J. Dairy Sci. 106:1441–1452
https://doi.org/10.3168/jds.2022-22338

Effects of dietary antioxidant supplementation on metabolism and inflammatory biomarkers in heat-stressed dairy cows

M. A. Abeyta,[1] M. Al-Qaisi,[1] E. A. Horst,[1] E. J. Mayorga,[1] S. Rodriguez-Jimenez,[1] B. M. Goetz,[1] S. Carta,[1] H. Tucker,[2] and L. H. Baumgard[1]*
[1]Department of Animal Science, Iowa State University, Ames 50011
[2]Novus International, St. Charles, MO 63304

ABSTRACT

Heat-stress-induced inflammation may be ameliorated by antioxidant supplementation due to the purported effects of increased production of reactive oxygen species or oxidative stress on the gastrointestinal tract barrier. Thus, study objectives were to evaluate whether antioxidant supplementation [AGRADO Plus 2.0 (AP); EW Nutrition] affects metabolism and inflammatory biomarkers in heat-stressed lactating dairy cows. Thirty-two mid-lactation multiparous Holstein cows were assigned to 1 of 4 dietary-environmental treatments: (1) thermoneutral (TN) conditions and fed a control diet (TN-CON; n = 8), (2) TN and fed a diet with AP (10 g antioxidant; n = 8), (3) heat stress (HS) and fed a control diet (HS-CON; n = 8), or (4) HS and fed a diet with AP (HS-AP; n = 8). The trial consisted of a 23-d prefeeding phase and 2 experimental periods (P). Respective dietary treatments were top-dressed starting on d 1 of the prefeeding period and continued daily throughout the duration of the experiment. During P1 (4 d), baseline data were collected. During P2 (7 d), HS was artificially induced using an electric heat blanket (Thermotex Therapy Systems Ltd.). During P2, the effects of treatment, day, and treatment-by-day interaction were assessed using PROC MIXED of SAS (SAS Institute Inc.). Heat stress (treatments 3 and 4) increased rectal, vaginal, and skin temperatures (1.2°C, 1.1°C, and 2.0°C, respectively) and respiration rate (33 breaths per minute) relative to TN cows. As expected, HS decreased dry matter intake, milk yield, and energy-corrected milk yield (32%, 28%, and 28% from d 4 to 7, respectively) relative to TN. There were no effects of AP on body temperature indices or production. Milk fat, protein, and lactose concentrations remained unaltered by HS or AP; however, milk urea nitrogen was increased during HS regardless of AP supplementation (26% relative to TN). Circulating glucose remained unchanged by HS, AP, or time. Additionally, HS decreased circulating glucagon (29% from d 3 to 7 relative to TN), but there was no additional effect of AP. There was a tendency for nonesterified fatty acid concentrations to be increased in HS-AP cows throughout P2 (60% relative to TN-CON), whereas it remained similar in all other treatments. Blood urea nitrogen increased for both HS treatments from d 1 to 3 before steadily decreasing from d 5 to 7, with the overall increase being most pronounced in HS-CON cows (27% relative to TN-CON). Further, supplementing AP decreased blood urea nitrogen in HS-AP on d 3 relative to HS-CON (15%). Circulating serum amyloid A tended to be and lipopolysaccharide binding protein was increased by HS, but neither acute-phase protein was affected by AP. Overall, AP supplementation appeared to marginally alter metabolism but did not meaningfully alter inflammation during HS.
Key words: leaky gut, ethoxyquin

INTRODUCTION

Heat stress (**HS**) occurs when an animal's ability to dissipate heat is outweighed by the combination of internal thermogenesis and environmental heat accumulation (Kadzere et al., 2002). Ultimately, HS has detrimental impacts on production [i.e., DMI and milk yield (**MY**)], morbidity, and mortality (Bernabucci et al., 2010; Burhans et al., 2022), and it thus presents a significant financial burden to livestock producers globally (approximately $1.5 billion/year in the US dairy industry alone; Key and Sneeringer, 2014). Projected rises in global ambient temperature, compounded with genetic selection for improved production performance (which inevitably increases metabolic heat production), create an increasingly urgent need to identify HS mitigation strategies (Kadzere et al., 2002; Brown-Brandl et al., 2004). Although several heat abatement approaches are commercially implemented (e.g., shade, sprinklers, and forced air movement), dairy cow productivity is still

Received May 25, 2022.
Accepted September 20, 2022.
*Corresponding author: baumgard@iastate.edu

compromised during the warm summer months (Baumgard and Rhoads, 2013; Collier et al., 2019). Thus, the endeavor to identify affordable and practical mitigation strategies to ameliorate HS remains pressing.

The multitude of physiological responses to HS are similar to those observed during immunoactivation, albeit with some unique to HS, and are often hypothesized to be a consequence of an energetically expensive inflammatory response secondary to HS-induced intestinal barrier dysfunction (Johnson, 2012; Baumgard and Rhoads, 2013; Kvidera et al., 2017a). Mechanistically, the cause of HS-induced intestinal hyperpermeability is not fully understood, but it is likely multifactorial, including redistribution of blood flow from the core to the periphery, rumen acidosis, and psychological stress (Lambert, 2009; Mayorga et al., 2020; Burhans et al., 2022). Intestinal hypoxia and ischemia are a consequence of the altered hierarchy of blood trafficking, and they contribute to increased intestinal production of reactive oxygen species and oxidative stress (**OS**) during HS (Hall et al., 1999, 2001; Cao et al., 2018). Although inconsistent (Di Costanzo et al., 1997; Ma et al., 2020), some evidence suggests that feeding supplemental antioxidants to heat-stressed farm animals can increase productivity (Aréchiga et al., 1998; Puthpongsiriporn et al., 2001; Sun et al., 2019). This appears to be mediated, at least in part, by their ability to improve intestinal barrier function during environmentally induced hyperthermia (Hall et al., 2001; Oliver et al., 2012; Liu et al., 2016). Thus, increased production of intestinal reactive oxygen species and OS may contribute to the HS-induced inflammatory milieu, and it presents a potential target for nutrition-focused mitigation strategies.

The antioxidant supplemented herein [AGRADO Plus 2.0 (**AP**); EW Nutrition] is a proprietary blend of primarily ethoxyquin, tertiary butyl hydroquinone, and citric acid, all 3 of which have been used as feed preservatives that are capable of retarding lipid oxidation during storage (Hawrysh et al., 1988; Błaszczyk et al., 2013; Pop and Mihalescu, 2017). In addition, these ingredients have increased plasma glutathione peroxidase activity in chicks, enhanced production in poultry and dairy cows, and improved cell viability in heat-stressed bovine mammary cells (Combs and Scott, 1974; Cabel et al., 1988; Vázquez-Añón et al., 2008; Jin et al., 2016). Under a variety of stressful circumstances (e.g., HS, high-altitude hypoxia, and feeding oxidized fat) in various species, antioxidant supplementation has improved intestinal barrier function (Xu et al., 2014; Liu et al., 2016) and production (Aréchiga et al., 1998; Sahin et al., 2002; Vázquez-Añón et al., 2008), but the production benefits are not always corroborated (Di Costanzo et al., 1997; Persson Waller et al., 2007), and excess supplementation has even been associated with increased risk or occurrence of mastitis in cows (Bouwstra et al., 2010a,b). Therefore, experimental objectives were to evaluate the effects of dietary AP supplementation on production, metabolism, and inflammation (as a proxy for intestinal barrier dysfunction) in heat-stressed lactating dairy cows. We hypothesized that supplementation of a dietary antioxidant blend would improve intestinal integrity during HS and thus result in decreased inflammation, altered metabolism, and improved production metrics in HS-AP cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Thirty-two mid-lactation multiparous lactating Holstein cows were balanced by MY, DIM, BW, parity, and presumed pregnancy status (699 ± 69 kg of BW; 139 ± 28 DIM; parity 2.4 ± 0.9) and used in an experiment conducted in 3 replications. Before the start of the experimental phase (i.e., before cows were moved into individual pens), cows were enrolled in a 23-d prefeeding period and assigned to 1 of 2 daily top-dressed dietary treatments: (1) control (**CON**; 500 g of ground corn) or (2) AP supplemented (490 g of ground corn + 10 g antioxidant; blend of ethoxyquin, tertiary butyl hydroquinone, citric acid, and calcium carbonate). After the prefeeding phase, cows were moved to individual box stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm (Ames, IA), where they continued to receive their respective dietary treatments daily. Cows were allowed 3 d to acclimate to new housing conditions, and during this time jugular catheters were implanted as previously described (Horst et al., 2020a). Following acclimation, the trial consisted of 2 experimental periods. Period 1 (**P1**) lasted 4 d, during which baseline data were obtained. Period 2 (**P2**) lasted 7 d, during which cows were assigned to 1 of 2 environmental treatments: (1) thermoneutral (**TN**) or (2) HS [HS conditions induced via an electric heat blanket (**EHB**)]. Environmental and dietary combinations resulted in 4 total treatments during P2: (1) TN + CON (**TN-CON**; n = 8), (2) TN + AP (**TN-AP**; n = 8), (3) HS + CON (**HS-CON**; n = 8), and (4) HS + AP (**HS-AP**; n = 8). One TN-CON and 2 TN-AP cows were removed from the final data set due to illness unrelated to treatment or environment. One TN-AP cow got mastitis before P2. The other 2 were hypophagic and had severely decreased milk production before P2. They were evaluated by a veterinarian, but a specific pathology was not obvious. Because our

primary objectives were related to immune activation during P2, we removed these 3 cows from the data set.

