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Effect of thermal amplitude on physiological parameters, ruminal fermentation, digestibility, health, and performance of Holstein dairy calves

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ABSTRACT

This study aimed to evaluate the effects of daily thermal amplitude during the first 28 d of age and its residual impact until 90 d of age on respiration rate (RR), heart rate, rectal temperature (RT), intake, ruminal fermentation, nutrient digestibility, blood metabolites, immune markers, health status, and performance of Holstein dairy calves. Thirty-four dairy calves were individually housed in a climate-controlled chamber and randomly assigned to 1 of 2 treatments: control (exposed to constant temperature and humidity index (THI) of 66), or thermal amplitude (TA) exposed daily to THI of 66 (0330 to 0630 h), 84 (0630 to 1530 h), 66 (1530 to 1830 h), and 54 (1830 to 0330 h) from birth to 28 d age (exposure period [EP]). From 29 to 90 d of age, all calves were maintained in identical housing conditions, spanning the postexposure period (**PEP**). Intake, physiological responses, and health outcomes were measured daily, performance, and ADG were evaluated weekly, whereas ruminal and blood parameters were assessed biweekly, and blood cytokines were evaluated on d 28. Apparent digestibility was measured at 9 to 12 d (digestibility 1) and 23 to 26 d of age (digestibility 2). During EP, TA calves exhibited increased RR, higher RT with a greater number of days in hyperthermia, and elevated milk and water intakes. Additionally, the TA showed a greater ruminal acetate: propionate ratio, and fecal nitrogen on digestibility 2. Interferon gamma induced protein (IP-10) and interleukin 8 (IL-8) were reduced in TA calves, and interleukin 4 (IL-4) increased. During PEP, residual effects in TA calves included increased RT, water intake, ruminal pH, blood cholesterol and creatinine, and reduced ruminal acetate and total short-chain fatty acids. Despite physiological and metabolic alterations, no differences were observed in health status or growth performance between treatments. These findings suggest that although thermal amplitude challenges thermoregulation in early life, it does not impair calf development under proper management. However, calves exposed to thermal amplitudes may not fully develop thermoregulatory mechanisms during periods of high temperatures, leading to occasional residual effects during PEP.

Key words: cold stress, dairy calves, heat stress, temperature, well-being

INTRODUCTION

Thermal stress, encompassing both cold and heat, significantly impairs cattle welfare and the economic viability of dairy operations (Roland et al., 2016; Lees et al., 2019). This stress occurs when environmental conditions deviate from the animals' thermoneutral zones, which for dairy calves typically range between 15°C and 25°C (NASEM, 2021). However, the precise lower and upper critical temperatures may vary considerably depending on a multitude of factors, including ambient conditions (bedding, wind, humidity, and so on) and physiological characteristics such as species, breed, age, weight, fat reserves, and nutritional status. When ambient temperatures extend beyond this range, thermoregulatory mechanisms are triggered, leading to metabolic and behavioral adjustments aimed at maintaining euthermia (Roland et al., 2016; Collier et al., 2019).

Due to their larger surface area relative to body mass and the lower metabolic heat production associated with a liquid diet compared with that generated through ruminal fermentation, young calves are generally more heat tolerant but less resistant to cold than adult animals (Gonzalez-Jimenez and Blaxter, 1962). Nevertheless, calves exposed to thermal stress exhibit various adverse effects, including alterations in physiological param-

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eters (Lim et al., 2021; Wang et al., 2023), reduced feed intake (Broucek et al., 2009; Rauba et al., 2019), and compromised nutrient digestibility (Christopherson and Kennedy, 1983; Mathers et al., 1989; Neves et al., 2025). These effects can ultimately detrimentally affect their health and performance (Roland et al., 2016; Fontoura et al., 2023). For instance, Wang et al. (2023) observed that adult cattle exposed to colder seasons had greater feed intake, but lower BW, ADG, nutrient digestibility, and ruminal short-chain fatty acids (**SCFA**) concentration. Similarly, Fontoura et al. (2023) reported that heat-stressed dairy calves experienced reduced feed intake, diminished ADG, and impaired feed efficiency.

Thermal amplitude, defined as the difference between maximum and minimum temperatures over a specific interval (Hijmans et al., 2005), is a common climatic feature in tropical, subtropical, and continental temperate climates, particularly during spring and autumn. Such daily fluctuations can expose calves to both cold and heat stress within the same 24-h period, posing a significant challenge to their capacity for maintaining thermal homeostasis.

