J. Dairy Sci. 106:2904–2918
https://doi.org/10.3168/jds.2021-21724

Effects of dietary organic acid and pure botanical supplementation on growth performance and circulating measures of metabolic health in Holstein calves challenged by heat stress

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ABSTRACT

To evaluate the effects of heat stress environmental conditioning and dietary supplementation with organic acid and pure botanicals (OA/PB) on growth in dairy calves, we enrolled 62 bull (noncastrated) and heifer calves in a study with a completely randomized design. Calves were assigned to 1 of 5 groups (n = 11 to 14/group): (1) thermoneutral conditions (TN-Con), (2) HS conditions (HS-Con), (3) thermoneutral conditions and pair-fed to match nutrient intake with HS-Con (TN-PF), (4) HS with low-dose OA/PB [75 mg/kg of body weight (BW); 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; HS-Low], or (5) HS with high-dose OA/PB (150 mg/kg of BW; HS-High). Supplements were delivered as a twice-daily bolus via the esophagus from wk 1 through 13 of life; all calves, including those on the control treatments, received an equivalent amount of triglyceride used for microencapsulation. Calves were raised in TN conditions from birth until weaning. After weaning, calves (62 ± 2 d; 91 ± 10.9 kg of BW) were transported to a new facility and remained in TN conditions [temperature-humidity index (THI): 60 to 69] for a 7-d covariate period. Thereafter, calves remained in TN or were moved to HS conditions (THI: diurnal change 75 to 83 during night and day, respectively) for 19 d. Clinical assessments were performed thrice daily, BW was recorded weekly, and blood was sampled on d 1, 2, 3, 8, 15, and 19. Upon experiment completion, calves from HS-Con and TN-Con were euthanized, and hot carcass and visceral organ weights were recorded. The mixed model included calf as a random effect; treatment, day, hour (when appropriate) as fixed effects, and the interactions of treatment × day and treatment × hour (when appropriate). Rectal and skin temperatures and respiration rates were greater in HS-Con than in TN-Con. During heat stress exposure, dry matter intake (DMI), average daily gain (ADG), and gain to feed (G:F) were lower in HS-Con relative to TN-Con. Comparing HS-Con and TN-PF, ADG and G:F were similar. Plasma fatty acid concentrations were elevated in TN-PF compared with HS-Con and TN-Con. Despite tendencies for increased aspartate aminotransferase, HS conditions did not overtly influence liver and inflammation markers. Liver weights were lower in HS-Con relative to TN-Con. During the first week of heat exposure, DMI was greater for HS-Low relative to HS-Con. Supplementation of OA/PB at low and high levels had a similar G:F to HS-Con. We conclude that reductions in DMI accounted for production losses during HS conditioning and that dietary OA/PB supplementation was not able to improve growth performance in heat-stressed calves.
Key words: calf, heat stress, organic acid, pure botanical

INTRODUCTION

Heat stress negatively affects the health and well-being of dairy cattle, and represents a major concern for dairy production systems (West, 2003). The physiological adaptations experienced by dairy cattle in response to heat stress are characterized by decreased feed intake, increased sweating and respiration rates, and elevated body temperature (Collier et al., 1982). In dairy cows, such changes contribute to increases in maintenance energy costs that can range from 25 to 30% (Fox and Tylutki, 1998). Blood supply is redirected from the visceral organs (i.e., intestines) toward the body periphery to improve heat dissipation and maintain thermoregulation (Hall et al., 1999). This provokes paracellular permeability and tight junction opening in murine models (Lambert, 2009), which may promote

Received December 17, 2021.
Accepted October 10, 2022.
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intestinal permeability and leakage of bacteria and their endotoxin into the circulation to stimulate local and systemic immune responses (Ghosh et al., 2020). It is also important to consider that growing animals experiencing heat stress not only have increased maintenance energy requirements but do so while experiencing decreases in feed intake (Nonaka et al., 2008; O'Brien et al., 2010; Yazdi et al., 2016), which compromises ADG. Nevertheless, O'Brien et al. (2010) determined that heat-stressed Holstein bull calves experienced a 12% reduction in feed intake during heat exposure (29.4 to 40°C for a period of 9 d), which completely accounted for the decrease in ADG; however, whether this occurs when longer periods of chronic heat exposure are experienced has not been completely explored.

Dietary therapies that enhance feed conversion and ADG, potentially by increasing DMI, deserve attention in dairy calves exposed to heat stress conditions. The concept of feeding acidifiers, such as organic acids and pure botanicals (**OA/PB**), to improve gut health and growth has been explored in swine, including piglets (Roth and Kirchgessner, 1998; Partanen and Mroz, 1999). The benefits and applications of OA in nonruminants involve improving nutrient digestibility, enhancing immune function, exerting antimicrobial effects against pathogenic bacteria, and increasing growth performance (Pearlin et al., 2020). Pure botanicals (i.e., single components of plant essential oils) have antimicrobial and anti-inflammatory properties as well as antioxidant and immune-modulatory effects in poultry and swine (Rossi et al., 2020). Bonetti et al. (2020) demonstrated that the combined dietary supplementation of sorbic acid and thymol reduces the growth and expression of the virulence genes of *Escherichia coli* K88, the etiological agent of postweaning diarrhea in pigs. Interestingly, dietary OA/PB supplementation (25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 56% triglyceride matrix) promoted greater ADG and BW in weaned piglets (Grilli et al., 2015). In an independent experiment, this mixture of OA/PB also improved intestinal barrier in human Caco-2 epithelial cells (Grilli et al., 2015).

Strategies such as dietary OA/PB supplementation that modulate the intestinal microbiota, restore the intestinal barrier, and improve ADG are likely to support growth in young dairy cattle experiencing challenging conditions such as heat stress and thus warrant investigation. Therefore, our objectives were to (1) assess the impact of moderate heat exposure on the growth and metabolic parameters of weaned dairy calves, and (2) evaluate the possible role of dietary OA/PB in supporting the growth and associated metabolic health of calves experiencing heat stress.

MATERIALS AND METHODS

Experimental Design

All experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee (IACUC; protocol #2018-0110). Starting in the first week of life, 62 bull (noncastrated; n = 29) and heifer (n = 33) calves were housed and enrolled in a study through weaning at the Cornell University Dairy Research Center (Harford, NY). Calves were randomly assigned, while balanced for sex and dam parity, to the treatments at 5 d of age. For enrollment, calves had a minimum BW of 34 kg at birth and consumed 6 L of colostrum. For the duration of the study (Figure 1), calves were unsupplemented or supplemented with OA/PB at 2 levels (75 or 150 mg/kg of BW; low or high OA/PB, respectively) divided equally over 2 esophageal boluses administered at 0800 and 1700 h daily. The low level of supplementation was designed to match previously used doses in weaned piglets, in which productive responses were observed (Grilli et al., 2015), and the high level of supplementation was designed so we could evaluate whether a dose response was present. The OA/PB supplement was composed of 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride (AviPlus R; Vetagro S.p.A.). All calves, including those on control treatment, received an equivalent amount of triglyceride (vegetable lipid encapsulate; Vetagro S.p.A.). The feeding levels of OA/PB and triglyceride were adjusted weekly based on the BW of each calf. Gelatin capsules (Torpac) were utilized for bolus administration. Calves were raised in thermoneutral (**TN**) conditions from birth to weaning [mean ± SD; 18.4 ± 2.2°C; 76 ± 0.5% relative humidity (**RH**), temperature-humidity index (**THI**) of 64 ± 2]. Before weaning, calves were individually housed in pens, reared on straw, and bottle-fed (at 0600 and 1600 h) a milk replacer (i.e., 150 g/L of water) consisting of 26% CP and 20% fat at 1.75% of BW on a DM basis. Milk replacer intake was recorded at every feeding. At d 42 of life, milk replacer intake was reduced by half and terminated on d 49. Starter grain (mean ± SD; 26.8 ± 1.14% CP; Table 1) and water were provided ad libitum daily from d 1 of life through trial completion (d 92 ± 2, mean ± SD). Starter intake and refusals were recorded daily in the morning.