EHB and Ambient Temperature

An EHB containing 12 infrared heating pads (Thermotex Therapy Systems Ltd.) was securely fitted to each cow using adjustable straps to account for cow size variation, as we have previously described (Al-Qaisi et al., 2019, 2020a,b), and this technique has been successfully used by other groups (Holdorf and White, 2021; Pate et al., 2021). In brief, a retractable 110-V electrical cord with auto-rewind was attached to the ceiling in the center of the box stall and to the EHB at the withers to power the blanket while simultaneously allowing for natural movement and behavior in the box stalls. The EHB remained at the same heat intensity (high) on the cows for the entirety of P2 and were monitored 3 times daily (0600, 1200, and 1800 h) to ensure they remained on and functional throughout the experiment. All cows were exposed to TN ambient conditions throughout the duration of the experiment (17°C ± 6°C, 63% ± 13% relative humidity, and 61 ± 8 temperature humidity index). Ambient temperature and humidity were monitored and recorded every 10 min by data loggers (Lascar EL-USB-2-LCD) and condensed into daily averages.

Feed Intake, MY, and Milk Composition

All cows were fed once daily (0800 h) a diet formulated to meet or exceed the predicted requirements (NRC, 2001; Table 1) of energy, protein, minerals, and vitamins. Orts were measured daily before feeding. Cows were milked twice daily (0600 and 1800 h), and yield was recorded. A sample for milk composition analysis was obtained at each milking during P1 and P2. Samples were stored at 4°C with a preservative (bronopol tablet; D & F Control System) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC-approved infrared analysis equipment and procedures (AOAC International, 1995).

Body Temperature Indices

Rectal temperature, skin temperature, and respiration rate were obtained 3 times daily during P1 and P2 (0600, 1200, and 1800 h) and condensed into daily averages. Rectal temperatures were measured using a digital thermometer (GLA M900 Digital Thermometer). Skin temperatures were measured on the neck in an area not covered by the EHB using an infrared thermometer (IRT207: The Heat Seeker 8:1 Mid-Range Infrared Thermometer, General Tool). Respiration rates were determined by counting flank movements for 15 s and multiplying the observed rate by 4 to obtain breaths per minute. Continuous vaginal temperatures were recorded every 10 min starting on d 3 of P1 through the end of P2 and were obtained using calibrated temperature loggers (iButton DS 1921, Maxim Integrated) that were fitted into the hollowed-out center of a blank controlled internal drug release (Zoetis) with a silicone aquarium sealant (Aqueon) and inserted into the vagina with an applicator as previously described (Al-Qaisi et al., 2020a,c). Time of controlled internal drug release insertion and removal were recorded, with the first hour of temperature data removed to allow for acclimation and to ensure data accuracy.

Blood Analysis

Blood samples were collected from the jugular catheter at 0600 h on d 4 of P1, and on d 1, 3, 5, and 7 of P2 in tubes containing K_2EDTA (BD) for plasma and glucagon analysis. For glucagon analysis, 3 mL of whole blood was collected and added to a K_2EDTA tube containing 150 μL of aprotinin (BP2503–10; Thermo Fisher Scientific). Plasma samples were harvested following centrifugation (immediately after collection from the catheter) at 1,500 × *g* for 15 min at 4°C and were subsequently frozen at −20°C until analysis. Samples for complete blood count analysis were collected at 0600 h from d 2 to 4 of P1 and daily during P2. Blood (4 mL) was collected from the catheter and placed into a tube containing K_2EDTA and stored at room temperature (approximately 3 h) before submitting to the Iowa

Table 1. Ingredients and composition of diet[1]

Ingredient	% of DM
Corn silage	30.1
Alfalfa hay	21.5
Ground corn	26.2
Mineral and protein mix	7.6
Corn gluten feed	6.0
Soybean meal	2.6
Soy Plus[2]	6.0
Chemical analysis[3] (% of DM unless noted)	
Starch	26.3
CP	16.6
NDF	30.6
ADF	21.5
NE_L (Mcal/kg of DM)	1.6

[1]Values represent an average of ration nutrient summary reports collected throughout the trial. Diet DM averaged 46.3%.

[2]Mechanically processed soybean meal (Dairy Nutrition Plus).

[3]Average nutrient levels: 4.61% fat, 0.84% Ca, 0.42% P, 0.33% Mg, 0.20% S, 1.34% K, 0.40% Na, 0.51% Cl, 77.66 mg/kg of Zn, 44.16 mg/kg of Mn, 3.50 mg/kg of Fe, 13.46 mg/kg of Cu, 0.75 mg/kg of Co, 0.32 mg/kg of Se, 0.75 mg/kg of I, 6,094.73 IU/kg of vitamin A, 1,218.95 IU/kg of vitamin D, and 24.38 IU/kg of vitamin E.

State University's Department of Veterinary Pathology for analysis.

Plasma insulin, nonesterified fatty acids (**NEFA**), BUN, glucose, glucagon, serum amyloid A (**SAA**), and lipopolysaccharide binding protein (**LBP**) concentrations were analyzed using commercially available kits according to manufacturers' instructions (insulin, Mercodia AB; NEFA, Wako Chemicals USA; BUN, Teco Diagnostics; glucose, Wako Chemicals USA Inc.; glucagon, R&D Systems Inc.; SAA, Tridelta Development Ltd.; LBP, Hycult Biotech). The inter- and intra-assay coefficients of variation for insulin, NEFA, BUN, glucose, glucagon, SAA, and LBP were 14.7% and 6.5%, 6.3% and 2.9%, 10.7% and 4.4%, 3.1% and 4.0%, 13.0% and 5.2%, 9.5% and 4.9%, and 9.5% and 4.2%, respectively.

Statistical Analysis

We did not conduct a sample size calculation, but based on expected variation (from our previous studies), and considering facility and logistical constraints, we determined that 8 animals per treatment would be sufficient to detect statistical significance in key metrics. Data were statistically analyzed using the MIXED procedure of SAS version 9.4 (SAS Institute Inc.). Each animal's respective parameter was analyzed using repeated measures (represented as day within the experiment) with an autoregressive covariance structure. The random effect was cow. The model included treatment, day, treatment by day, and replication as fixed effects and P1 and P2 were analyzed separately from each other, and these multiple comparisons were not mathematically adjusted for (Rothman, 1990). Data are reported as least squares means ± standard error and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

RESULTS

Parity, Body Temperature Indices, and Production

Parity for treatments 1 through 4 did not differ ($P = 0.40$) and was 2.7, 2.2, 2.1, and 2.7, respectively. During P2, rectal temperature and vaginal temperature gradually increased from d 1 to 3 in HS cows, whereas in TN cows they remained unchanged ($P < 0.01$), with the most pronounced increases being from d 3 to 7 of HS (1.2°C and 1.1°C, respectively; $P < 0.01$; Figure 1). Additionally, skin temperature and respiration rate were elevated in HS cows throughout P2 (2.0°C and 33 breaths per minute, respectively; $P < 0.01$; Figure 1) relative to TN cows. No body temperature metric was influenced by AP. As expected, HS decreased DMI relative to TN cows; the largest decrease occurred from d 4 to 7 of P2 (32%; $P < 0.01$; Table 2), and this was not affected by feeding AP. Regardless of dietary treatment, MY and ECM progressively decreased and tended to decrease throughout P2 ($P \leq 0.06$; Table 2) in HS cows, whereas it remained steady in TN, resulting in production decreases that were most pronounced from d 4 to 7 of HS (both 28%; $P < 0.01$). Overall, there was no effect of HS or AP on feed efficiency during P2 ($P > 0.25$; Table 2). Further, milk fat, lactose, and protein concentrations were not affected by environment or AP supplementation ($P > 0.31$; Table 2). Milk urea nitrogen concentration increased (26%; $P < 0.01$; Table 2) in HS relative to TN cows, and there were no effects of AP.

