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Short Communication

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Effects of an Electrolyte, Energy and Osmolyte Compound and Heat Stress on Productivity in Early Lactation Holstein Cows

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Abstract

Heat stress (**HS**) affects milk production and intake of dairy cattle. Strategies to mitigate the effects of HS suggest dietary supplements including electrolyte, energy and osmolyte compounds (such as Bovine BlueLite, TechMix, Stewart, MN, which is a blend of these compounds), but direct and long-term evaluation of this supplement in lactating cows is needed. We hypothesized that the supplement would improve productivity of dairy cows (~52 DIM) under HS. Cows (n = 52) were enrolled into a 4-wk 2x2 factorial arrangement as follows; 1) heat stress (HT-CON, n = 13), 2) heat stress plus supplement (HT-SUP, n = 13), 3) cooling (CL-CON, n = 13) and 4) cooling plus supplement (CL-SUP, n = 13). Cows were housed in a free-stall barn. Supplement treatment was top dressed on TMR (56.5g/cow in the morning and 56.5g/cow in the afternoon feeding). Evaporative cooling system was provided to CL cows whereas HT cows only had shade. Milk yield, DMI, rectal temperature (**RT**), and respiration rate (**RR)** of individual cows were recorded during the study.

Furthermore, milk contents of fat, protein and lactose were measured weekly. Heat stress increased RR and RT relative to CL. Heat stress reduced DMI (HT = 20.3, CL = 24.5 ± 0.4 kg/d), and SUP reversed some of the effects in HT cows (HT-SUP = 20.9, HT-CON = 19.6 ± 0.5 kg/d) but did not affect CL cows. ECM yield was also decreased by HT (HT = 38.1, CL = 42.6 ± 0.8 kg/d), and SUP partially reversed that effect in HT-SUP relative to HT-CON (HT-SUP = 39.6, HT-CON = 36.5 ± 1.1 kg/d). No main effects were observed for milk components, cortisol or HSP plasma concentration. Results suggest that SUP partially mitigates the impact of HT on DMI and ECM in lactating dairy cows under HT.

Heat stress (**HS**) is a major issue for animal production especially in tropical and subtropical areas and is increasing due to global warming (Chen et al., 2021). Homeotherms need to release metabolic heat to the environment to maintain normal body core temperature. When heat production exceeds heat dissipation, the ability to release heat is limited, leading to heat stress which affects metabolism and performance (Collier et al., 2006). Humidity is a major contributor to heat stress; therefore, the temperature-humidity index (**THI**) has been considered a more accurate tool to determine the heat stress load of livestock in a confinement setting (Collier et al., 2006). In dairy cattle, the threshold THI associated with production losses is as low as 64 (Ji et al., 2020). All southern regions of the US have high average temperatures, and in Florida, THI levels easily surpass the 64 THI threshold even during the coldest months of the year.

Negative effects due to exposure of dairy cattle to heat stress include reduced DMI, lower milk yield, body condition loss, and reduced feed efficiency, resulting in significant economic losses (Brown-Brandl, 2018). Reduction of DMI is an adaptive mechanism to minimize heat production from feed fermentation and metabolism (Chang-Fung-Martel et al., 2021). Moreover, HS induces a reduction in peristalsis in the digestive system that delays digestive function and feed metabolism, and thus, feed intake (Chen et al., 2021). As a result, HS negatively impacts milk yield, thereby representing a major issue for dairy producers. Indeed, milk production losses can exceed 30% under severe HS and milk yield declines an average of 0.2 kg for every THI unit increment once threshold is overpassed (West, 2003).

In an attempt to mitigate HS, cooling systems that include shade, fans and soakers have been deployed heavily in the dairy industry. These systems prevent drops in cow's feed intake and milk yield especially when THI exceeds 68 (Chang-Fung-Martel et al., 2021). Additionally, dietary strategies have been employed to mitigate the negative impacts of HS, with differing levels of effectiveness. These approaches are oriented to support water balance, ensure adequate intake of nutrients and electrolytes, and address specific nutritional requirements during periods of HS, such as increased needs for vitamins and minerals (Renaudeau et al., 2012). Supplementation with products containing electrolytes, osmolytes, and energetic compounds have shown to have potential metabolic support in lactating cattle under HS (Al-Qaisi et al., 2020). Therefore, we hypothesized that supplementation of electrolyte and energetic compounds (BlueLite pellets, TechMix, Stewart, Minnesota) during extended heat stress of early lactation cows will reduce the negative effects on productive parameters and circulating indicators of heat stress.