Two weeks after weaning, bull and heifer calves (mean ± SD; 62 ± 2 d of age, 91 ± 10.9 kg of BW) remained on starter and their respective dietary treatments but were transported to the Cornell University Block Barn (Ithaca, NY) for facility acclimation in thermoneutrality for 7 d (mean ± SD; 20 ± 1.7°C; 68 ± 0.4% RH;

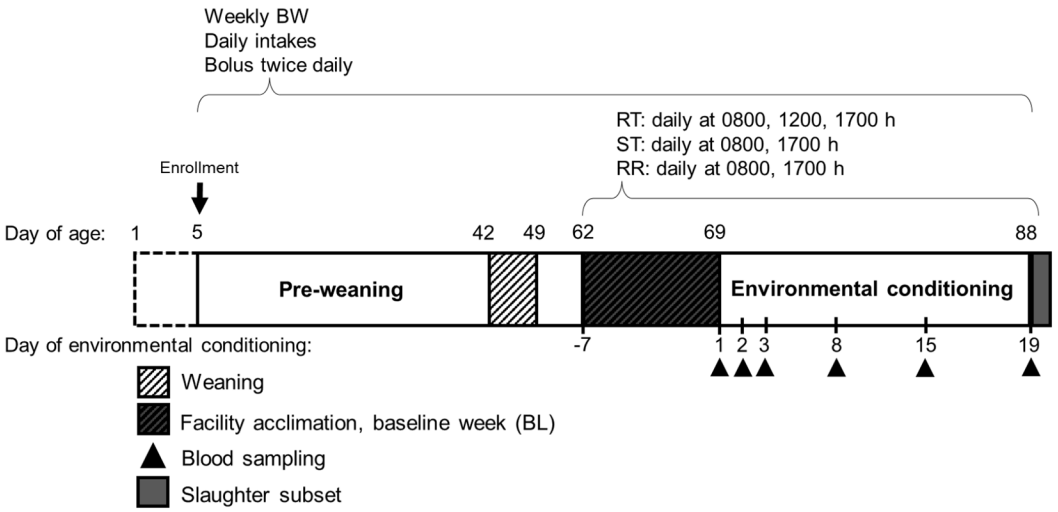

Figure 1. Experimental timeline. Sixty-two bull and heifer calves were enrolled and randomly assigned to treatments through weaning. For the duration of the study, calves were unsupplemented or supplemented with organic acid and pure botanicals (OA/PB) at 2 feeding levels (75 or 150 mg/kg of BW; low or high OA/PB, respectively) divided equally over 2 esophageal boluses administered at 0800 and 1700 h daily. The OA/PB supplement was composed of 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride (AviPlus R; Vetagro S.p.A.). All calves including those unsupplemented with OA/PB received an equivalent amount of triglyceride (vegetable lipid encapsulate; Vetagro S.p.A.). Milk replacer intake was reduced by half at 42 d of age, and terminated on d 49. After weaning (62 ± 2 d of age), calves remained on starter and their respective dietary treatments but were transported to a new facility for acclimation in thermoneutrality for 7 d [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were subject to environmental conditions: thermoneutrality and unsupplemented (TN-Con; n = 12, 6 heifers and 6 bulls), heat-stressed and unsupplemented (HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutrality and unsupplemented but pair-fed to match the intake of heat-stressed and unsupplemented calves (TN-PF; n = 11, 6 heifers and 5 bulls), heat-stressed and supplemented with low-dose OA/PB (HS-Low; n = 14, 8 heifers and 6 bulls), and heat-stressed and supplemented with high-dose OA/PB (HS-High; n = 13, 7 heifers and 6 bulls) for 19 d. RT = rectal temperature; ST = skin temperature; RR = respiration rate.

63 ± 2 THI). Calves were housed individually in pens and reared on wood shavings. Acclimated calves were subject to environmental conditions assigned at enrollment as follows: thermoneutrality and unsupplemented (**TN-Con**; n = 12, 6 heifers and 6 bulls), heat-stressed and unsupplemented (**HS-Con**; n = 12, 6 heifers and 6 bulls), thermoneutrality and unsupplemented but pair-fed to match the intake of heat-stressed and unsupplemented calves (**TN-PF**; n = 11, 6 heifers and 5 bulls), heat-stressed and supplemented with low-dose OA/PB (**HS-Low**; n = 14, 8 heifers and 6 bulls), and heat-stressed and supplemented with high-dose OA/PB (**HS-High**; n = 13, 7 heifers and 6 bulls) for 19 d. We did not perform a power analysis for the effects of OA/PB on calves, considering that this was original research. Thus, we established the sample size per treatment based on documented evidence for the effects of heat stress or pair-feeding on changes in intake and growth performance (i.e., n = 6 to 8 was adequate; O'Brien et al., 2010; Yazdi et al., 2016). Regarding OA/PB, the low feeding level described was investigated in swine before utilizing n = 10 per group (Grilli et al., 2015). Those authors were able to see an increase in ADG; thus, we deemed our sample size per treatment adequate considering that the duration of OA/PB feeding was longer (i.e., 2 vs. 13 wk) and our HS-High feeding level greater. Temperature and RH for each environment were monitored using 2 HOBO loggers located in TN and heat stress conditions (model MX2300; Onset Computer Corp.). Ambient temperature was adjusted manually by thermostatic control of a centralized gas heater. For thermoneutral conditions, daily ambient temperature was kept at ~18 to 22°C. For heat stress conditions, ambient temperature increased at 0700 h from 27 to 34°C and decreased at 1800 h from 34 to 27°C. The goal was to maintain a THI ≤68.0 in TN and 76 (night) to 83 (day) for heat stress conditioning. For TN-PF, calves were maintained in a thermoneutral environment and pair-fed daily with HS-Con on a per kilogram of metabolic BW ($BW^{0.75}$) basis, after starter intake of HS-Con pair for the previous day was recorded, to eliminate confounding effects of dissimilar nutrient intake. Thus, by accounting for the lowered DMI in an animal that is kept in thermoneutrality, we can assess the direct effect of heat exposure. In addition, pair-feeding was only performed within sex (i.e., heifers to heifers and bulls to bulls) using the intake from the previous day of the HS-Con pair.

Table 1. Nutrient composition (% of DM unless otherwise noted) of experimental starter diet (mean ± SD) fed to weaned Holstein calves[1]

Item	Starter[2]
DM, %	87.0 ± 0.50
CP	26.8 ± 1.14
Soluble protein	3.45 ± 0.44
NDF	23.3 ± 0.75
ADF	10.3 ± 0.82
TDN	75.6 ± 0.61
Ash	7.92 ± 0.53
Crude fat (ether extract)	3.16 ± 0.17
Lignin	2.51 ± 0.19
ME, Mcal/kg of DM	2.86 ± 0.00
NE_M, Mcal/kg of DM	1.78 ± 0.01
NE_G, Mcal/kg of DM	1.14 ± 0.01

[1]In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB; HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with low-dose OA/PB (75 mg/kg of BW; AviPlus R; Vetagro S.p.A.; HS-Low; n = 14; 8 heifers and 6 bulls), and HS with a high-dose of OA/PB (150 mg/kg of BW; AviPlus R; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing a matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI).

[2]Starter samples were collected weekly and composited by month. A total of 5 composites were submitted for nutrient composition analysis.