Metabolism and Immune Activation Biomarkers

Circulating glucose concentrations did not change over time, regardless of environmental or dietary treatments ($P > 0.11$; Table 3). Further, circulating insulin concentrations increased for HS-CON cows, with the most pronounced increase being from d 3 to 7 of P2 (53% relative to TN-CON; $P = 0.05$; Table 3), whereas it remained unchanged in HS-AP. The insulin-to-DMI ratio gradually increased from d 1 to 5 in HS animals ($P < 0.01$), with a tendency for a 2.4-fold increase relative to TN treatments detected with post hoc analysis on d 5 ($P = 0.06$; data not shown). Interestingly, HS decreased glucagon concentrations in both treatments, with the most pronounced difference occurring from d 3 to 7 (29% relative to TN cows; $P = 0.01$; Table 3). There was a tendency for circulating NEFA concentrations to be increased in HS-AP cows throughout P2 (60% relative to TN-CON; $P = 0.10$; Table 3), whereas in all other treatments they remained relatively similar. Blood urea nitrogen concentrations increased for both HS treatments from d 1 to 3 of P2 before steadily decreasing from d 5 to 7, with the overall increase being primarily driven by HS-CON cows (27% relative to TN-CON; $P = 0.01$; Table 3). Additionally, supplementing AP decreased circulating BUN on d 3 of HS relative to HS-CON (15%; $P = 0.05$; data not shown). No overall treatment effects were observed in circulating white blood cells, neutrophils, platelets, monocytes, lymphocytes, eosinophils, basophils, or SAA concentrations ($P > 0.12$; Table 4). However, the concentration of circulating LBP was increased by HS, and this was primarily driven by the HS-AP treatment (64% relative to TN-CON; $P = 0.03$; Table 4). Post hoc analysis indicated HS also tended to increase SAA (140 vs. 250 μg/mL; $P = 0.08$; in TN vs. HS, respectively).

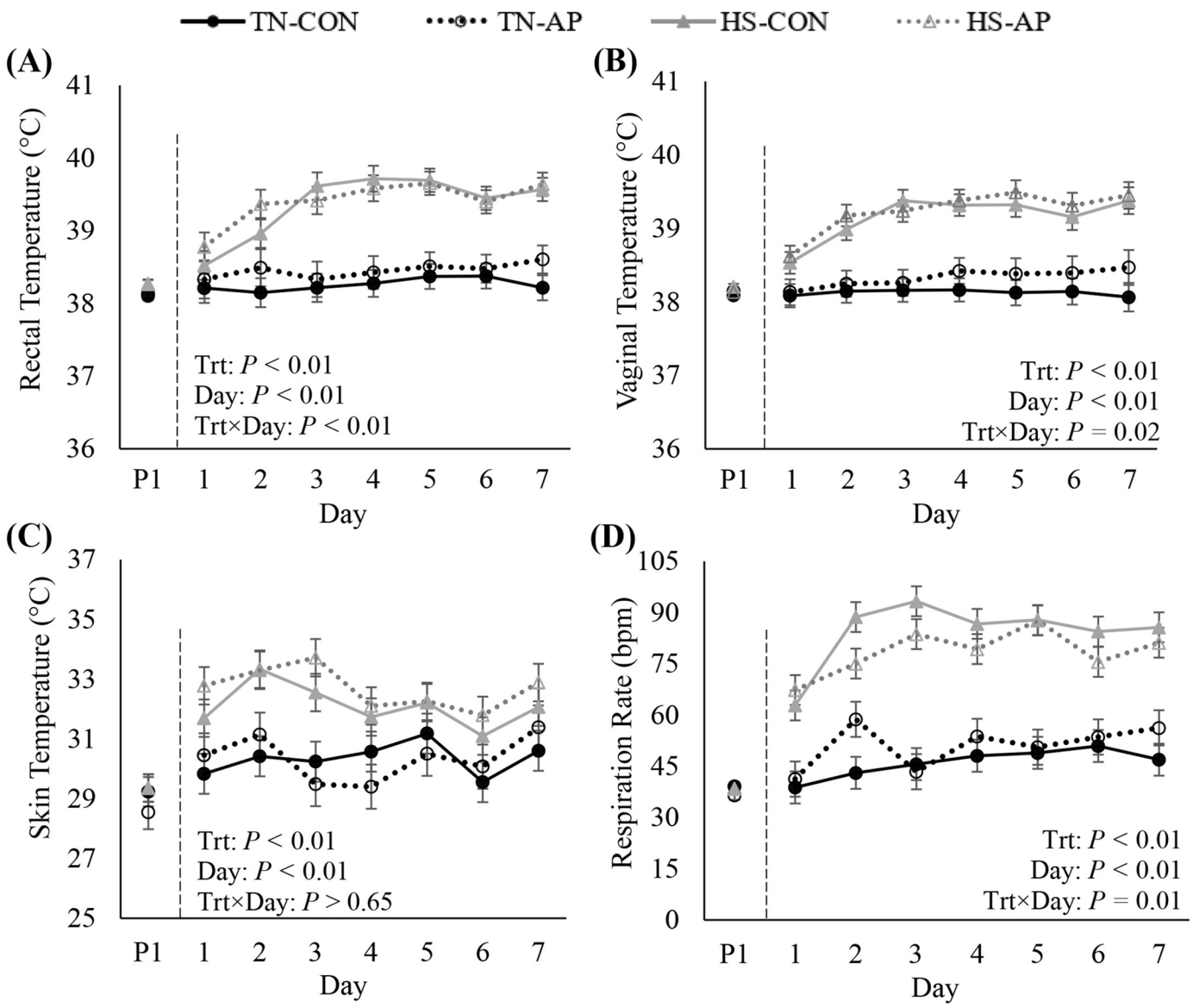

Figure 1. Effects of antioxidant supplementation on (A) rectal temperature, (B) vaginal temperature, (C) skin temperature, and (D) respiration rate (bpm = breaths per minute) in heat-stressed or thermoneutral (TN) lactating dairy cows. Data were analyzed using PROC MIXED and included fixed effects of treatment, time, and their interaction. Period 1 (P1) represents an average of measurements obtained before cows were exposed to their respective environmental treatment during period 2 (TN vs. heat stressed; d 1 to 4 of P1). Results are expressed as least squares means ± standard error of the mean and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$. Trt = treatment; TN-CON = TN and fed a control diet; TN-AP = TN and fed a diet with AGRADO Plus 2.0 (AP; EW Nutrition); HS-CON = heat stress (HS) and fed a control diet; HS-AP = HS and fed a diet with AP.

DISCUSSION

Livestock producers are challenged to identify affordable and practical mitigation strategies that ameliorate HS and its associated sequelae. In addition to obvious phenotypes (e.g., reduced MY, DMI, and daily gain), a more inconspicuous consequence of HS is intestinal hyperpermeability, which has been described in multiple species (Oliver et al., 2012; Pearce et al., 2013; Koch et al., 2019). Mechanistically, the cause of HS-induced intestinal hyperpermeability is seemingly multifactorial, including the diversion of splanchnic blood flow toward peripheral surfaces (Lambert, 2009), rumen acidosis (Burhans et al., 2022), and psychological stress (Mayorga et al., 2020; Xu et al., 2021). Additionally, OS (which increases with tissue hypoxia or ischemia; Hall et al., 2001; Lambert, 2009), or at least an increase in reactive oxygen species, may also be an important

Table 2. Effects of antioxidant supplementation on production parameters in heat-stressed or thermoneutral lactating dairy cows[1]

	Treatment					*P*-value		
Parameter	TN-CON	TN-AP	HS-CON	HS-AP	SEM	Trt	Time	Trt × time
DMI (kg/d)	22.2[a]	20.7[a]	16.8[b]	15.0[b]	0.8	<0.01	<0.01	0.14
Milk yield (kg/d)	36.9[a]	33.1[ab]	27.7[b]	27.9[b]	2.4	0.02	0.14	0.01
ECM (kg/d)	41.9[a]	35.9[ab]	31.8[b]	30.4[b]	2.3	<0.01	0.04	0.06
FE, ECM:DMI	1.91	1.81	1.97	2.11	0.11	0.25	0.03	0.66
Milk composition								
Fat (%)	4.50	4.25	4.67	4.43	0.16	0.31	<0.01	0.44
Lactose (%)	4.73	4.74	4.70	4.62	0.05	0.38	<0.01	0.39
Protein (%)	3.18	3.11	3.16	3.03	0.09	0.65	<0.01	0.41
MUN (mg/dL)	12.5[b]	12.1[b]	15.9[a]	15.1[a]	0.7	<0.01	<0.01	0.13

[a,b]Values within a row with differing superscripts denote differences ($P \leq 0.05$) between treatments.