Our study was conducted during the summer of 2022 at the University of Florida Dairy Unit with the approval of all procedures by the Institutional Animal Care and Use Committee (IACUC). A total of 52 lactating Holstein cows with an average of 52 d in milk (DIM) (SD = 28 d) were enrolled in a 2x2 factorial arrangement in a completely randomized design yielding 4 groups of 13 cows each with individual cow as the experimental unit. Each cow was randomly assigned to one of the combinations of environment (heat stress (**HT**) vs cooling (**CL**)) and supplement (supplement (**SUP**) vs control (**CON**)) treatments as the main factors as follows: 1) Heat stress-Control (HT-CON), 2) Heat stress-Supplement (HT-SUP), 3) Cooling-Control (CL-CON), and 4) Cooling-Supplement (CL-SUP) during a period of 28 d, balancing each group based on their PTA for milk yield, DIM and parity. The CL treatment consisted of shade, fans (located ~3 m on top of beds line and activated automatically at ~20°C. Wind speeds from fans was ~3.6 km/h) and soakers (located on top of feed line and activated automatically at ~20°C for cycles of 30 s on and 5 min off and 1 min on and 5 min off after ~24°C) whereas the HT treatment was only shade, and the supplement was offered top-dressed with the total mixed ration (TMR) at a rate of 113 g/d (per manufacturer recommendation) divided in 56.5 g at morning (0800 h) and afternoon (1300 h) feedings for the SUP cows whereas the CON cows did not receive any supplementation or carrier. The basal TMR consisted of (%, DM basis): corn silage, 37.3; oat silage, 3.9; ground corn grain, 23.2; soybean meal, 9.8; AminoPlus (Ag Processing Inc., Omaha, Nebraska), 6.0; fresh cow mineral, 5.5; Nurisol (Global Agri-Trade, Rancho, California), 1.0; citrus pulp, 9.4 and a molasses, 1.9. The TMR had the following calculated nutrient analysis (DM basis): Crude protein, 15.71%; M.E. allowable milk 43.5 kg/d; aNDFom, 21.29%; starch, 31.47%; ether extract, 3.62% and DCAD, 226.99 mEq/kg. Additionally, the ingredients of the supplement include electrolytes (Na, K, Cl, S, Mg, Ca), vitamins (B, A, D, E), energetic compounds (dextrose and sucrose) and an osmolyte (betaine). The calculated analysis of the supplement shows a DCAD of 58.36 mEq/kg. The study was conducted during the summer when THI was on average 77.2 ± 1.3, a THI that induces HS in cows in the absence of fans and soakers. Cows were housed in a free stall barn equipped with automatic individually activated gates for each feeder (Calan Gate System) to measure individual feed intake. Cows were fed ad libitum calculating their individual feed intake by offering feed in the morning and afternoon and measuring residual feed after 24 h relative to the morning feeding. Milk yield was recorded throughout the study period after each morning and evening milking. In addition, milk samples were collected weekly from consecutive morning and evening milkings from each cow, and milk component analysis for fat, protein, and lactose as well as MUN and SCC was subsequently performed at Southeast Milk, Inc. Laboratory (Belleview, Florida). Milk and component values were then converted to ECM using the following formula: ECM, kg/d = [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)] and SCC were transformed to SCS as follows SCS = [Log10(SCC/12.5)] / Log10(2).

Rectal temperature (**RT**) was measured using a digital thermometer (Pavia rectal temp, Pavia Sales

Group Inc., Minnetonka, MN) and respiration rate (**RR**) was measured by counting thoracic movements for one minute. Vitals were assessed every 2 d in all cows at 1400 h to confirm the effect of heat stress.