Data and Sample Collection

Starter grain was collected weekly throughout the experiment and composited by month. Clinical assessments were performed thrice daily from acclimation until conclusion of the experiment. During these assessments, rectal temperatures were recorded at 0800, 1200, and 1700 h. Skin temperatures and respiration rates were recorded at 0800 and 1700 h daily. Rectal temperatures were measured using a large-animal digital rectal thermometer (model GLA M900; GLA Agricultural Electronics). Skin temperatures were measured using a noncontact infrared temperature gun (model 586; Fluke Corp.) on a shaved area of the left flank. Respiration rates were determined by counting flank movements for a 15-s period and then multiplying by 4 to obtain movements per minute. Body weights were recorded weekly using a scale mat (model VS-2000; A and A Scales LLC). If calves in the heat stress environment had a rectal temperature >40.8°C, they were temporarily removed from heat stress and hosed down with water in thermoneutrality per IACUC guidelines. Once rectal temperature returned to <40.8°C (~10–15 min), calves were returned to their individual pens. In addition, calves were scored daily for signs of diarrhea, respiratory disease, and fever using the instructions from the calf health scorer (University of Wisconsin-Madison, 2015). Preprandial blood (i.e., ~30 min before morning feeding) was collected in the morning (d 1, 2, 3, 8, 15, and 19) by jugular venipuncture into evacuated 10-mL blood tubes, which contained 15% K_3 EDTA (Cardinal Health Inc.) as an anticoagulant when plasma was collected. No anticoagulant was used when collecting serum samples into evacuated sterile glass tubes (Cardinal Health Inc.). Plasma and serum samples were separated using centrifugation (3,400 × *g* for 20 min). Separated plasma or serum samples were initially stored at −20°C and then transferred to −80°C for long-term storage within 2 wk of collection. At the end of the 19-d period, bull and heifer calves in the TN-Con and HS-Con groups were euthanized by captive bolt stunning followed by exsanguination. At slaughter, hot carcass weight and weights of small intestine empty, reticulo-rumen empty, liver, pancreas, heart, lungs with the trachea, kidneys without perirenal fat, and spleen were recorded for each calf after evisceration.

Sample Analyses

Feed samples were analyzed for DM (AOAC International, 2000), CP (AOAC International, 2000), soluble protein (Krishnamoorthy et al., 1982), NDF (Van Soest et al., 1991), ADF (AOAC International, 2000), TDN (sum of digestible protein, digestible carbohydrate and fat), ash (Thiex et al., 2012), ether extract (Thiex, 2009), and lignin (AOAC International, 2000) by Cumberland Valley Analytical Services Inc. (Cumberland, MD).

Serum concentrations of albumin (bromocresol green method #AB3800), aspartate aminotransferase (**AST**; IFCC method #AS3876), total protein (Biuret method #TP4001), triglycerides (glycerine phosphate oxidase peroxidase method #TR3823), urea (kinetic method #UR3825), glucose (UV method #GL3816), BHB (enzymatic method #RB1007), and total calcium (arsenazo method #CA3871) were measured using an automated clinical chemistry analyzer (Daytona; Randox Laboratories Ltd.) using reagents provided by Randox. Plasma total fatty acids (**FA**; HR series NEFA-HR #999-34691, 995-34791, 991-34891, and 993-35191; Wako Chemicals USA Inc.) and serum amyloid A (**SAA**; #TP-802; Tridelta Development Ltd.) were quantified according to the manufacturers' instructions. All spectrophotometric measurements were conducted using a SpectraMax Plus 384 Microplate Reader (Molecular Devices). Serum insulin concentrations were determined using RIA (#PI-12K Porcine Insulin RIA Kit; EMD Millipore Corp.) on a LKB-Wallac CliniGamma

Counter (Beckman Coulter) as described previously (Krumm et al., 2019). Intra- and interassay coefficients of variation were 3.27 and 10.9%, 5.10 and 10.6%, and 8.99 and 10.4% for total fatty acids, SAA, and insulin, respectively.

Calculations and Statistical Analyses

The THI was calculated according to the equation reported by Kendall et al. (2008): THI = (1.8 × T + 32) − [(0.55 − 0.0055 × RH) × (1.8 × T − 26)], where T = air temperature (°C) and RH = relative humidity (%). Weekly BW difference was divided by the days in the week to calculate ADG. Gain to feed ratio (**G:F**) was computed by dividing ADG by DMI.

Statistical analyses were carried out using the mixed model procedure of SAS (version 9.4, SAS Institute Inc.) according to the following model:

$$Y_{ijklmn} = \mu + C_i(T_k) + D_j + T_k + S_l + D_j \times T_k + pVar_m + BW_n + e_{ijklmn},$$

where Y_{ijklmn} = dependent variable; μ = overall mean effect for the measure; $C_i(T_k)$ = random effect of calf nested within treatment ($i = 1$ to 62); D_j = fixed effect of day ($j = 1$ to 19); T_k = fixed effect of treatment ($k = 1$ to 5); S_l = fixed effect of sex ($l = 1$ to 2); $D_j \times T_k$ = fixed effect of the interaction between day and treatment; $pVar_m$ = baseline measurement for each variable described used as a covariate; BW_n = BW at birth used as a covariate; and e_{ijklmn} = residual error. This model was used to evaluate DMI, BW, ADG, G:F, and blood metabolites for each calf's respective response variable, using day as the repeated measure. The covariance structures used to model correlation on the repeated measures taken on calf within day included variance components, compound symmetry, autoregressive one, unstructured, and ante-dependence one. Smaller fit values (Bayesian information criterion, BIC) were always selected.

Differences between rectal and skin temperatures and respiration rates were tested using the mixed model procedure of SAS according to the following model:

$$Y_{ijklmno} = \mu + C_i(T_l) + C_i(D_j) + D_j + H_k + T_l + S_m + D_j \times T_l + H_k \times T_l + D_j \times H_k + D_j \times H_k \times T_l + pVar_n + BW_o + e_{ijklmno},$$

where $Y_{ijklmno}$ = dependent variable; μ = overall mean effect for the measure; $C_i(T_l)$ = random effect of calf nested within treatment ($i = 1$ to 62); $C_i(D_j)$ = random effect of calf nested within day ($j = 1$ to 19); D_j = fixed effect of day ($j = 1$ to 19); H_k = fixed effect of hour [$k = 1$ to 3 (rectal temperature) or 1 to 2 (skin temperature and respiration rate)]; T_l = fixed effect of treatment ($l = 1$ to 5); S_m = fixed effect of sex ($m = 1$ to 2); $D_j \times T_l$ = fixed effect of the interaction between day and treatment; $H_k \times T_l$ = fixed effect of the interaction between hour and treatment; $D_j \times H_k$ = fixed effect of the interaction between day and hour; $D_j \times H_k \times T_l$ = fixed effect of the 3-way interaction between hour, day, and treatment; $pVar_n$ = baseline measurement for each variable described used as a covariate; BW_o = BW at birth used as a covariate; and $e_{ijklmno}$ = residual error. The covariance structures used to model correlation on the repeated measures taken on calf within time within day included variance components, compound symmetry, autoregressive one, unstructured, and ante-dependence one. Smaller fit values (BIC) were always selected.

Differences between carcass and visceral organ weights were tested using the general linear model procedure of SAS according to the following model:

$$Y_{lmn} = \mu + T_l + S_m + BW_n + e_{lmn},$$

where Y_{lmn} = dependent variable; μ = overall mean effect for the measure; T_l = fixed effect of treatment ($l = 1$ to 2); S_m = fixed effect of sex ($m = 1$ to 2); BW_n = BW at birth used as a covariate; and e_{lmn} = residual error.

Observations were deemed outliers if Studentized residuals were >4.0 or < −4.0, and the effect of their removal was evaluated (≤1 per variable). Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values to ensure no violation of model assumptions. The least squares means comparisons for analysis conducted using both the general linear model and mixed model were performed using the Tukey-Kramer test. Main effects were declared significant at $P \leq 0.05$ and trending toward significance at $0.05 < P \leq 0.10$. Interactions were declared significant at $P \leq 0.05$, and tendencies were declared at $P \leq 0.15$. Results are expressed as least squares means ± standard errors of the mean (LSM ± SEM), unless otherwise noted.