[1]Trt = treatment; TN-CON = thermoneutral control; TN-AP = thermoneutral supplemented with 10 g/d AGRADO Plus 2.0 (AP; EW Nutrition); HS-CON = heat stressed via an electric heat blanket and control; HS-AP = heat stressed via an electric heat blanket + 10 g/d AP; FE = feed efficiency. Data are reported as LSM ± SEM.

pathophysiological contributor to HS-induced barrier dysfunction (Rao et al., 2002; Cao et al., 2018; Ortega and Szabó, 2021), a concept reinforced with intervening experimentation; administering antioxidants improved barrier function and reduced histological tissue damage in HS experiments (Hall et al., 2001; Oliver et al., 2012). Inflammation that occurs secondary to intestinal barrier dysfunction and paracellular antigen passage is energetically expensive and diverts nutrients away from productive purposes (Johnson, 2012; Kvidera et al., 2017a). Thus, targeting reactive oxygen species presents a potential mitigation strategy that may improve intestinal barrier function and ameliorate inflammation during HS. Therefore, objectives were to evaluate the effects of AP (a commercial dietary antioxidant) on production, metabolism, and inflammatory metrics in heat-stressed lactating dairy cows.

Heat stress was successfully induced herein, as indicated by increased rectal temperature, vaginal temperature, skin temperature, and respiration rate in HS cows relative to TN controls. Euthermia is paramount for optimal corporeal functioning; thus, heat-stressed animals employ robust physiological, metabolic, and behavioral adaptations to prevent lethal hyperthermia, but these survival strategies come at the expense of production (Silva, 2006; Cook et al., 2007). Limiting feed intake is a well-conserved behavioral response to HS that transcends species as means to reduce metabolic heat production (Baumgard and Rhoads, 2013). Herein, HS decreased DMI, MY, and ECM as expected, but this was not ameliorated by feeding AP. It is likely the reduction in MY and ECM is explained by both decreased DMI and, at least in part, HS-induced barrier dysfunction (discussed below). Previous experiments have observed increased MY and DMI with AP feeding in TN dairy cows (Vázquez-Añón et al., 2008; Wang et al., 2010), but these production benefits are inconsistent (Boerman et al., 2014a,b), and AP did not improve DMI in heat-stressed laying hens (Felver-Gant et al., 2014). Thus, further work evaluating the effects of AP on production metrics in heat-stressed cows and other farm animals is warranted.

Heat-stressed cows displayed evidence of intestinal hyperpermeability as indicated by elevations in meta-

Table 3. Effects of antioxidant supplementation on metabolic parameters in heat-stressed or thermoneutral lactating dairy cows[1]

	Treatment					*P*-value		
Parameter	TN-CON	TN-AP	HS-CON	HS-AP	SEM	Trt	Time	Trt × time
Glucose (mg/dL)	73.9	74.1	74.0	72.9	2.1	0.97	0.11	0.43
Insulin (μg/L)	0.73[b]	0.82[ab]	1.12[a]	0.71[b]	0.12	0.05	0.26	0.48
Insulin:DMI	0.04	0.04	0.07	0.06	0.01	0.12	<0.01	0.25
Glucagon (pg/mL)	176[b]	183[a]	141[bc]	125[c]	13	0.01	0.03	0.13
NEFA (μEq/L)	132[y]	136[xy]	126[y]	212[x]	28	0.10	0.60	0.89
BUN (mg/dL)	10.3[b]	10.2[b]	13.1[a]	11.7[ab]	0.7	0.01	<0.01	<0.01

[a–c]Values within a row with differing superscripts denote differences ($P \leq 0.05$) between treatments.

[x,y]Values within a row with differing superscripts denote a tendency for a difference ($0.05 < P \leq 0.10$) between treatments.

[1]Trt = treatment; TN-CON = thermoneutral control; TN-AP = thermoneutral supplemented with 10 g/d AGRADO Plus 2.0 (AP; EW Nutrition); HS-CON = heat stressed via an electric heat blanket and control; HS-AP = heat stressed via an electric heat blanket + 10 g/d AP; NEFA = nonesterified fatty acids. Data are reported as LSM ± SEM.

Table 4. Effects of antioxidant supplementation on leukocyte dynamics and inflammatory biomarkers in heat-stressed or thermoneutral lactating dairy cows[1]

	Treatment					*P*-value		
Parameter	TN-CON	TN-AP	HS-CON	HS-AP	SEM	Trt	Time	Trt × time
WBC (× 10^3/μL)	7.7	8.5	7.8	7.2	0.7	0.66	0.08	0.16
Neutrophils (× 10^3/μL)	3.1	3.8	3.2	3.1	0.3	0.27	0.09	0.47
Platelets (× 10^3/μL)	368	331	361	338	34	0.85	0.05	0.35
Monocytes (× 10^3/μL)	0.38	0.39	0.38	0.36	0.02	0.82	0.46	0.81
Lymphocytes (× 10^3/μL)	3.8	3.7	3.6	3.3	0.6	0.92	0.99	0.82
Eosinophils (× 10^3/μL)	0.31	0.37	0.51	0.30	0.07	0.13	0.31	0.89
Basophils (× 10^3/μL)	0.07	0.07	0.06	0.06	0.01	0.68	0.67	0.58
SAA (μg/mL)	123.2	157.0	184.1	314.8	61.4	0.12	0.55	0.36
LBP (μg/mL)	2.2[b]	2.0[b]	3.0[ab]	3.6[a]	0.4	0.03	0.11	0.30

[a,b]Values within a row with differing superscripts denote differences ($P \leq 0.05$) between treatments.

[1]Trt = treatment; TN-CON = thermoneutral control; TN-AP = thermoneutral supplemented with 10 g/d AGRADO Plus 2.0 (AP; EW Nutrition); HS-CON = heat stressed via an electric heat blanket and control; HS-AP = heat stressed via an electric heat blanket + 10 g/d AP; WBC = white blood cells; SAA = serum amyloid A; LBP = lipopolysaccharide binding protein. Data are reported as LSM ± SEM.

bolic and inflammatory metrics relative to TN counterparts. Hyperinsulinemia is a well-conserved response to immunoactivation in several species (Zarrin et al., 2014; Kvidera et al., 2017b; Horst et al., 2020a) and plays a central role in optimizing glucose supply and utilization in activated immune cells (Helderman, 1981; Walrand et al., 2004). Despite their catabolic state, heat-stressed animals frequently have elevated insulin concentrations, and this is especially apparent when compared with pair-fed thermal neutral controls (Wheelock et al., 2010; Gangloff et al., 2016; Barnes et al., 2019), purportedly in response to immunoactivation following HS-induced barrier dysfunction (Baumgard and Rhoads, 2013; Mayorga et al., 2020). Interestingly, circulating insulin increased in HS-CON but not HS-AP cows, but both HS treatments had an increased insulin-to-DMI ratio above TN animals. Reasons why AP blunted the insulin response during HS are not clear, but the increased insulin-to-DMI ratio in both HS treatments agrees with previous experiments (Wheelock et al., 2010; Al-Qaisi et al., 2020b). Heat stress also increased BUN and MUN concentrations, which is consistent with other HS experiments (Baumgard et al., 2011; Al-Qaisi et al., 2020a,b,c), and i.v. lipopolysaccharide infusion trials in cows (Kvidera et al., 2017a; Horst et al., 2020a). As previously mentioned, immunoactivation requires a substantial quantity of nutrients, and this occurs simultaneously with illness-induced hypophagia (Johnson, 2012; Kvidera et al., 2017a). Thus, skeletal muscle is ostensibly catabolized to supply amino acids for gluconeogenesis, positive acute-phase protein synthesis, and leukocyte proliferation (Mayorga et al., 2020; Ma et al., 2021), resulting in elevated blood and MUN. Therefore, the altered insulin-to-DMI ratio and BUN and MUN concentrations suggest homeorhetic adaptations to support a HS-induced activated immune system.

Along with the observed metabolic changes, circulating LBP increased and SAA tended to increase during HS. This agrees with other experiments where acute-phase proteins increased in heat-stressed pigs (Cui et al., 2019), rabbits (Madkour et al., 2020), goats (Al-Dawood, 2017), and lactating dairy cows (Al-Qaisi et al., 2020b) and suggests the heat load was sufficient to induce intestinal barrier dysfunction and its associated sequelae. Serum amyloid A responses are inconsistent in heat-stressed cows, and though it increased in some experiments (Al-Qaisi et al., 2020b; Pate et al., 2021), it did not in others (Opgenorth et al., 2021; S. Rodriguez-Jimenez and L. H. Baumgard, unpublished). Interestingly, inflammatory metrics (cytokines and acute-phase proteins) are sometimes blunted during the actual heat load and then exacerbated upon HS recovery and the return to TN conditions in multiple species (Leon et al., 2006; Abuajamieh et al., 2018; S. Rodriguez-Jimenez and L. H. Baumgard, unpublished). In fact, exaggerated inflammation during HS recovery may play a pathophysiological role in the development of heat stroke (Leon, 2007). Although HS increased LBP and SAA, circulating leukocytes were largely unaffected by HS or dietary treatment. This conflicts with prior reports by Al-Qaisi et al. (2020b), where circulating lymphocytes and basophils were decreased by HS, potentially due to leukocyte migration into the gastrointestinal tract (Koch et al., 2019). However, the lack of perturbations herein may reflect the transient nature of leukocyte dynamics and the ability of bone marrow to maintain leukocytosis during the gradual onset of HS-induced immunoactivation (Scheiermann et al., 2015).