In addition, a single blood sample was collected from a subset of 40 cows (10 cows randomly selected per treatment group) via the coccygeal venipuncture on d 0, 7, 14, and 28 relative to the beginning of the treatments with all collections performed at 0600 h, using heparinized vacutainers (BD Vacutainer heparin tubes, Franklin Lakes, NJ). After collection, blood samples were centrifuged at 1,006g for 15 min at 4°C, and plasma was separated and stored at −80°C until analysis of cortisol and heat shock protein (**HSP**). Cortisol concentration in samples was measured using an enzyme-linked immunosorbent assay (**ELISA**) commercial kit with an intra- and inter-assay coefficient of variation of 8.33 and 8.78% respectively (K003-H1/H5, DetectX Cortisol, Arbor Assays, Ann Arbor, Michigan) and HSP70 concentration in samples was measured using an ELISA commercial kit for cattle with an intra- and inter-assay coefficient of variation of 4.82 and 18.72% respectively (SEA873Mi, Cloud-clone Corp. Katy, Texas).

Data collected were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model considers fixed effects of environment (HT vs CL), supplement (SUP vs CON), time, environment by supplement interaction and environment by supplement by time interaction and the random effect of cow nested withing treatment. PTA for milk yield was included as a covariate in the analysis for DMI, milk yield and milk components; however, it was not significant, therefore it was removed. In addition, values of one week before starting treatments were included as covariates for DMI, milk yield, milk components, cortisol and HSP. Post hoc pairwise comparisons were performed using the Tukey-Kramer adjustment. Finally, residuals were tested for normality using the plots = studentpanel option in SAS. Statistical significance was considered when $P < 0.05$ and tendency when $P < 0.10$.

Respiration rate showed an environment by supplement interaction ($P = 0.04$) where SUP reduced RR in cows under HT but did not affect RR of cows under CL (Table 1). Moreover, main effects of environment showed that RR was higher for HT cows relative to CL cows (92.6 ± 1.0 vs. 82.2 ± 1.0, breaths/minute; $P < 0.01$) whereas main effect of supplement showed that SUP tended to reduce respiration rate relative to CON cows (86.0 ± 1.0 vs. 88.9 ± 1.0, breaths/minute; $P = 0.06$). A similar pattern was observed for RT (°C) where a significant environment by supplement interaction was observed ($P = 0.01$); SUP reduced RT in cows under HT but did not affect RT in cows under CL (Table 1). An environment main effect was detected where HT cows had a higher RT than CL cows (39.83 ± 0.07 vs. 38.80 ± 0.07°C; $P < 0.01$) yet there was no main effect of supplement (39.30 ± 0.07 vs. 39.34 ± 0.07°C; $P = 0.74$).

Table 1. Effects of environment (CL or HT) and supplement (SUP or CON) on respiration rate, rectal temperature (HT-CON, n = 13; HT-SUP, n = 13; CL-CON, n = 13; and CL-SUP, n = 13) and blood cortisol

and blood HSP (CL-CON, n = 8; CL-SUP, n = 7; HT-CON, n = 10; and HT-SUP n = 9). including *P*-values for main effects of environment, supplement and environment by supplement interaction

	Treatments				**SEM**	***P* -value**		
	CL-CON	**CL-SUP**	**HT-CON**	**HT-SUP**		**Enviro**[1]	**Supple**[2]	**Enviro x Supple**
Respiration rate, (breaths/min)	82.1^{b}	82.4^{b}	95.6^{a}	89.7^{c}	1.4	<0.01	0.06	0.04
Rectal temperature, °C	38.70^{b}	38.92^{b}	39.98^{a}	39.69^{c}	0.10	<0.01	0.74	0.01
Cortisol, ng/mL [3]	10.99	8.42	8.67	11.01	1.07	0.90	0.91	0.03
HSP, ng/mL	74.75	76.40	77.70	75.76	10.7	0.91	0.99	0.86

[1] Main effect of environment (CL or HT).

[2] Main effect of supplement (SUP or CON).

[3] Despite the environment by supplement interaction, post hoc analyses indicated that after Tukey-Kramer adjustment of pairwise differences, treatment combinations were not different.

[a–c] LSM values in the same row with different superscripts are different ($P < 0.05$). Comparisons were performed follow the Tukey-Kramer adjustment.