RESULTS

No morbidities or mortalities were observed in calves (n = 62). However, sample size divergence by treatment occurred because we had to remove a bull calf from the TN-PF group due to pre-existing health conditions

unrelated to the treatment (i.e., malformation of the distal esophageal sphincter). We also had the opportunity to add extra heifers to the experiment (n = 2 for HS-Low and n = 1 for HS-High).

Figure 2 depicts the environmental conditions (i.e., temperature, RH, and THI) achieved during the trial. Temperature peaked at 22.1 and 36.3°C and THI at 69 and 83 for thermoneutral and heat stress conditions, respectively; RH fluctuated from 67 to 77% in thermoneutral conditions, and from 35 to 46% in heat stress conditions. Clinical responses of body temperatures and respiration rates are described in Table 2 and Supplemental Figure S1 (https://doi.org/10.17632/ydg73pfv38.1; Fontoura et al., 2022c). As expected, housing weaned Holstein calves in moderate heat stress conditions for 19 d markedly increased rectal (39.9 ± 0.08, 39.9 ± 0.07, 40.0 ± 0.08 vs. 38.9 ± 0.08 and 38.7 ± 0.08°C; LSM ± SEM; treatment, $P < 0.01$) and skin (38.7 ± 0.36, 38.7 ± 0.36, 38.8 ± 0.36 vs. 32.8 ± 0.36 and 31.8 ± 0.36°C; treatment, $P < 0.01$) temperatures, as well as respiration rates (104.0 ± 2.61, 104.0 ± 2.50, 101.0 ± 2.60 vs. 64.0 ± 2.66 and 58.0 ± 0.263 breaths/min; treatment, $P \leq 0.05$) of calves in heat stress conditions (HS-Con, HS-Low, and HS-High, respectively) compared with calves housed in thermoneutrality (TN-Con and TN-PF, respectively). Thus, the observed increased rectal and skin temperatures and respiration rates in calves maintained in moderate heat stress compared with calves in thermoneutrality were maintained throughout the 19 d of environmental conditioning (Supplemental Figure S1; Fontoura et al., 2022c).

Exposure to high ambient temperatures significantly decreased DMI of heat-stressed calves (treatment, $P < 0.01$; Table 3). Calves in HS-Con consumed less DM than calves in TN-Con for most of the study (i.e., at d 3, 4, 5, 7, 9, 11, 12, 13, 14, 16, 17, and 19; treatment × day, $P < 0.05$; Figure 3A; Supplemental Table S1; https://data.mendeley.com/datasets/rb46jf9k6m/1; Fontoura et al., 2022b). In accordance with our experimental design, TN-PF had similar DMI to HS-Con (treatment, $P = 0.99$). Although DMI was similar between HS-Low and HS-Con and between HS-High and HS-Con (treatment, $P = 0.77$), we did observe a tendency for a treatment × day effect ($P = 0.14$). Specifically, during the first week of environmental conditioning (i.e., d 4, 5, and 7), HS-Low calves had greater intake than HS-Con calves (e.g., 3.37 ± 0.15 vs. 2.81 ± 0.15 kg on d 4, respectively; $P \leq 0.05$; Figure 3A). Although BW was not overtly modified by treatment, TN-Con calves were heavier in the last week of the experiment compared with all other treatments (Figure 3B; treatment × day, $P < 0.05$). In addition, HS-Con calves had lower ADG relative to TN-Con (0.84 ± 0.08 vs. 1.32 ± 0.08 kg/d, respectively; treatment, $P < 0.05$). We did not observe changes in G:F when comparing TN-Con, TN-PF, or OA/PB-supplemented groups with HS-Con (Table 3).

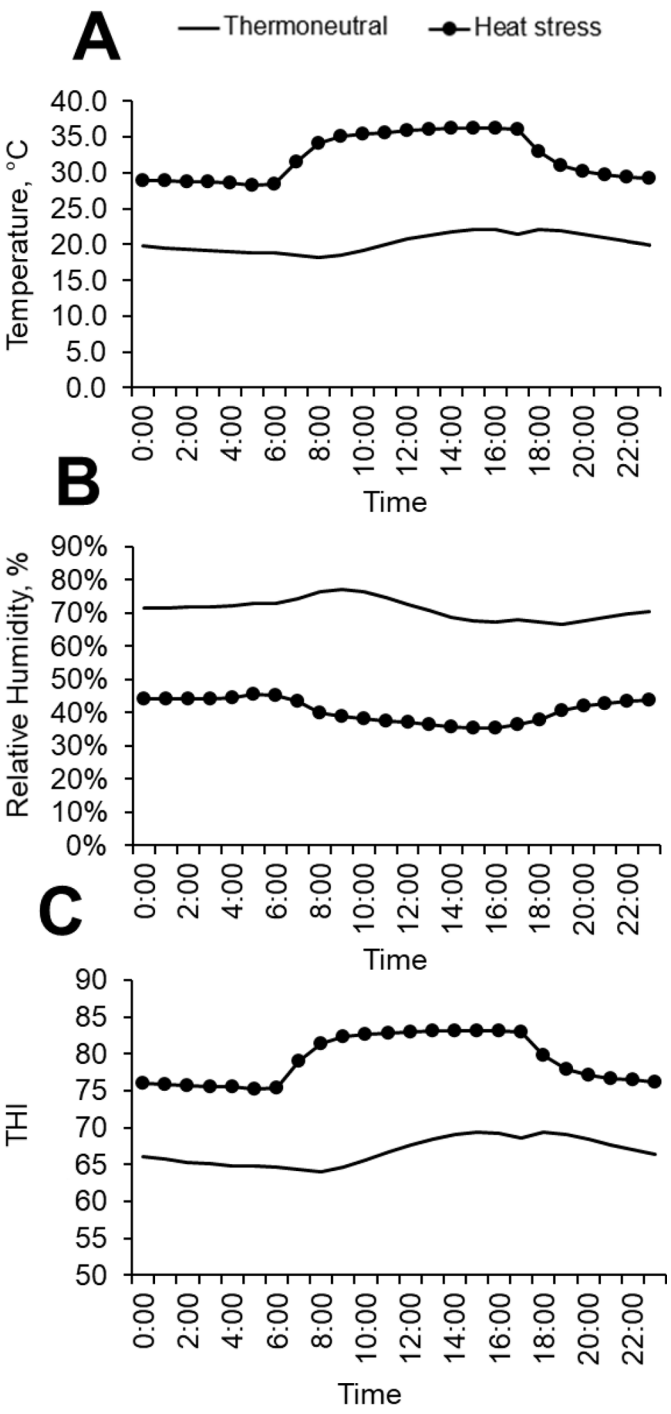

Figure 2. Average environmental conditions exposed to weaned calves for 19 d. Data were averaged hourly for (A) ambient temperature, (B) relative humidity, and (C) temperature-humidity index (THI).