Circulating LBP and SAA increased in both HS treatments, but this was more pronounced in the HS-AP cows, suggestive of increased inflammation from

AP supplementation. However, this contradicts the insulin and BUN responses and is not supported by other phenotypic data (e.g., MY and ECM). Further, tertiary butyl hydroquinone and citric acid (ingredients included in AP) have decreased intestinal and peripheral inflammation in various animal models of immunoactivation (Jin et al., 2010; Abdel-Salam et al., 2014; Zhao et al., 2019; Rahman et al., 2020). Interestingly, feeding excessive antioxidants can potentially act as a prooxidant and negatively affect overall health (Sotler et al., 2019). Regardless, given the purported anti-inflammatory properties of AP and inconsistencies with other metrics observed herein, it is unlikely the increase in LBP in HS-AP cows was biologically meaningful. However, differences in model, species, and dosage may explain discrepancies between studies; thus, further work evaluating AP supplementation and inflammatory metrics during HS is needed.

As reviewed by Mayorga et al. (2020) and Baumgard and Rhoads (2013), heat-stressed animals have a metabolically perplexing phenotype as they tend to retain adipose tissue in the face of reduced nutrient intake. This blunted lipolytic response to negative energy balance is even more striking when compared with pair-fed animals kept in TN conditions (Wheelock et al., 2010; Sanz Fernandez et al., 2015a,b) and is likely the result of HS-induced hyperinsulinemia, as insulin is intensely antilipolytic (Brockman and Laarveld, 1986). Despite marked reductions in feed intake in the HS cows (approximately 33%), NEFA concentrations were either maintained at similar concentrations to (HS-CON) or slightly elevated above (HS-AP) ad-libitum-fed TN animals. The discrepancy between HS-CON and HS-AP animals is likely explained by differences in circulating insulin concentrations, as HS-AP cows had lower insulin relative to HS-CON cows, allowing for further adipose tissue mobilization. Regardless, the NEFA response observed in HS-AP animals remained blunted relative to what would be expected in TN lactating cows on a similar plane of nutrition (Wheelock et al., 2010), and it is consistent with prior HS experiments (Al-Qaisi et al., 2020b,c).

Heat stress decreased circulating glucagon, and this was especially apparent in the HS-AP cows. In contrast to most monogastrics, glucagon increases postprandially (Sano et al., 1993; Mineo et al., 1994) but decreases during feed restriction in ruminants (Carruthers et al., 1974; Vicini et al., 1988; Horst et al., 2020b), suggesting the reduction may reflect concurrent heat-induced anorexia. However, HS may impose further reductions in glucagon as a protective strategy to reduce internal thermogenesis. Interestingly, circulating glucagon increases in response to cold stress (Kuroshima et al., 1978; Seitz et al., 1981; Doi et al., 1982), and exogenous glucagon administration increases energy expenditure and thermogenesis in humans (Nair, 1987), mice (Beaudry et al., 2019), dogs (Weiser and Grande, 1974), and pigs (Ingram and Kaciuba-Uscilko, 1980). Thus there is potential that a reduction in glucagon, in combination with other thermogenic hormones (e.g., thyroid-stimulating hormone or thyroxine and triiodothyronine; Pereira et al., 2008; Kahl et al., 2015; Sanz Fernandez et al., 2015a), may aid in minimizing endogenous heat production during HS. However, the effects of HS on this key metabolic hormone are unclear, as some have reported decreased (Kuroshima et al., 1978; Doi et al., 1982; Tang et al., 2013), increased (Tatár et al., 1986; Kappel et al., 1997), or unchanged (Koch et al., 2016) glucagon concentrations in response to HS in various species. Therefore, further evaluation of the relationship between HS and glucagon secretion is needed, especially considering how important glucose is to both the immune system and milk synthesis (Baumgard et al., 2017; Horst et al., 2021).

There are some limitations that need considering. Notably, markers of OS and reactive oxygen species were not measured, and thus we are not certain that HS actually increased the production of reactive oxygen species or OS. Consequently, it is possible that AP did not have an opportunity to express its antioxidant benefit. We are also presuming that the markers of immune activation (LBP, insulin) are originating from HS-induced gastrointestinal tract hyperpermeability, but this cannot be confirmed with the variables measured herein.

CONCLUSIONS

Heat stress threatens animal production and well-being, and its effects may intensify as global temperatures continue to rise. Thus, it is imperative to identify practical and effective mitigation strategies to combat heat-induced intestinal barrier dysfunction and its associated corollaries (e.g., decreased production and inflammation). Because antioxidant supplementation has previously improved production and intestinal health during a variety of stressors (including heat), it was of interest to evaluate the effects of AP supplementation on production, metabolism, and inflammatory metrics (as a proxy for intestinal hyperpermeability) in heat-stressed lactating dairy cows. Although AP marginally altered metabolic parameters, it did not meaningfully alter the inflammatory profile or production metrics in heat-stressed animals. Given the continued relevance of gut health and thermal stress to the dairy industry, further work evaluating the effects of antioxidant supplementation during HS in dairy cows is warranted.

ACKNOWLEDGMENTS

Results described herein were funded in part by Novus International (St. Charles, MO) and the Norman Jacobson Endowed Professorship. H. Tucker is an employee of Novus International and was involved with the experimental design, data interpretation, and manuscript preparation. The authors have not stated any other conflicts of interest.

REFERENCES

Abdel-Salam, O. M. E., E. R. Youness, N. A. Mohammed, S. M. Y. Morsy, E. A. Omara, and A. A. Sleem. 2014. Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. J. Med. Food 17:588–598. https://doi.org/10.1089/jmf.2013.0065.

Abuajamieh, M., S. K. Kvidera, E. J. Mayorga, A. Kaiser, S. Lei, J. T. Seibert, E. A. Horst, M. V. Sanz Fernandez, J. W. Ross, J. T. Selsby, A. F. Keating, R. P. Rhoads, and L. H. Baumgard. 2018. The effect of recovery from heat stress on circulating bioenergetics and inflammatory biomarkers. J. Anim. Sci. 96:4599–4610. https://doi.org/10.1093/jas/sky345.

Al-Dawood, A. 2017. Acute phase proteins as indicators of stress in Baladi goats from Jordan. Acta Agric. Scand. A Anim. Sci. 67:58–65. https://doi.org/10.1080/09064702.2017.1363815.

Al-Qaisi, M., E. A. Horst, S. K. Kvidera, E. J. Mayorga, L. L. Timms, and L. H. Baumgard. 2019. Technical note: Developing a heat stress model in dairy cows using an electric heat blanket. J. Dairy Sci. 102:684–689. https://doi.org/10.3168/jds.2018-15128.

Al-Qaisi, M., E. J. Mayorga, E. A. Horst, S. K. Kvidera, C. S. McCarthy, M. A. Abeyta, B. M. Goetz, H. A. Ramirez-Ramirez, L. L. Timms, and L. H. Baumgard. 2020a. Validating a heat stress model: The effects of an electric heat blanket and nutritional plane on lactating dairy cows. J. Dairy Sci. 103:5550–5560. https://doi.org/10.3168/jds.2019-17543.

Al-Qaisi, M., E. A. Horst, E. J. Mayorga, B. M. Goetz, M. A. Abeyta, I. Yoon, L. L. Timms, J. A. Appuhamy, and L. H. Baumgard. 2020b. Effects of a *Saccharomyces cerevisiae* fermentation product on heat-stressed dairy cows. J. Dairy Sci. 103:9634–9645. https://doi.org/10.3168/jds.2020-18721.

Al-Qaisi, M., E. A. Horst, S. K. Kvidera, C. S. McCarthy, E. J. Mayorga, M. A. Abeyta, B. Goetz, N. C. Upah, D. M. McKilligan, B. W. Kolstad, L. L. Timms, and L. H. Baumgard. 2020c. Effects of dietary electrolytes, osmolytes, and energetic compounds on body temperature indices in heat-stressed lactating cows. Res. Vet. Sci. 132:42–48. https://doi.org/10.1016/j.rvsc.2020.05.012.