For DMI, a significant environment by supplement interaction was observed ($P = 0.05$) (Table 2), yet pairwise comparison after Tukey adjustment indicated that differences were driven by the main effect of environment as HT cows had decreased DMI compared with CL cows (20.3 ± 0.4 vs. 24.5 ± 0.4; kg/d SEM; $P < 0.01$) and no main effect of supplement was observed (22.5 ± 0.4 vs. 22.3 ± 0.4; kg/d SEM; $P = 0.60$).

Table 2. Effects of environment (CL or HT) and supplement (SUP or no SUP) on, DMI, Milk yield, ECM, Milk components % and yield (HT-CON, n = 13; HT-SUP, n = 13; CL-CON, n = 13; and CL-SUP, n = 13) including *P*-values for main effects of environment, supplement and environment by supplement interaction

Response	**Treatments**				**SEM**	***P* -value**		
	CL-CON	**CL-SUP**	**HT-CON**	**HT-SUP**		**Enviro**[1]	**Supple**[2]	**Enviro x Supple**

DMI, kg/d[3]	24.9	24.1	19.6	20.9	0.5	<0.01	0.60	0.05
Milk yield, kg/d	40.4	40.6	35.0	37.7	1.1	<0.01	0.21	0.26
ECM, kg/d	42.6	42.6	36.5	39.6	1.1	<0.01	0.15	0.17
Efficiency, milk yield/DMI	1.59	1.71	1.87	1.79	0.07	<0.01	0.73	0.12
Efficiency, ECM/DMI	1.70	1.79	1.91	1.89	0.06	0.01	0.65	0.36
Milk components, %								
Fat [3]	3.99	3.79	3.81	3.92	0.09	0.56	0.49	0.05
Protein	3.30	3.32	3.18	3.29	0.06	0.20	0.23	0.48
Lactose	4.85	4.84	4.93	4.85	0.05	0.41	0.56	0.52
MUN, ng/dL	13.9	14.1	14.6	14.6	0.38	0.09	0.75	0.73
SCS	3.39	3.36	2.75	2.54	0.28	<0.01	0.74	0.66
Milk components yield, kg/d								
Fat	1.63[a]	1.52[ab]	1.32[b]	1.47[ab]	0.06	<0.01	0.81	0.05
Protein	1.41	1.40	1.17	1.30	0.06	<0.01	0.27	0.17
Lactose	1.97	1.97	1.74	1.81	0.09	0.03	0.66	0.64

[1] Main effect of environment (CL or HT).

[2] Main effect of supplement (SUP or CON).

[3] Despite the environment by supplement interaction, post hoc analyses indicated that after Tukey adjustment of pairwise differences, treatment combinations were not different.

[a–b] LSM values in the same row with different superscripts differ ($P < 0.05$) for the Environment x Supplement interaction. Comparisons were performed following the Tukey-Kramer adjustment.

As expected, the main effect of environment was observed where HT decreased milk yield relative to CL cows (36.4 ± 0.8 vs. 40.5 ± 0.8; $P < 0.01$). Supplement or the interaction were not significant ($P = 0.21$ and $P = 0.26$ respectively). Milk yield was converted to ECM and cows under HT produced significatively less ECM than cows in the CL group (38.1 ± 1.8 vs. 42.6 ± 1.8; $P < 0.01$). Supplement and environment by supplement interaction were not significant ($P = 0.15$ and $P = 0.17$). A triple interaction (environment by supplement by week interaction) was observed for ECM ($P = 0.02$) where the slice by week function with Tukey adjustment showed that when comparing HT-SUP vs HT-CON, the HT-SUP combination tended to have an increased ECM in wk 4 ($P = 0.09$) relative to HT-CON (Figure 1).