Table 4 describes the changes in circulating plasma and serum markers of metabolic health in response to

Table 2. Effects of heat stress and dietary organic acid and pure botanical supplementation on clinical parameters of weaned Holstein calves

	Treatment[1]						*P*-value						
Item	TN-Con	HS-Con	TN-PF	HS-Low[2]	HS-High[2]	SEM	Treatment (T)	Day (D)	Hour (H)	T × D	T × H	D × H	T × D × H
Rectal temperature, °C													
0800 h	38.8[b]	39.3[a]	38.6[b]	39.4[a]	39.4[a]	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.83
1200 h	38.9[b]	39.9[a]	38.6[b]	39.9[a]	39.9[a]	0.08							
1700 h	38.9[b]	39.9[a]	38.7[b]	39.9[a]	40.0[a]	0.08							
Skin temperature, °C													
0800 h	31.8[b]	37.7[a]	31.5[b]	37.7[a]	37.9[a]	0.36	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.15
1700 h	32.8[b]	38.7[a]	31.8[b]	38.7[a]	38.8[a]	0.36							
Respiration rate, breaths/min													
0800 h	60.0[b]	91.0[a]	54.0[b]	94.0[a]	89.0[a]	2.58	<0.01	<0.01	<0.01	<0.01	0.05	<0.01	0.18
1700 h	64.0[b]	104.0[a]	58.0[b]	104.0[a]	101.0[a]	2.66							

[a,b]Least squares means in the same row with different superscripts differ ($P \leq 0.05$).

[1]In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB; HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with low-dose OA/PB (75 mg/kg of BW; AviPlus R; Vetagro S.p.A.; HS-Low; n = 14; 8 heifers and 6 bulls), and HS with a high-dose of OA/PB (150 mg/kg of BW; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing a matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI).

[2]AviPlus R contains 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride.

environmental conditions and diet. Relative to TN-Con calves, heat-stressed calves had lower serum glucose concentrations (HS-Con vs. TN-Con; treatment, $P < 0.01$) and this effect was more pronounced after 1 wk of heat exposure (Figure 4A). Calves in the TN-PF group had higher circulating plasma total FA concentrations relative to both TN-Con and HS-Con (106.5 ± 5.73 vs. 69.2 ± 5.13 and 69.9 ± 5.66 μmol/L, respectively; treatment, $P < 0.01$). This response peaked during the second week of the trial (Figure 4B). Pair-feeding calves in thermoneutrality also decreased serum BHB concentrations relative to all other treatments from d 8 until the end of the experiment (Figure 4C). In addition, we detected a treatment × day tendency for modified serum urea concentrations in TN-Con (Figure 4D; $P = 0.06$). Circulating insulin concentrations were reduced by heat stress conditioning and pair-feeding compared with thermoneutrality (treatment, $P < 0.01$). Serum AST concentrations increased over time in the HS-Con group (Figure 4E; i.e., from 78.9 ± 7.19 to 111.9 ± 7.5 U/L; treatment × day, $P = 0.01$). In addition, HS-Con calves had increased AST concentrations relative to TN-PF calves (Figure 4E; treatment × day, $P = 0.01$). We also observed that circulating total calcium concentrations tended to be lower for HS-Con calves than for TN-Con calves (10.4 ± 0.11 vs. 10.7 ± 0.11 mmol/L; treatment, $P = 0.06$). The levels of circulating albumin, SAA, total protein, and triglycerides were not affected by heat stress conditioning or OA/PB supplementation. The responses in metabolic and inflammation markers of calves supplemented with OA/PB were comparable to those of HS-Con animals.

Heat stress conditions affected visceral organ weights (Table 5). Specifically, heat-stressed calves had lighter livers (2.74 ± 0.12 vs. 3.11 ± 0.11 kg; treatment, $P = 0.03$) and tended to have heavier kidneys (0.686 ± 0.04 vs. 0.589 ± 0.04 kg; treatment, $P = 0.09$) compared with calves maintained in thermoneutrality. The gross weights for small intestine, pancreas, rumen, lungs, heart, and spleen were unaffected by heat stress conditioning compared with those of thermoneutral calves.

DISCUSSION

Dairy production is a top commodity affected by the changing climate (St-Pierre et al., 2003; West, 2003). The combination of escalating global temperatures (Schär et al., 2004; NOAA, 2021) and the elevation in internal body heat in *Bos taurus* breeds with high genetic potential (Aguilar et al., 2010) are contributing factors to the shifting zone of thermal comfort in modern dairy herds. Previous studies have proposed that the thermal comfort zone for dairy cattle, utilizing the dairy cow as a model, is at THI <68 (Hahn et

Table 3. Effects of heat stress and dietary organic acid and pure botanical supplementation on DMI and growth of weaned Holstein calves

	Treatment[1]						*P*-value		
Item	TN-Con	HS-Con	TN-PF	HS-Low[2]	HS-High[2]	SEM	Treatment	Time	Treatment × Time
DMI, kg/d	3.81[a]	3.13[b]	3.12[b]	3.34[ab]	3.18[b]	0.15	<0.01	<0.01	0.14
BW, kg	106.9	105.6	104.4	104.8	105.1	0.81	0.22	<0.01	<0.01
DMI per unit of $BW^{0.75}$, g/$BW^{0.75}$	112.4[a]	98.2[b]	95.4[b]	103.1[ab]	98.4[b]	3.23	<0.01	<0.01	0.44
ADG, kg/d	1.32[a]	0.84[b]	1.04[ab]	0.93[b]	0.96[ab]	0.08	<0.01	0.66	0.44
Gain to feed, kg/kg	0.31	0.23	0.30	0.25	0.28	0.02	0.11	0.91	0.56

[a,b]Least squares means in the same row with different superscripts differ ($P \leq 0.05$).

[1]In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB; HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with low-dose OA/PB (75 mg/kg of BW; AviPlus R; Vetagro S.p.A.; HS-Low; n = 14; 8 heifers and 6 bulls), and HS with a high-dose of OA/PB (150 mg/kg of BW; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing a matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI).

[2]AviPlus R contains 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride.

al., 2009). Mild signs of heat stress can be observed at THI of 68 to 74, moderate signs of heat stress with noticeable decreases in performance observed at THI 75 to 80, severe signs of heat stress at THI 81 to 84, and threats to life when THI >85 (Hahn et al., 2009; De Rensis et al., 2015). Dairy calves, however, generate less metabolic heat and have greater body surface area relative to body mass, allowing more efficient heat dissipation and thereby making these animals more resistant to heat stress compared with lactating cows (West, 2003). Indeed, recent work proposed that heat stress signs (i.e., respiratory rate, rectal temperature, heart rate) are absent if THI ≤78, with signs observed when THI is between 79 and 90 in dairy calves (Kovács et al., 2020). In our study, we were able to achieve a maximum THI of 83 during the day and 75 at night, resulting in conditions that allow calves to experience moderate heat stress. Our conditions were milder than those described by Yazdi et al. (2016), in which calves were exposed to increasing THI of 77 to 90 for a period of 9 d. We were able to expose our calves to a longer duration of elevated heat. Collectively, we were able to observe increased rectal and skin temperatures and respiration rates, which are classical physiological adaptations caused by heat exposure in mammals (Gaughan et al., 2000). Importantly, the observed elevations in rectal and skin temperatures and respiration rates were maintained throughout the environmental conditioning period.