AOAC International. 1995. AOAC official method 972.16. Fat, lactose, protein, and solids in milk. Mid infrared spectroscopic method. Pages 23–26 in Official Methods of Analysis. 16th ed. Vol. 2. AOAC International, Arlington, VA.

Aréchiga, C. F., C. R. Staples, L. R. McDowell, and P. J. Hansen. 1998. Effects of timed insemination and supplemental β-carotene on reproduction and milk yield of dairy cows under heat stress. J. Dairy Sci. 81:390–402. https://doi.org/10.3168/jds.S0022-0302(98)75589-4.

Barnes, T. L., C. N. Cadaret, K. A. Beede, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2019. Hypertrophic muscle growth and metabolic efficiency were impaired by chronic heat stress, improved by zilpaterol supplementation, and not affected by ractopamine supplementation in feedlot lambs. J. Anim. Sci. 97:4101–4113. https://doi.org/10.1093/jas/skz271.

Baumgard, L. H., R. J. Collier, and D. E. Bauman. 2017. A 100-year review: Regulation of nutrient partitioning to support lactation. J. Dairy Sci. 100:10353–10366. https://doi.org/10.3168/jds.2017-13242.

Baumgard, L. H., and R. P. Rhoads Jr.. 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:311–337. https://doi.org/10.1146/annurev-animal-031412-103644.

Baumgard, L. H., J. B. Wheelock, S. R. Sanders, C. E. Moore, H. B. Green, M. R. Waldron, and R. P. Rhoads. 2011. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. J. Dairy Sci. 94:5620–5633. https://doi.org/10.3168/jds.2011-4462.

Beaudry, J. L., K. D. Kaur, E. M. Varin, L. L. Baggio, X. Cao, E. E. Mulvihill, J. H. Stern, J. E. Campbell, P. E. Scherer, and D. J. Drucker. 2019. The brown adipose tissue glucagon receptor is functional but not essential for control of energy homeostasis in mice. Mol. Metab. 22:37–48. https://doi.org/10.1016/j.molmet.2019.01.011.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4:1167–1183. https://doi.org/10.1017/S175173111000090X.

Błaszczyk, A., A. Augustyniak, and J. Skolimowski. 2013. Ethoxyquin: An antioxidant used in animal feed. Int. J. Food Sci. 2013:585931. https://doi.org/10.1155/2013/585931.

Boerman, J. P., C. L. Preseault, and A. L. Lock. 2014a. Effect of dietary antioxidant and increasing corn oil inclusion on milk fat yield and fatty acid composition in dairy cattle. J. Dairy Sci. 97:7697–7705. https://doi.org/10.3168/jds.2013-7701.

Boerman, J. P., C. L. Preseault, J. Kraft, H. M. Dann, and A. L. Lock. 2014b. Short communication: Effect of antioxidant supplementation on milk production, milk fat synthesis, and milk fatty acids in dairy cows when fed a diet designed to cause milk fat depression. J. Dairy Sci. 97:1077–1081. https://doi.org/10.3168/jds.2013-7442.

Bouwstra, R. J., M. Nielen, J. A. Stegeman, P. Dobbelaar, J. R. Newbold, E. H. J. M. Jansen, and T. van Werven. 2010a. Vitamin E supplementation during the dry period in dairy cattle. Part I: Adverse effect on incidence of mastitis postpartum in a double-blind randomized field trial. J. Dairy Sci. 93:5684–5695. https://doi.org/10.3168/jds.2010-3159.

Bouwstra, R. J., M. Nielen, J. R. Newbold, E. H. J. M. Jansen, H. F. Jelinek, and T. van Werven. 2010b. Vitamin E supplementation during the dry period in dairy cattle. Part II: Oxidative stress following vitamin E supplementation may increase clinical mastitis incidence postpartum. J. Dairy Sci. 93:5696–5706. https://doi.org/10.3168/jds.2010-3161.

Brockman, R. P., and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants; a review. Livest. Prod. Sci. 14:313–334. https://doi.org/10.1016/0301-6226(86)90012-6.

Brown-Brandl, T. M., J. A. Nienaber, H. Xin, and R. S. Gates. 2004. A literature review of swine heat production. Trans. ASAE 47:259–270. https://doi.org/10.13031/2013.15867.

Burhans, W. S., C. A. Rossiter Burhans, and L. H. Baumgard. 2022. Invited review: Lethal heat stress: The putative pathophysiology of a deadly disorder in dairy cattle. J. Dairy Sci. 105:3716–3735. https://doi.org/10.3168/jds.2021-21080.

Cabel, M. C., P. W. Waldroup, W. D. Shermer, and D. F. Calabotta. 1988. Effects of ethoxyquin feed preservative and peroxide level on broiler performance. Poult. Sci. 67:1725–1730. https://doi.org/10.3382/ps.0671725.

Cao, S., H. Wu, C. Wang, Q. Zhang, L. Jiao, F. Lin, and C. H. Hu. 2018. Diquat-induced oxidative stress increases intestinal permeability, impairs mitochondrial function, and triggers mitophagy in piglets. J. Anim. Sci. 96:1795–1805. https://doi.org/10.1093/jas/sky104.

Carruthers, T. C., J. G. Manns, and S. J. Quesnel. 1974. The effect of prostaglandin E_1 on lipid metabolism and hormone secretion in sheep. Can. J. Physiol. Pharmacol. 52:319–322. https://doi.org/10.1139/y74-043.

Collier, R. J., L. H. Baumgard, R. B. Zimbelman, and Y. Xiao. 2019. Heat stress: Physiology of acclimation and adaptation. Anim. Front. 9:12–19. https://doi.org/10.1093/af/vfy031.

Combs, G. F. Jr., and M. L. Scott. 1974. Antioxidant effects on selenium and vitamin E function in the chick. J. Nutr. 104:1297–1303. https://doi.org/10.1093/jn/104.10.1297.

Cook, N. B., R. L. Mentink, T. B. Bennett, and K. Burgi. 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. J. Dairy Sci. 90:1674–1682. https://doi.org/10.3168/jds.2006-634.

Cui, Y., C. Wang, Y. Hao, X. Gu, and H. Wang. 2019. Chronic heat stress induces acute phase responses and serum metabolome changes in finishing pigs. Animals (Basel) 9:395. https://doi.org/10.3390/ani9070395.

Di Costanzo, A., J. N. Spain, and D. E. Spiers. 1997. Supplementation of nicotinic acid for lactating Holstein cows under heat stress conditions. J. Dairy Sci. 80:1200–1206. https://doi.org/10.3168/jds.S0022-0302(97)76048-X.

Doi, K., T. Ohno, and A. Kuroshima. 1982. Role of endocrine pancreas in temperature acclimation. Life Sci. 30:2253–2259. https://doi.org/10.1016/0024-3205(82)90252-1.

Felver-Gant, J. N., R. L. Dennis, J. Zhao, and H. W. Cheng. 2014. Effects of dietary antioxidant on performance and physiological responses following heat stress in laying hens. Int. J. Poult. Sci. 13:260–271. https://doi.org/10.3923/ijps.2014.260.271.

Gangloff, E. J., K. G. Holden, R. S. Telemeco, L. H. Baumgard, and A. M. Bronikowski. 2016. Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. J. Exp. Biol. 219:2944–2954. https://doi.org/10.1242/jeb.143107.

Hall, D. M., K. R. Baumgardner, T. D. Oberley, and C. V. Gisolfi. 1999. Splanchnic tissues undergo hypoxic stress during whole body hyperthermia. Am. J. Physiol. Gastrointest. Liver Physiol. 276:G1195–G1203. https://doi.org/10.1152/ajpgi.1999.276.5.G1195.

Hall, D. M., G. R. Buettner, L. W. Oberley, L. Xu, R. D. Matthes, and C. V. Gisolfi. 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280:H509–H521. https://doi.org/10.1152/ajpheart.2001.280.2.H509.

Hawrysh, Z. J., P. J. Shand, B. Tokarska, and C. Lin. 1988. Effects of tertiary butylhydroquinone on the stability of canola oil. I. Accelerated storage. Can. Inst. Food Sci. Technol. J. 21:549–554. https://doi.org/10.1016/S0315-5463(88)71037-8.

Helderman, J. H. 1981. Role of insulin in the intermediary metabolism of the activated thymic-derived lymphocyte. J. Clin. Invest. 67:1636–1642. https://doi.org/10.1172/JCI110199.

Holdorf, H. T., and H. M. White. 2021. Effects of rumen-protected choline supplementation in Holstein dairy cows during electric heat blanket-induced heat stress. J. Dairy Sci. 104:9715–9725. https://doi.org/10.3168/jds.2020-19794.