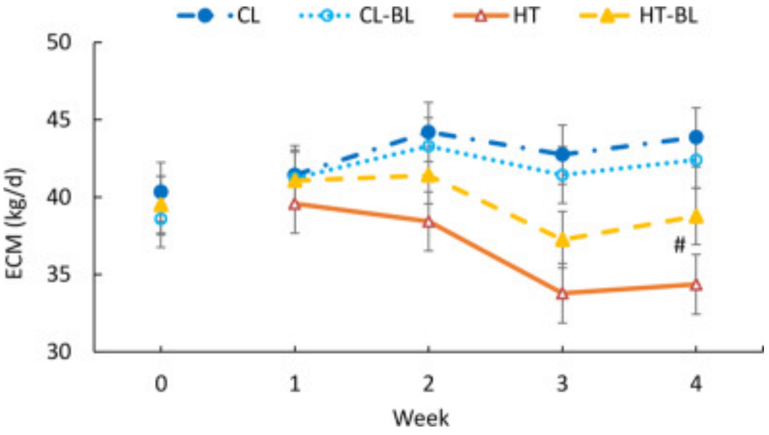

Figure 1. Energy corrected milk of cows exposed to cooling system alone (CL-CON, solid circles with dash dotted dark-blue line), cooling system and supplement (CL-SUP, open circles with dotted light-blue line), heat stress alone (HT-CON, open triangles with solid orange line) and heat stress and supplement (HT-SUP, solid triangle with continues line) (HT-CON, n = 13; HT-SUP, n = 13; CL-CON, n = 13; and CL-SUP, n = 13). Effect of environment ($P < 0.01$), supplement ($P = 0.15$), environment by supplement interaction ($P = 0.17$) and environment by supplement by time interaction ($P = 0.02$). Week 0 measurements were taken before starting treatments and were included in the model as covariate. The SLICE function of SAS was utilized to analyze individual week pairwise differences with Tukey adjustment. # $P \leq 0.10$.

Feed efficiency was calculated by dividing milk yield by DMI, where HT cows showed higher feed efficiency compared with CL (1.83 ± 0.05 vs. 1.65 ± 0.05; $P < 0.01$), however supplement was not significant ($P = 0.73$). In addition, feed efficiency calculated with ECM was also increased by HT relative to CL (1.90 ± 0.04 vs. 1.74 ± 0.04; $P = 0.01$), nonetheless, supplement was not significant ($P = 0.65$). In both, feed efficiency calculated by milk yield and by ECM, there was no environment by supplement interaction (Table 2). Milk components including the percentage of fat, protein, and lactose were not affected by the main effects of environment nor supplement ($P > 0.10$), however an environment by supplement interaction was found for fat percentage ($P = 0.05$; Table 2). Despite this interaction, pairwise comparisons adjusted by Tukey-Kramer did not reach statistical significance.

HT tended to increase MUN ($P = 0.09$) while SCS was lower ($P < 0.01$) in HT cows relative to CL and no supplement nor interaction effects were observed for MUN or SCS (Table 2). Milk component yields were lowered by HT compared with CL (i.e., lactose, $P < 0.01$; protein, $P < 0.01$; and fat, $P = 0.03$; kg/d), no supplement effect was observed, and only fat yield showed an environment by supplement interaction ($P = 0.05$; Table 2). Post hoc comparisons using the Tukey-Kramer adjustment identified a significant difference only between CL-CON and HT-CON ($P = 0.008$).

Plasma cortisol and HSP concentration from a subset of cows was evaluated. Initially, samples from 10 cows of each group were randomly selected for cortisol and HSP analysis, however, some samples were discarded due to hemolysis resulting in sample number as follows: CL-CON, n = 8; CL-SUP, n = 7; HT-CON, n = 10; and HT-SUP, n = 9. In addition, values from d 0 were included in the model as covariate. Cortisol showed an environment by supplement interaction ($P = 0.03$); however, Tukey's adjusted pairwise comparisons showed no significant differences among main factor levels combinations. Additionally, there was no environment ($P = 0.90$) nor supplementation effects ($P = 0.90$; Table 1) for cortisol. HSP was not influenced by environment ($P = 0.91$), supplement ($P = 0.99$) nor was an interaction present ($P = 0.86$; Table 1).

Average THI in the barn during the experiment was 77.2 ± 1.3, which is expected to induce HS, especially in the absence of an effective evaporative cooling system. A THI of 64 is considered the borderline between thermoneutral zone and heat stress for lactating cows producing 31 kg of milk or more (Ji et al., 2020). RR and RT increased in HT cows due to an imbalance between heat production and dissipation, reflecting that cows carry more heat load. However, the environment by supplement interaction for RR and RT shows that supplementation improved RR and RT in the HT cows. This effect may be attributed to enhanced fluid retention and heat exchange, likely driven by betaine's role in maintaining intracellular fluid balance (Abhijith et al., 2024).