Our findings of reduced feed intake during heat stress conditioning have been observed previously in studies using growing as well as lactating dairy cattle (Baumgard and Rhoads, 2013; Wang et al., 2020). It is widely accepted that the reduction in voluntary feed intake is driven by homeorhetic adaptations to survive an increased heat load (Collier et al., 2017). The feed intake reduction in our study was comparable to that of previous studies utilizing Holstein bulls, which reduced voluntary feed intake when subjected to heat stress conditions for 9 d (O'Brien et al., 2010; Yazdi et al., 2016). However, it is important to highlight that our model of heat stress allowed for comparison of TN-Con, TN-PF, and HS-Con groups in a longitudinal manner rather than utilizing each animal as its own control in different periods (i.e., a TN period followed by either an HS or a PF period). We did not observe differences in BW across the treatment groups. Similarly, Nonaka et al. (2008), while evaluating the effects of heat stress on weight gain of prepubertal Holstein heifers, observed a lack in BW difference across heifers maintained at 20°C, 28°C, and 33°C. Importantly, ADG and G:F were reduced in our heat-stressed calves compared with TN-Con calves. The TN-PF group in our study had an intermediate response in ADG and G:F and did not differ from TN-Con or HS-Con calves. This is in contrast to previous studies, where TN-PF bull calves had reduced ADG and G:F relative to the HS-Con group after 9 d of heat exposure (O'Brien et al., 2010; Yazdi et al., 2016), as opposed to the 19-d period in our study. Those authors reason that this is due to higher fat deposition in heat-stressed animals. This seems plausible considering previous studies that have reported increases in circulating insulin concentrations (O'Brien et al., 2010; Wheelock et al., 2010) and abdominal fat deposition in heat-stressed animals (Baumgard and Rhoads, 2013). We attribute some of the divergences (i.e., BW, ADG, and G:F) found in our study compared with others to (1) the age of calves enrolled in the studies (2 to 3 mo

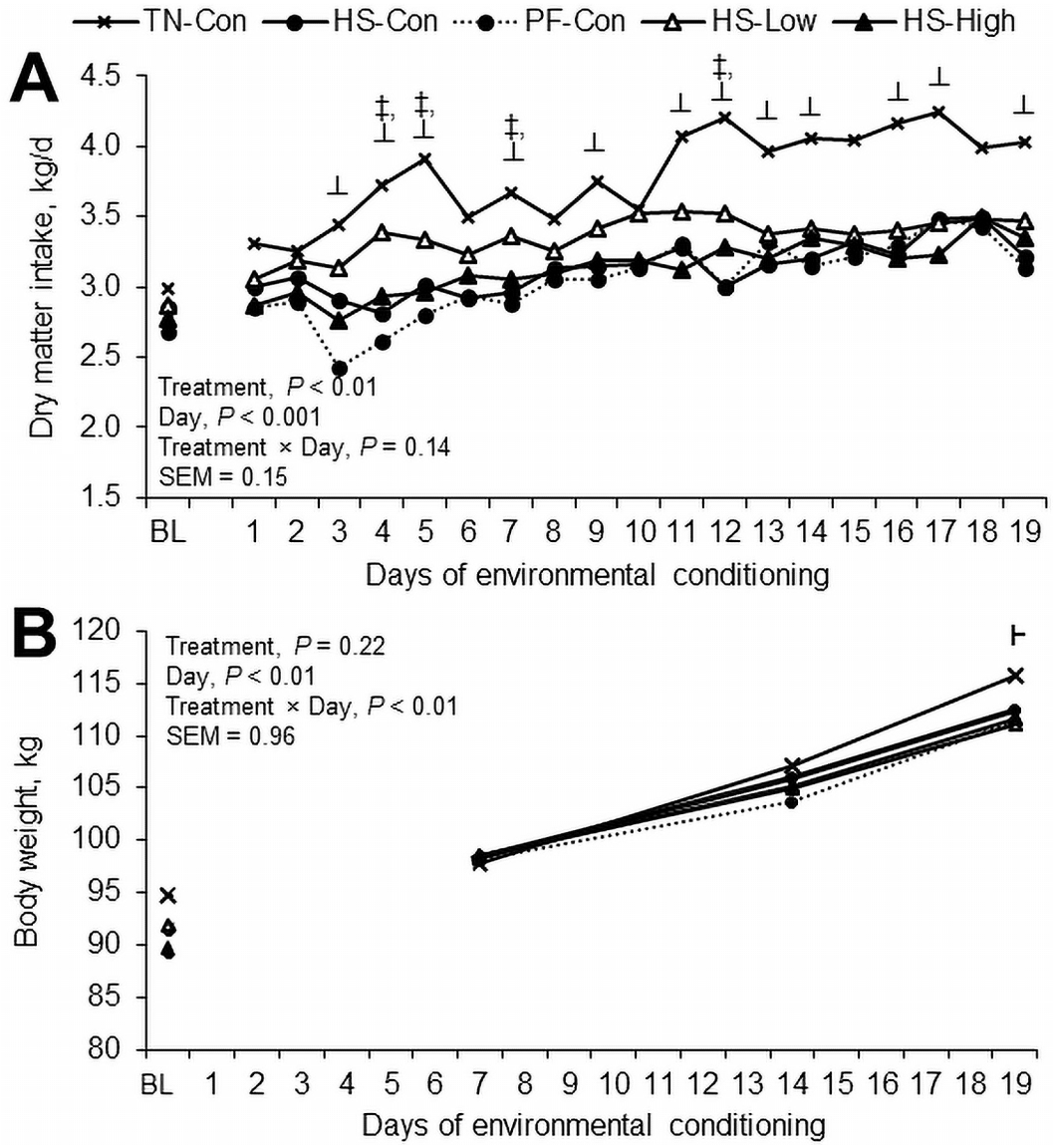

Figure 3. Effects of environmental conditioning and dietary organic acid and pure botanical on (A) daily DMI and (B) BW of weaned Holstein calves (62 ± 2 d of age). Bull and heifer calves were randomly assigned during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB) (HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with a low dose of OA/PB (75 mg/kg of BW; AviPlus R, Vetagro S.p.A.; HS-Low; n = 14, 8 heifers and 6 bulls), and HS with a high dose of OA/PB (150 mg/kg of BW; AviPlus R; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI). BL = average values for baseline acclimation period. ⊥HS-Con vs. TN-Con, $P \leq 0.05$; ‡HS-Con vs. HS-Low, $P \leq 0.05$; †TN-Con vs. all treatments. For Figure 3A, not all significant comparisons are shown but are provided in Supplemental Table S1 (https://data.mendeley.com/datasets/rb46jf9k6m/1; Fontoura et al., 2022b).

in the present study vs. 5 to 7 mo in previous studies), (2) the inclusion of both heifer and bull calves, which intrinsically brings more variation to the present data set, and (3) the environmental conditions achieved (i.e., lower overall THI).

The reduced liver weight in heat-stressed calves could partially explain the reductions in productive performance observed between HS-Con and TN-Con calves, considering that the liver is energetically demanding and important for growth and performance (Ferrell, 1988). This may represent an adaptive mechanism by which animals endure heat stress conditioning because this organ has the flexibility to alter mass and metabolism in accordance with level of dietary intake within

Table 4. Effects of heat stress and dietary organic acid and pure botanical supplementation on blood serum and plasma metabolites in weaned Holstein calves

	Treatment[1]						*P*-value		
Item	TN-Con	HS-Con	TN-PF	HS-Low[2]	HS-High[2]	SEM	Treatment	Day	Treatment × Day
Metabolic marker									
Glucose, mmol/L	127.1[a]	117.1[b]	128.1[a]	115.7[b]	114.2[b]	2.17	<0.01	<0.01	0.11
Total fatty acids, μmol/L	69.2[b]	69.9[b]	106.5[a]	66.3[b]	80.6[b]	5.73	<0.01	<0.01	<0.01
BHB, mmol/L	0.36[a]	0.37[a]	0.25[b]	0.40[a]	0.37[a]	0.02	<0.01	<0.01	0.02
Urea, mmol/L	33.0	34.3	34.6	34.5	35.3	0.95	0.56	<0.01	0.06
Insulin, ng/mL	1.49[a]	0.88[b]	0.70[b]	0.98[b]	0.89[b]	0.14	<0.01	0.61	0.34
Liver function and inflammation marker									
Serum amyloid A, μg/mL	67.1	55.7	64.3	46.6	43.2	12.9	0.61	0.09	0.90
Albumin, g/L	3.79	3.74	3.89	3.74	3.77	0.04	0.14	0.21	0.46
Total protein, g/L	6.96	6.83	6.94	6.92	6.82	0.09	0.74	<0.01	0.87
Aspartate aminotransferase, U/L	90.6	94.0	75.6	94.4	92.8	5.86	0.11	<0.01	0.01
Triglycerides, mmol/L	31.4	27.6	30.1	30.1	29.8	1.47	0.49	0.03	0.87
Calcium, mmol/L	10.7[x]	10.4[y]	10.7[x]	10.4[y]	10.4[y]	0.11	0.06	0.27	0.33

[a,b]Least squares means in the same row with different superscripts differ ($P \leq 0.05$).