Horst, E. A., S. K. Kvidera, and L. H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance—a critical evaluation of traditional dogmas. J. Dairy Sci. 104:8380–8410. https://doi.org/10.3168/jds.2021-20330.

Horst, E. A., E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, P. J. Gorden, S. K. Kvidera, and L. H. Baumgard. 2020b. Evaluating effects of zinc hydroxychloride on biomarkers of inflammation and intestinal integrity during feed restriction. J. Dairy Sci. 103:11911–11929. https://doi.org/10.3168/jds.2020-18860.

Horst, E. A., L. M. van den Brink, E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, S. K. Kvidera, L. S. Caixeta, R. P. Rhoads, and L. H. Baumgard. 2020a. Evaluating acute inflammation's effects on hepatic triglyceride content in experimentally induced hyperlipidemic dairy cows in late lactation. J. Dairy Sci. 103:9620–9633. https://doi.org/10.3168/jds.2020-18686.

Ingram, D. L., and H. Kaciuba-Uscilko. 1980. Metabolic effects of glucagon in the young pig. Horm. Metab. Res. 12:430–433. https://doi.org/10.1055/s-2007-999167.

Jin, W., H. Ni, Y. Dai, H. Wang, T. Lu, J. Wu, J. Jiang, and W. Liang. 2010. Effects of tert-butylhydroquinone on intestinal inflammatory response and apoptosis following traumatic brain injury in mice. Mediators Inflamm. 2010:502564. https://doi.org/10.1155/2010/502564.

Jin, X. L., K. Wang, L. Liu, H. Y. Liu, F.-Q. Zhao, and J. X. Liu. 2016. Nuclear factor-like factor 2-antioxidant response element signaling activation by *tert*-butylhydroquinone attenuates acute heat stress in bovine mammary epithelial cells. J. Dairy Sci. 99:9094–9103. https://doi.org/10.3168/jds.2016-11031.

Johnson, R. W. 2012. Fueling the immune response: What's the cost? Pages 211–223 in Feed Efficiency in Swine. J. Patience, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. Livest. Prod. Sci. 77:59–91. https://doi.org/10.1016/S0301-6226(01)00330-X.

Kahl, S., T. H. Elsasser, R. P. Rhoads, R. J. Collier, and L. H. Baumgard. 2015. Environmental heat stress modulates thyroid status and its response to repeated endotoxin challenge in steers. Domest. Anim. Endocrinol. 52:43–50. https://doi.org/10.1016/j.domaniend.2015.02.001.

Kappel, M., A. Gyhrs, H. Galbo, and B. K. Pedersen. 1997. The response on glucoregulatory hormones of *in vivo* whole body hyperthermia. Int. J. Hyperthermia 13:413–421. https://doi.org/10.3109/02656739709046542.

Key, N., and S. Sneeringer. 2014. Potential effects of climate change on the productivity of U.S. dairies. Am. J. Agric. Econ. 96:1136–1156. https://doi.org/10.1093/ajae/aau002.

Koch, F., O. Lamp, M. Eslamizad, J. Weitzel, and B. Kuhla. 2016. Metabolic response to heat stress in late-pregnant and early lactation dairy cows: Implications to liver-muscle crosstalk. PLoS One 11:e0160912. https://doi.org/10.1371/journal.pone.0160912.

Koch, F., U. Thom, E. Albrecht, R. Weikard, W. Nolte, B. Kuhla, and C. Kuehn. 2019. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. Proc. Natl. Acad. Sci. USA 116:10333–10338. https://doi.org/10.1073/pnas.1820130116.

Kuroshima, A., K. Doi, and T. Ohno. 1978. Role of glucagon in metabolic acclimation to cold and heat. Life Sci. 23:1405–1409. https://doi.org/10.1016/0024-3205(78)90401-0.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2017a. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360–2374. https://doi.org/10.3168/jds.2016-12001.

Kvidera, S. K., E. A. Horst, E. J. Mayorga, M. V. Sanz-Fernandez, M. Abuajamieh, and L. H. Baumgard. 2017b. Estimating glucose requirements of an activated immune system in growing pigs. J. Anim. Sci. 95:5020–5029. https://doi.org/10.2527/jas2017.1830.

Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87(Suppl. 14):E101–E108. https://doi.org/10.2527/jas.2008-1339.

Leon, L. R. 2007. Heat stroke and cytokines. Prog. Brain Res. 162:481–524. https://doi.org/10.1016/S0079-6123(06)62024-4.

Leon, L. R., M. D. Blaha, and D. A. DuBose. 2006. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. J. Appl. Physiol. 100:1400–1409. https://doi.org/10.1152/japplphysiol.01040.2005.

Liu, F., J. J. Cottrell, J. B. Furness, L. R. Rivera, F. W. Kelly, U. Wijesiriwardana, R. V. Pustovit, L. J. Fothergill, D. M. Bravo, P. Celi, B. J. Leury, N. K. Gabler, and F. R. Dunshea. 2016. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. Exp. Physiol. 101:801–810. https://doi.org/10.1113/EP085746.

Ma, B., L. Zhang, J. Li, T. Xing, Y. Jiang, and F. Gao. 2021. Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. Poult. Sci. 100:215–223. https://doi.org/10.1016/j.psj.2020.09.090.

Ma, F. T., Q. Shan, Y. H. Jin, D. Gao, H. Y. Li, M. N. Chang, and P. Sun. 2020. Effect of *Lonicera japonica* extract on lactation performance, antioxidant status, and endocrine and immune function in heat-stressed mid-lactation dairy cows. J. Dairy Sci. 103:10074–10082. https://doi.org/10.3168/jds.2020-18504.

Madkour, M., M. M. Aboelenin, E. Younis, M. A. Mohamed, H. Hassan, M. Alagawany, and M. Shourrap. 2020. Hepatic acute-phase

response, antioxidant biomarkers and DNA fragmentation of two rabbit breeds subjected to acute heat stress. Ital. J. Anim. Sci. 19:1568–1576. https://doi.org/10.1080/1828051X.2020.1861993.

Mayorga, E. J., J. W. Ross, A. F. Keating, R. P. Rhoads, and L. H. Baumgard. 2020. Biology of heat stress; the nexus between intestinal hyperpermeability and swine reproduction. Theriogenology 154:73–83. https://doi.org/10.1016/j.theriogenology.2020.05.023.

Mineo, H., Y. Hashizume, Y. Hanaki, K. Murata, H. Maeda, T. Onaga, S. Kato, and N. Yanaihara. 1994. Chemical specificity of short-chain fatty acids in stimulating insulin and glucagon secretion in sheep. Am. J. Physiol. Endocrinol. Metab. 267:E234–E241. https://doi.org/10.1152/ajpendo.1994.267.2.E234.

Nair, K. S. 1987. Hyperglucagonemia increases resting metabolic rate in man during insulin deficiency. J. Clin. Endocrinol. Metab. 64:896–901. https://doi.org/10.1210/jcem-64-5-896.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Nat. Acad. Press.

Oliver, S. R., N. A. Phillips, V. L. Novosad, M. P. Bakos, E. E. Talbert, and T. L. Clanton. 2012. Hyperthermia induces injury to the intestinal mucosa in the mouse: Evidence for an oxidative stress mechanism. Am. J. Physiol. Regul. Integr. Comp. Physiol. 302:R845–R853. https://doi.org/10.1152/ajpregu.00595.2011.

Opgenorth, J., M. Abuajamieh, E. A. Horst, S. K. Kvidera, J. S. Johnson, E. J. Mayorga, M. V. Sanz-Fernandez, M. A. Al-Qaisi, J. M. DeFrain, D. H. Kleinschmit, P. J. Gorden, and L. H. Baumgard. 2021. The effects of zinc amino acid complex on biomarkers of gut integrity, inflammation, and metabolism in heat-stressed ruminants. J. Dairy Sci. 104:2410–2421. https://doi.org/10.3168/jds.2020-18909.

Ortega, A. D. S. V., and C. Szabó. 2021. Adverse effects of heat stress on the intestinal integrity and function of pigs and the mitigation capacity of dietary antioxidants: A review. Animals (Basel) 11:1135. https://doi.org/10.3390/ani11041135.

Pate, R. T., D. Luchini, J. P. Cant, L. H. Baumgard, and F. C. Cardoso. 2021. Immune and metabolic effects of rumen-protected methionine during a heat stress challenge in lactating Holstein cows. J. Anim. Sci. 99:skab323. https://doi.org/10.1093/jas/skab323.

Pearce, S. C., V. Mani, R. L. Boddicker, J. S. Johnson, T. E. Weber, J. W. Ross, R. P. Rhoads, L. H. Baumgard, and N. K. Gabler. 2013. Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. PLoS One 8:e70215. https://doi.org/10.1371/journal.pone.0070215.

