	0.799	0.409	0.331	0.950	
Glucose (mg/dL)	81.0	74.2	78.8	77.7	74.4	71.5	3.4	0.262	0.201	0.479	0.327	0.466	0.678	0.326	
ACTH (pg/mL)	0.8	1.1	0.8	1.9	2.1	1.7	0.2	0.0009	0.385	0.197	0.004	0.655	0.349	0.847	
Cortisol (ng/mL)	3.4	6.1	5.3	5.2	5.5	6.3	0.1	0.304	0.0009	0.121	0.641	0.493	0.467	0.004	

¹ENV = environment; TRT = treatment.
²REC = recovery.
³NEFA = nonesterified fatty acids.

Figure 4. Mean daily water consumption in cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during the Agriculture Research Complex phase of the study. Water consumption differed by environment ($P = 0.01$), and there was a treatment $\times$ environment interaction ($P < 0.01$). Water intake was lower in thermoneutral (TN) in OmniGen-fed animals ($P < 0.007$) but did not differ between treatment groups in heat stress (HS) or recovery.

on insulin concentrations since the other studies cited evaluated insulin after several days of heat exposure and in our study insulin did indeed rise across the HS period after an initial abrupt drop on the first day of heat shock.

Plasma cortisol, an important indicator of the physiological status of the cow in HS, was measured during the ARC phase at d 7 (TN), d 8, 14, and 17 (HS) and d 18 (REC; Table 4, Figure 9). Cortisol levels ($\mu\text{g}/\text{dL}$) were only observed to be different between the cows fed

Figure 5. Mean daily milk yield for cows fed OmniGen-AF (Phibro Animal Health, Teaneck, NJ) and control during the Agriculture Research Complex phase of the study. Milk yield differed by environment ($P < 0.001$), but there was no treatment or treatment $\times$ environment interaction on milk yield. TN = thermoneutral; HS = heat stress.

Figure 6. Milk SCC by day in cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during thermoneutral (TN; d 1 to 7), heat stress (HS; d 8 to 17), and recovery (d 18 to 20) periods during the Agriculture Research Complex phase of the study. Somatic cell counts only differed by day within environment [day (environment), $P < 0.03$] as SCC spiked during recovery in both treatment groups.

CON and OG on d 8 (HS; Table 4, $P < 0.004$, Figure 9), which is the first day of HS and therefore is associated with the acute response to HS. No overall TRT, ENV, or TRT $\times$ ENV effect on plasma cortisol was detected (Table 4, Figure 9). Plasma cortisol is known to be elevated during acute but not chronic HS (Christison

and Johnson, 1972; Johnson and Vanjonack, 1976; Collier and Gebremedhin, 2015). This is the first report to show that feeding OG reduced acute cortisol response to HS.

Adrenocorticotrophic hormone is important in regulating the stress response via the adrenal axis. Cortisol is

Figure 7. Plasma glucose concentrations in plasma of cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during thermoneutral (TN), heat stress (HS), and recovery periods of the Agriculture Research Complex phase of the study. No differences were associated with environment, treatment or day, or their interactions. Pooled SE = 5.2 mg/dL.

Figure 8. Plasma insulin concentrations in plasma of cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during thermoneutral (TN) and heat stress (HS) periods during the Agriculture Research Complex phase of the study. Insulin concentrations were affected by environment (ENV, $P < 0.001$) but not treatment (TRT), and no TRT $\times$ ENV interactions were present. Pooled SE = 0.6 μ IU/mL.

a corticosteroid whose secretion from the adrenal gland is regulated by ACTH. Cortisol levels typically increase with the immediate insult of HS. We observed this response in the mean plasma cortisol concentrations of the CON-fed cows on d 8, the first day of HS, whereas OG-fed cows did not have an increase in plasma corti-

sol levels on d 8. No overall treatment or period effect for cortisol between groups was detected. Despite the fact that cortisol concentrations were not elevated in OG-fed cows, plasma ACTH was significantly elevated compared with the CON ($P = 0.004$, Table 4) across all sample days. In addition, an ENV effect was also

Figure 9. Mean plasma cortisol concentrations in cows fed control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) during thermoneutral (TN), heat stress (HS), and recovery periods of the Agriculture Research Complex phase of the study. Cortisol concentrations were not affected by environment (ENV) or treatment (TRT), but a day $\times$ TRT (ENV) interaction was observed as cortisol concentrations were increased the first day of HS ($P < 0.004$). Pooled SE = 0.1 ng/mL.

Figure 10. Mean plasma ACTH concentration in control and OmniGen-AF (Phibro Animal Health, Teaneck, NJ) fed cows during thermo-neutral (TN), heat stress (HS), and recovery periods of the controlled Agriculture Research Complex phase of the study. The ACTH concentrations were elevated in cows fed OmniGen-AF (treatment, $P < 0.004$) and also by HS (environment, $P < 0.0009$), but no treatment $\times$ environment interaction was present. Pooled SE = 4.9 pg/mL.

detected ($P = 0.0009$, Table 4), with higher levels of ACTH during HS in both CON and OG-fed cows, especially on the first day of heat stress (d 8, Figure 10). The surprising finding in this study of elevated ACTH concentrations in animals fed OG during the entire sampling period in the ARC phase of the study is unexpected and suggests that negative feedback dynamics for adrenal cortisol regulation are altered in animals fed OG. Plasma ACTH concentrations in cattle injected with corticotropin-releasing hormone ranged from 20 to 120 pg/mL before and after ACTH stimulation (Tančin et al., 2000). Concentrations of ACTH in plasma of cows in this study ranged from 15 to 55 pg/mL, which is in the range of normal values for ACTH reported by Tančin et al. (2000). More research focused on the hypothalamic-pituitary-adrenal axis response to OG could help define the mechanism associated with the altered cortisol response to acute HS detected in animals fed OG.

In conclusion, supplementing lactating dairy cows with OG beginning 45 d before and during exposure to moderate HS resulted in a reduction of the typical physiological and production responses associated with HS as measured by feed intake, RT, RR, and cortisol secretion but was paradoxically associated with increased ACTH concentrations in OG-fed animals. The reason for the elevated ACTH and lower cortisol response to acute HS in OG-fed animals may lie in altered pituitary or adrenal response to factors controlling cortisol secretion.

ACKNOWLEDGMENTS

The authors acknowledge support from Phibro Animal Health to conduct this study.

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