[x,y]Least squares means in the same row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

[1]In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB; HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with low-dose OA/PB (75 mg/kg of BW; AviPlus R; Vetagro S.p.A.; HS-Low; n = 14; 8 heifers and 6 bulls), and HS with a high-dose of OA/PB (150 mg/kg of BW; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing a matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI).

[2]AviPlus R contains 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride.

and across differing physiological stages (Johnson et al., 1990). However, we recognize that the absence of TN-PF animals from slaughter limits the power of our observations and adds difficulty in differentiating the effects caused by heat exposure or feed intake (Baumgard and Rhoads, 2013) in relation to carcass and organ weights.

One of the hallmarks of heat stress adaptations in dairy cattle is the restrained capability for body fat mobilization (Baumgard and Rhoads, 2013). Observations of increased concentrations of plasma FA in pair-fed controls, relative to their heat-stressed counterparts, have been reported not only in lactating cows, but also in growing animals (Rhoads et al., 2009; O'Brien et al., 2010; Yazdi et al., 2016). These changes in plasma FA concentrations are in conjunction with a decrease or tendency for a reduction in circulating glucose concentrations in heat-stressed animals (O'Brien et al., 2010; Yazdi et al., 2016). Previous observations of increased circulating insulin concentrations in heat-stressed animals (O'Brien et al., 2010) support the divergence in postabsorptive metabolism between heat-stressed and pair-fed animals; although the mechanistic cause for increased circulating insulin is not entirely clear. Although we observed similar patterns related to plasma FA and glucose concentrations, we did not observe differences relative to insulin concentrations between HS-Con and TN-PF calves in the present study. An emerging hypothesis explaining the insulin peak in heat-stressed animals is the activation of the immune system in response to the increased heat load that locally damages the small intestine, causing inflammation (Baumgard and Rhoads, 2013; Koch et al., 2019). Insulin exerts important modulating effects that allow inflammatory immune cells greater capability to use glucose as a metabolic fuel through upregulated glycolysis (Kornberg, 2020). This not only supports rapid clonal expansion of immune cells (van Niekerk et al., 2020) but also enhances their bactericidal actions, because it provides a means of increasing the flux through the pentose phosphate pathway, yielding more NADPH required for the redox reactions that generate reactive oxygen species via the NADPH-oxidase system (Ganeshan and Chawla, 2014). We hypothesize that the immune status between HS-Con and TN-PF calves was comparable due to the absence of an insulin response before feeding.

It is important to highlight that liver and inflammation markers were within normal ranges for our study calves, and all animals were clinically healthy during the environmental conditioning phase of our experiment. However, we did observe tendencies in liver function and inflammation markers that deserve consideration.

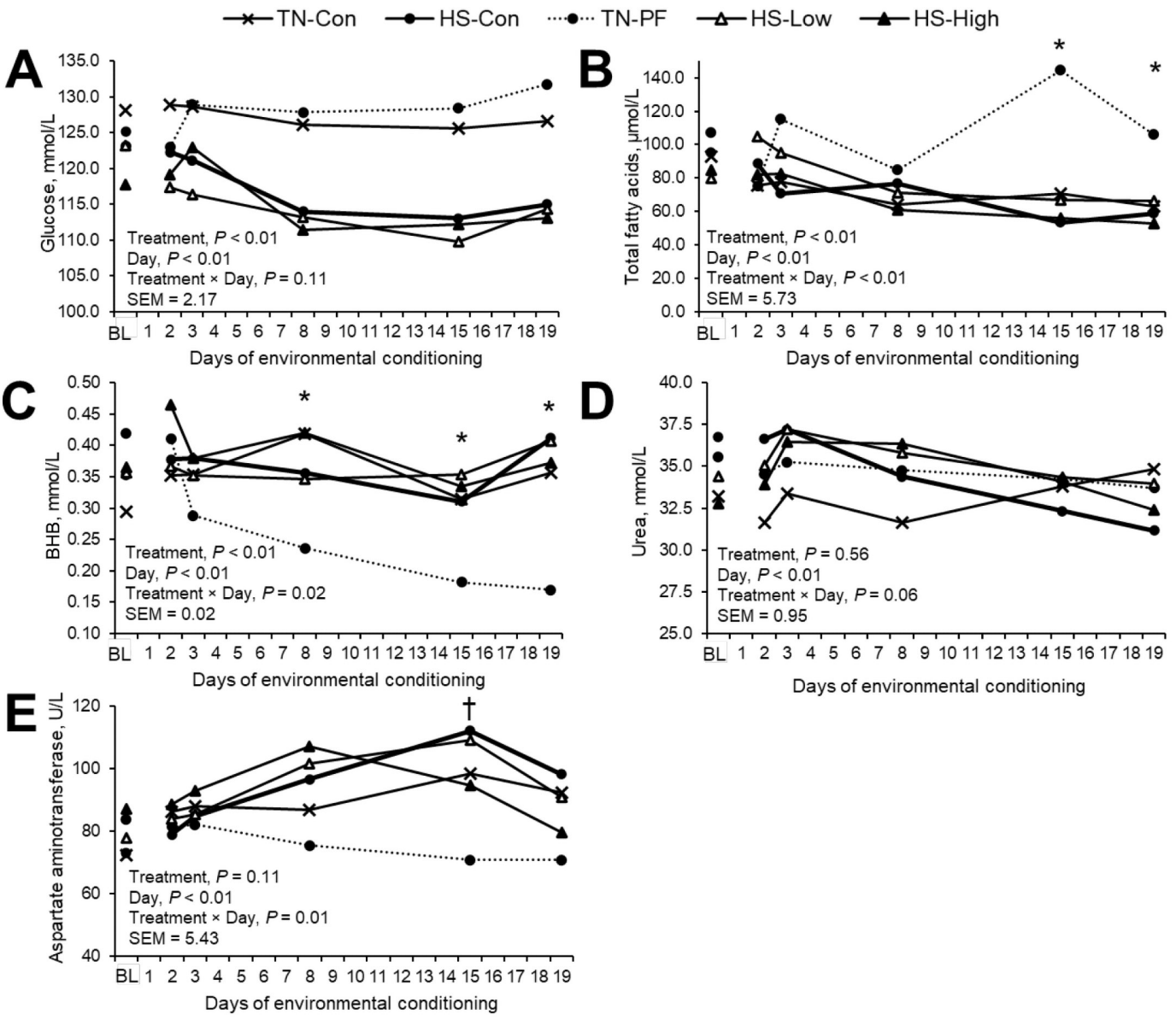

Figure 4. Effects of environmental conditioning and dietary organic acid and pure botanical on serum metabolic markers of weaned Holstein calves (62 ± 2 d of age). Preprandial (A) serum glucose, (B) nonesterified fatty acids, (C) BHB, (D) urea, and (E) aspartate aminotransferase levels of bull and heifer calves randomly assigned during the first week of life: thermoneutral conditions (TN-Con; n = 12, 6 heifers and 6 bulls), heat stress with no organic acids and pure botanicals (OA/PB) (HS-Con; n = 12, 6 heifers and 6 bulls), thermoneutral conditions pair-fed to HS-Con (TN-PF; n = 11, 6 heifers and 5 bulls), HS with a low dose of OA/PB (75 mg/kg of BW; AviPlus R, Vetagro S.p.A.; HS-Low; n = 14, 8 heifers and 6 bulls), and HS with a high dose of OA/PB (150 mg/kg of BW; AviPlus R; HS-High; n = 13, 7 heifers and 6 bulls). Calves unsupplemented with OA/PB received a placebo containing matching dose of triglyceride. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI). BL = average values for baseline acclimation period. *TN-PF vs. all treatments, $P \leq 0.05$; †HS-Con vs. TN-PF, $P \leq 0.05$.