Pereira, A. M. F., F. Baccari Jr., E. A. L. Titto, and J. A. A. Almeida. 2008. Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in Alentejana, Mertolenga, Frisian and Limousine cattle breeds. Int. J. Biometeorol. 52:199–208. https://doi.org/10.1007/s00484-007-0111-x.

Persson Waller, K., C. Hallén Sandgren, U. Emanuelson, and S. K. Jensen. 2007. Supplementation of RRR-alpha-tocopheryl acetate to periparturient dairy cows in commercial herds with high mastitis incidence. J. Dairy Sci. 90:3640–3646. https://doi.org/10.3168/jds.2006-421.

Pop, F., and L. Mihalescu. 2017. Effects of α-tocopherol and citric acid on the oxidative stability of alimentary poultry fats during storage at low temperatures. Int. J. Food Prop. 20:1085–1096. https://doi.org/10.1080/10942912.2016.1199037.

Puthpongsiriporn, U., S. E. Scheideler, J. L. Sell, and M. M. Beck. 2001. Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult. Sci. 80:1190–1200. https://doi.org/10.1093/ps/80.8.1190.

Rahman, Z., D. K. Dwivedi, and G. B. Jena. 2020. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum. Exp. Toxicol. 39:547–562. https://doi.org/10.1177/0960327119895559.

Rao, R. K., S. Basuroy, V. U. Rao, K. J. Karnaky Jr., and A. Gupta. 2002. Tyrosine phosphorylation and dissociation of occluding—ZO-1 and E-cadherin—β-catenin complexes from the cytoskeleton by oxidative stress. Biochem. J. 368:471–481. https://doi.org/10.1042/bj20011804.

Rothman, K. J. 1990. No adjustments are needed for multiple comparisons. Epidemiology 1:43–46. https://doi.org/10.1097/00001648-199001000-00010.

Sahin, K., N. Sahin, and M. Onderci. 2002. Vitamin E supplementation can alleviate negative effects of heat stress on egg production, egg quality, digestibility of nutrients and egg yolk mineral concentrations of Japanese quails. Res. Vet. Sci. 73:307–312. https://doi.org/10.1016/S0034-5288(02)00126-1.

Sano, H., N. Hattori, Y. Todome, J. Tsuruoka, H. Takahashi, and Y. Terashima. 1993. Plasma insulin and glucagon responses to intravenous infusion of propionate and their autonomic control in sheep. J. Anim. Sci. 71:3414–3422. https://doi.org/10.2527/1993.71123414x.

Sanz Fernandez, M. V., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. C. Isom, R. P. Rhoads, and L. H. Baumgard. 2015a. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. Physiol. Rep. 3:e12315. https://doi.org/10.14814/phy2.12315.

Sanz Fernandez, M. V., S. K. Stoakes, M. Abuajamieh, J. T. Seibert, J. S. Johnson, E. A. Horst, R. P. Rhoads, and L. H. Baumgard. 2015b. Heat stress increases insulin sensitivity in pigs. Physiol. Rep. 3:e12478. https://doi.org/10.14814/phy2.12478.

Scheiermann, C., P. S. Frenette, and A. Hidalgo. 2015. Regulation of leucocyte homeostasis in the circulation. Cardiovasc. Res. 107:340–351. https://doi.org/10.1093/cvr/cvv099.

Seitz, H. J., W. Krone, H. Wilke, W. Tarnowski, D. Carsten, B. Dunkelmann, and A. Harneit. 1981. Rapid rise in plasma glucagon induced by acute cold exposure in man and rat. Pflugers Arch. 389:115–120. https://doi.org/10.1007/BF00582100.

Silva, J. E. 2006. Thermogenic mechanisms and their hormonal regulation. Physiol. Rev. 86:435–464. https://doi.org/10.1152/physrev.00009.2005.

Sotler, R., B. Poljsak, R. Dahmane, T. Jukic, D. P. Jukic, C. Rotim, P. Trebse, and A. Starc. 2019. Prooxidant activities of antioxidants and their impact on health. Acta Clin. Croat. 58:726–736. https://doi.org/10.20471/acc.2019.58.04.20.

Sun, L. L., S. T. Gao, K. Wang, J. C. Xu, M. V. Sanz-Fernandez, L. H. Baumgard, and D. P. Bu. 2019. Effects of source on bioavailability of selenium, antioxidant status, and performance in lactating dairy cows during oxidative stress-inducing conditions. J. Dairy Sci. 102:311–319. https://doi.org/10.3168/jds.2018-14974.

Tang, S., J. Yu, M. Zhang, and E. Bao. 2013. Effects of different heat stress periods on various blood and meat quality parameters in young Arbor Acer broiler chickens. Can. J. Anim. Sci. 93:453–460. https://doi.org/10.4141/cjas2013-041.

Tatár, P., M. Vigaš, J. Jurčovičová, R. Kvetňanský, and V. Štrec. 1986. Increased glucagon secretion during hyperthermia in a sauna. Eur. J. Appl. Physiol. Occup. Physiol. 55:315–317. https://doi.org/10.1007/BF02343805.

Vázquez-Añón, M., J. Nocek, G. Bowman, T. Hampton, C. Atwell, P. Vázquez, and T. Jenkins. 2008. Effects of feeding a dietary antioxidant in diets with oxidized fat on lactation performance and antioxidant status of the cow. J. Dairy Sci. 91:3165–3172. https://doi.org/10.3168/jds.2007-0737.

Vicini, J. L., W. S. Cohick, J. H. Clark, S. N. McCutcheon, and D. E. Bauman. 1988. Effects of feed intake and sodium bicarbonate on milk production and concentrations of hormones and metabolites in plasma of cows. J. Dairy Sci. 71:1232–1238. https://doi.org/10.3168/jds.S0022-0302(88)79678-2.

Walrand, S., C. Guillet, Y. Boirie, and M. P. Vasson. 2004. In vivo evidences that insulin regulates human polymorphonuclear neutrophil functions. J. Leukoc. Biol. 76:1104–1110. https://doi.org/10.1189/jlb.0104050.

Wang, Y. M., J. H. Wang, C. Wang, B. Chen, J. X. Liu, H. Cao, F. C. Guo, and M. Vázquez-Añón. 2010. Effect of different rumen-inert fatty acids supplemented with a dietary antioxidant on performance and antioxidative status of early-lactation cows. J. Dairy Sci. 93:3738–3745. https://doi.org/10.3168/jds.2010-3054.

Weiser, P. C., and F. Grande. 1974. Calorigenic effects of glucagon and epinephrine in anesthetized dogs. Exp. Biol. Med. (Maywood) 145:912–917. https://doi.org/10.3181/00379727-145-37923.

Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644–655. https://doi.org/10.3168/jds.2009-2295.

Xu, C., R. Sun, X. Qiao, C. Xu, X. Shang, W. Niu, and Y. Chao. 2014. Effect of vitamin E supplementation on intestinal barrier function in rats exposed to high altitude hypoxia environment. Korean J. Physiol. Pharmacol. 18:313–320. https://doi.org/10.4196/kjpp.2014.18.4.313.

Xu, W., J. Lu, Y. Chen, Z. Wang, J. Cao, and Y. Dong. 2021. Impairment of CRH in the intestinal mucosal epithelial barrier of pregnant Bama miniature pig induced by restraint stress. Endocr. J. 68:485–502. https://doi.org/10.1507/endocrj.EJ20-0332.

Zarrin, M., O. Wellnitz, H. A. van Dorland, J. J. Gross, and R. M. Bruckmaier. 2014. Hyperketonemia during lipopolysaccharide-induced mastitis affects systemic and local intramammary metabolism in dairy cows. J. Dairy Sci. 97:3531–3541. https://doi.org/10.3168/jds.2013-7480.

Zhao, S., Z. Chen, J. Zheng, J. Dai, W. Ou, W. Xu, Q. Ai, W. Zhang, J. Niu, K. Mai, and Y. Zhang. 2019. Citric acid mitigates soybean meal induced inflammatory response and tight junction disruption by altering TLR signal transduction in the intestine of turbot, *Scophthalmus maximus*. Fish Shellfish Immunol. 92:181–187. https://doi.org/10.1016/j.fsi.2019.06.004.

ORCIDS

M. A. Abeyta https://orcid.org/0000-0002-1625-5310
M. Al-Qaisi https://orcid.org/0000-0002-0020-3428
E. J. Mayorga https://orcid.org/0000-0001-7673-5191
S. Carta https://orcid.org/0000-0002-5778-2627
H. Tucker https://orcid.org/0000-0001-6375-4465
L. H. Baumgard https://orcid.org/0000-0002-3077-5996