Because of HT, cows reduced their DMI by 4.2 kg/d compared with CL cows. When under HS, the cooling center located in the hypothalamus signals the appetite center to decrease intake resulting in lower heat production from fermentation (Albright and Alliston, 1971). Consequently, milk yield, ECM and milk components yield in HT cows also decreased, due to both lower intake and negative effects of HS on mammary epithelial cells (i.e., increased apoptosis rate) due to HS (Tao et al., 2018) as shown in the HT cows where they produced 4.5 kg of ECM less than the CL group. Collier et al. (1982) showed that HS decreases sodium (Na) and potassium (K) concentration in the rumen, moreover, sweating and panting induced by HS causes loss of electrolytes further disrupting electrolyte balance. Indeed, supplementation of electrolyte compounds have demonstrated to reduce negative effects of HS on milk production as reported by West (2003). Betaine is an osmolyte compound that contributes to maintain intracellular electrolyte and fluid balance in many species including cattle (Abhijith et al., 2024). BlueLite is a combination of energy, osmolyte and electrolyte compounds including betaine, K and Na, and thus is expected that its supplementation ameliorates HT effects like in previous studies where individual components were tested (Collier et al., 1982; Abhijith et al., 2024). The present study clearly shows the negative effects of HT on DMI and ECM compared with CL cows. Although supplementation did not fully reverse the effects of HT to the same level of CL cows, supplement did ameliorate a portion of the negative effects of HT on ECM in the fourth week of the experiment. The impact of supplementation on ECM in HT-SUP cows relative to HT-CON cows may be explained by the replacement of lost dietary electrolytes, the contribution of the supplement to an increased DCAD, plus the positive effects of betaine may all help maintain electrolyte balance. Al-Qaisi et al. (2020) did not observe difference on DMI and milk yield by this supplement, however, in their trial cows were only exposed to a few days of HS. Conversely, in our experiment cows were exposed to HS for 4 weeks which allows for analysis of long-term effects of the supplement. Indeed, relative to HT-CON cows, HT-SUP cows benefited from SUP as the trial advanced, which indicates that benefits of the supplement might increase as the heat stress insult lengthens although supplementation does not allow for full replacement of active cooling conditions.

Feed efficiency is a key indicator of the effectiveness with which dairy cows convert feed into milk. We calculated efficiency based on milk yield and ECM and found an increase in feed efficiency in HT cows compared with CL, but supplement did not show any significant effect. Our results match with Hill and Wall (2017) study, where they collected a large data set from cows at different THI levels and found that as THI increased, feed efficiency also increased. However, a meta-analysis by Chen et al. (2024) concluded that feed efficiency did not change due to HS. In our study, as opposed to Chen et al. (2024) but similar to Hill and Wall (2017), we enrolled early-lactation cows, which are known to mobilize body energy reserves to meet the increasing demand of milk production (Bernabucci et al., 2010). Consequently, in early lactation, a reduction in feed intake induces greater body energy mobilization to maintain milk yield, thereby resulting in an apparent increase in feed efficiency. Moreover, the composition of the supplement includes energy components such as dextrose, sucrose and fructose. However, the supplement was administered at a rate of 113 g/cow/d, which is insufficient to offset the reduction in TMR intake induced by HT (4.2 kg/d reduction), therefore not significantly impacting feed efficiency.

The percentage of milk components (i.e., fat, protein and lactose) were not affected by the main effects of environment nor supplement. Although an interaction was observed for milk fat, Tukey-Kramer adjusted pairwise comparisons did not reach statistical significance suggesting high variability likely due to insufficient samples size to detect differences in this variable. Studies evaluating milk component changes due to HS have yielded variable results. Al-Qaisi et al. (2020b) found an increase in milk fat percentage due to HS whereas (Bouraoui et al., 2002) reported decreased milk fat percentage during hotter months. Similarly, some studies indicate that milk protein percentage is reduced due to lower nutrient intake, thereby limiting milk protein synthesis under HS (Bernabucci et al., 2010; Al-Qaisi et al. 2020b), but like milk fat, differences in our study were not observed. In general, lactose percentage suffers little if any change with heat stress or other dietary manipulation, which is consistent with its function as the osmotic regulation of milk secretion (Al-Qaisi et al. 2020b; Cowley et al., 2015). No differences of milk components have consistently been found with electrolyte supplementation when under HS (Al-Qaisi et al., 2020; West 2003; Cabrera, 2014) which aligns with our findings. More research is needed to clarify the variability in fat and protein percentage under HS and supplementation with products with similar composition.