The trend toward increased circulating urea concentrations in HS and PF groups, relative to TN-Con animals, is a potentially important observation because it may infer changes in ruminal fermentation, skeletal muscle protein breakdown, and impaired growth (Schneider et al., 1988; Kamiya et al., 2006). Another likelihood is that HS animals were more prone to muscle degradation because of the observation that increases in circulating AST concentrations, which were observed in HS-Con versus TN-PF on d 15, are correlated with

Table 5. Effects of heat stress on carcass and organ weights in weaned Holstein calves

Item[1]	Treatment[2]		SEM	*P*-value
	TN-Con	HS-Con		Treatment
Hot carcass weight, kg	85.9	81.9	2.53	0.29
Gross weight, kg				
Small intestine	3.03	2.80	0.15	0.25
Pancreas	0.124	0.122	0.01	0.87
Liver	3.11	2.74	0.12	0.03
Reticulo-rumen	3.00	2.89	0.14	0.59
Heart	0.879	0.963	0.09	0.50
Lungs	1.59	1.67	0.09	0.57
Kidneys	0.589	0.686	0.04	0.09
Spleen	0.350	0.397	0.03	0.24

[1]Weights for hot carcass organ weights of small intestine empty, reticulo-rumen empty, liver, pancreas, heart, lungs with the trachea, kidneys without peri-renal fat, and spleen were recorded for each calf after evisceration.

[2]In a completely randomized design, bull and heifer calves were assigned to treatments during the first week of life: thermoneutral (TN-Con; n = 12, 6 heifers and 6 bulls) or heat stress (HS-Con; n = 12, 6 heifers and 6 bulls) conditions. After weaning, calves (62 ± 2 d of age) were transported to a new facility where they were acclimated for 7 d in thermoneutrality [20 ± 1.7°C; 68 ± 0.4% relative humidity; 63 ± 2 temperature-humidity index (THI)]. Thereafter, calves were exposed to environmental conditions of TN (20 ± 1.3°C; 71 ± 0.3% relative humidity; 67 ± 2 THI) or HS (32 ± 3.3°C; 41 ± 0.4% relative humidity; 79 ± 2 THI). At the end of the 19-d period, calves (88 ± 2 d of age) were euthanized.

muscle breakdown (Hashim, 2010; Ramachandran and Sajith, 2014).

Dietary supplementation with OA/PB is common practice in poultry and swine production (Tugnoli et al., 2020). Remarkably, a plethora of compounds can be classified as OA/PB (Pearlin et al., 2020). Although their mode of action most certainly varies, these nutritional strategies aim to protect against pathogenic bacteria and immune modulation of the gastrointestinal tract, thereby improving intestinal health. We chose the current OA/PB mixture (i.e., 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride matrix) for its bactericidal and anti-inflammatory properties. Thymol and citric and sorbic acids can have their undissociated forms freely enter bacterial cells, at neutral pH, and dissociate, causing a reduction in intracellular pH, consequently inhibiting enzymatic reactions and nutrient transport (Mroz et al., 2006). Vanillin possesses anti-inflammatory properties that can attenuate cytokine production in LPS-activated cells (Zhao et al., 2019). Thymol also possesses anti-inflammatory properties that can reduce the levels of inflammatory cytokines (i.e., IL-6 and IL-1) in rats with ulcerative colitis (Tahmasebi et al., 2019).

To our knowledge, this is the first study to evaluate the current mixture of OA/PB in young ruminant species. Because a previous study alluded to the possibility of improved intestinal health after long-term dietary OA/PB supplementation (i.e., swine species; Tugnoli et al., 2020), we opted for a long supplementation feeding period starting at d 5 of age. Compared with the HS-Con group, calves that were receiving a low dietary level of OA/PB supplementation had increased DMI, during the first week of heat conditioning. It is important to note there are contrasting reports on the effects of different blends of OA/PB on feed intake in nonruminant species (Ettle et al., 2004; Pearlin et al., 2020). Attributing a reason for the increase in DMI becomes cumbersome, especially because of the variety of OA, PB, and OA/PB blends that are available for use in farm animal species, and differing routes of OA/PB delivery (i.e., supplemented in feed vs. bolus administration). It is important to highlight that we also observed increases in DMI of heat-stressed lactating cows supplemented with OA/PB (i.e., 75 mg/kg top dressed daily; Fontoura et al., 2022a). Although the reason for the increased DMI in OA/PB-supplemented animals remains unknown, earlier studies suggest that sorbic acid supplementation may improve piglet DMI and growth performance through stimulation of the insulin growth factor (IGF) system in the liver and higher IGF-1 circulating levels in supplemented piglets (Luo et al., 2011). Evidence also supports the DMI of HS-Low calves from the enhanced palatability effects caused by the addition of vanillin (Harper et al., 2016). Citric acid has been reported to increase feed intake in broiler chicks (Chowdhury et al., 2009), although the mechanistic reason behind this observation remains unclear. A possibility for the lack of DMI response in the HS-High group, relative to HS-Low, is supported by the higher inclusion of thymol, which is known to suppress feed intake in broilers (Hashemipour et al., 2013) and pigs (Trevisi et al., 2007).

Despite previous reports of improvements in BW gain in weaned piglets supplemented with OA/PB (Grilli et al., 2015), we did not observe differences when comparing OA/PB-supplemented groups with HS-Con calves. It has been hypothesized that a shift in the gut microbiome explains improvements in performance of OA/PB-supplemented piglets (Wei et al., 2021). However, it is unclear whether heat stress or dietary OA/PB feeding modified the gut microbiome in the present study because we did not measure this outcome. The overall responses related to inflammation markers in the heat stress groups, including OA/PB-supplemented groups, followed similar patterns observed in calves kept in thermoneutrality. This evidence supports our interpretation that moderate heat stress exposure was not enough to trigger inflammation in calves, underpinning the importance of future work that explores the effects of dietary OA/PB supplementation in calves that are experiencing severe heat stress or an alterna-

tive state that has potential to promote inflammation and compromised immunity. Such efforts may reveal improved immune function and growth performance in response to OA/PB feeding.

CONCLUSIONS

Heat exposure reduced growth performance (i.e., ADG and G:F) in calves. It is likely that reduced liver weight supported this decreased growth performance. It is important to note that the reduction in DMI explained the overall reduction in growth performance of heat-stressed calves. Even though our clinical observations (i.e., body temperature and respiration rates) indicated that calves were heat stressed, we did not observe an increase in disease incidence or extreme changes in liver and inflammation markers during heat stress conditioning or with OA/PB treatment. The low level of supplementation of microencapsulated OA/PB partially restored DMI after heat exposure in weaned calves. Thus, we conclude that the OA/PB supplementation strategy may represent a potential means to improve intake in dairy calves exposed to acute bouts of heat stress. However, dietary OA/PB supplementation does not appear to be a strategy to improve growth performance in calves experiencing moderate heat stress.

ACKNOWLEDGMENTS

We acknowledge the financial support from the Foundation for Food and Agriculture (FFAR; Washington, DC) Fellows Program, which partnered with Vetagro S.p.A. (Reggio Emilia, Italy) as the industry sponsor supporting A. B. P. Fontoura's PhD program. We also thank Ada Zhu, Arabella Park, Bruce Berggren-Thomas, Crystal Chang, Martina Cortese, and Margaret Bryce (all of Department of Animal Science, Cornell University) for sample collection support, and the assistance from Jessica Waltemeyer, Jeff Jebbett, and the farm staff at the Cornell University Large Animal Research and Teaching Unit. The authors have not stated any conflicts of interest.

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