Milk fat yield also showed an environment by supplement interaction. Pairwise comparisons after Tukey adjustment only indicated differences between CL-CON and HT-CON reinforcing the negative effects of HT. The lack of difference between HT-SUP cows and both groups of CL cows suggests that supplementation under heat stress conditions might help maintain fat yield similar to that of cows under cooling conditions which also aligns with the observed improvement in ECM.

HT tended to increase MUN, potentially due to lower DMI which limits protein supply; additionally, studies have shown that plasma urea nitrogen also increases under HS, indicating muscle protein breakdown as a compensatory response to decreased protein intake (Bernabucci et al., 2010). Furthermore, in contrast to expected, HT decreased SCS relative CL. Different results are found on SCC and SCS across literature due to HS. Al-Qaisi et al. (2020a) found an increase in SCC while Al-Qaisi et al. (2020b) did not observe such differences due to HS. Similar to ours, earlier research that included a large data set also found that HS decreased SCS (Smith et al., 2013). More research is needed to clarify the effects of HS on SCC. Additionally, as in Al-Qaisi et al. (2020) study, no effect of supplementation was detected in our study.

Cortisol showed an environment by supplement interaction (Table 1); however, Tukey adjusted pairwise comparison showed no significant differences which might suggest high variability. Studies in beef (Kim et al., 2022) and dairy cattle (Marins et al., 2021) have shown that acute HS increases plasma cortisol concentrations, and animals exhibit a decrease in cortisol pattern back toward normal as they adapt to chronic HS exposure which might contribute to explain the lack of differences in cortisol since we sampled at d 7 relative to beginning of treatments when cortisol levels might have gone down after adaptation. Similarly, HSP plasma concentration is highly variable, especially in dairy breeds and within individuals of a homogeneous breed (Rakib et al., 2024). Responses of HSP to HS stimulus in in vitro studies with cell cultures seem to be more evident than in vivo studies (Collier et al., 2018). In the present study, no main effects of environment, supplement or their interactions were observed for HSP. The lack of difference in HSP plasma concentrations might be explained by the fact that even when CL cows are provided with evaporative cooling system, they still express signs of HS due to challenging environmental conditions during the summer in Florida. Indeed, the CL group showed an elevated respiration rate in the present study (i.e., ~82.2 breaths/min) relative to a normal respiration rate for lactating cows (i.e., 60 breaths/min), meaning that all cows were experiencing some level of HS likely including before the initiation of treatments which could have triggered some adaptative physiological changes that masked any increase in plasma cortisol and HSP during our study.

In the present study, environmental conditions were sufficient to induce heat stress in the absence of active cooling, as shown by the response of rectal temperature and respiration rate of HT cows, confirming the effectiveness of our HT model. Intake and milk yield were heavily compromised due to HT and the supplement partially reversed those effects of HT. Moreover, the effects of SUP become more evident after supplementation during several weeks of HT. While HT had no effect on milk components overall, SUP appeared to rescue some impacts of HT on metabolism and allow for greater milk fat percentage. Furthermore, cortisol and HSP plasma concentration were not affected by either the main effects of environment or supplement in our experiment; however, caution is warranted with the interpretation of these outcomes as some level of heat stress was observed even with active cooling.

Notes

Funding was provided by TechMix, LLC. (Stewart, Minnesota).

All animal procedures were conducted in accordance with the University of Florida institutional animal care and use committee (IACUC) with approval before beginning the experiment under the IACUC protocol # 202200000272.

N.C. Upah is an employee of TechMix, LLC. None of the remaining authors have any conflict of interest.

List of non-standard abbreviations: HS = Heat stress, HT = Heat stress treatment, CL = Cooling treatment, CON = Supplement control treatment, SUP = Supplement treatment, THI = Temperature-humidity index, RR = Respiration rate, RT = Rectal temperature, HSP = Heat shock protein, ELISA = enzyme-linked immunosorbent assay

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