Despite the prevalence of thermal amplitudes, there remains a notable lack of specific studies evaluating their effects on young calves. Therefore, the objective of this study was to evaluate the effects of daily thermal amplitude on physiological parameters, feed intake, ruminal fermentation, apparent nutrient digestibility, health status, and performance of Holstein dairy calves until 28 d of age under controlled climatic conditions, as well as to evaluate residual effects up to 90 d of age. We hypothesized that exposure to daily thermal amplitudes in Holstein dairy calves would compromise physiological parameters, reduce feed intake, negatively affect health status, and impair growth performance. However, given the paucity of neonatal data on combined cold–heat oscillations, detecting either impairment or resilience would be decision-relevant for calf management.

MATERIALS AND METHODS

The Animal Ethics Committee of Embrapa Dairy Cattle (protocol no. 4115231121) approved all procedures. We conducted the experiment at the Multiuser Laboratory of Bioefficiency and Sustainability in Livestock Farming at Embrapa Dairy Cattle in Coronel Pacheco, MG, Brazil.

Animals, Facilities, and Treatments

The study enrolled 34 Holstein calves, 13 males and 21 females, all born in a compost barn equipped with a wind tunnel. Calves were enrolled from January to early July 2022, totaling 6 mo for the complete cohort. Only calves from multiparous healthy cows housed in this compost barn during the dry period were included. During the birth period, the average environmental conditions were 23.1°C ± 2.67°C, 80.3% ± 4.13% relative humidity (**RH**), and a wind speed of 1.3 m/s, resulting in a temperature-humidity index (**THI**) of 69.7 ± 3.63. Immediately after birth, each calf was identified, and navel care was performed using 10% iodine tincture.

Colostrum was administered via oresophageal tube in 2 stages: the first within 2 h after birth, providing 10% of the calf's BW standardized to 25% Brix, and the second 6 to 8 h later, providing an additional 5% of the BW at the same Brix level.

The calves were randomly assigned into 1 of 2 treatments: (1) control (**CON**; 6 males and 11 females), maintained at a constant THI of 66 (22°C and 65% RH) for 24 h; and (2) thermal amplitude (**TA**; 7 males and 10 females), exposed daily to THI of 66 (22°C and 65% RH) from 0330 to 0630 h; THI of 84 (32°C and 65% RH) from 0630 to 1530 h; THI of 66 (22°C and 65% RH) from 1530 to 1830 h, and THI of 54 (14°C and 65% RH) from 1830 to 0330 h (Figure 1).

The trial was conducted from January to October 2022. Calves entered the climate chamber on the day of birth and remained there until 28 d of age (wk 1–4, exposure period [**EP**]). Because calvings were distributed across time, it took approximately 1 mo to complete enrollment for each chamber group. The chamber accommodated up to 9 calves at a time, so calves from each treatment were housed sequentially, with treatment periods alternating throughout the study to minimize temporal bias. After completing the EP, calves from both treatments were moved to the same barn for the postexposure period (**PEP**; 29–90 d of age [wk 5–13]). This resulted in a staggered but overlapping design: Not all calves were in the chamber or barn simultaneously, but there was a substantial overlap period in the barn when calves from both treatments were co-housed under identical conditions. So, to minimize potential confounding, we (1) randomized calf allocation to treatments, (2) alternated treatment periods to avoid systematic bias, and (3) included the housing period as a random effect in our statistical models. We also performed sensitivity analyses treating period as a fixed blocking factor.

Inside the climate chamber, calves were housed in individual pens (1.78 × 1.14 m) with floors covered by WingFlex rubber mats (Kraiburg TPE GmbH & Co., Waldkraiburg, Germany) and wood shavings as bedding. Thermometers (FEPRO-MUT600S, Exbom, São Paulo, Brazil) with maximum and minimum temperature recording and thermo-hygrometers (AK28 new, AKSO, Rio Grande do Sul, Brazil) were positioned at animal height and used throughout the experimental period. Maximum, minimum, and average temperatures, as well as RH, were recorded at 0300, 0600, 1000, 1400, 1600, and 1800 h.

These environmental data were used to calculate and regulate the THI according to Dikmen and Hansen (2009):

$$HI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)].$$

During PEP, all calves were housed in individual pens (1.20 × 1.67 m) with similar flooring (WingFlex rubber mats, Kraiburg TPE GmbH & Co., Waldkraiburg, Germany) and bedding of wood shavings. The barn was equipped with fans that were activated when the ambient temperature exceeded 22°C. Because calves exited the climate chamber at different calendar weeks, their entry into the barn during PEP was staggered. However, there was a substantial overlap period during which calves from both treatments were co-housed under the same management and environmental conditions. The daily barn THI was continuously monitored using thermo-hygrometers placed within the facility with an average THI of 71.1 ± 5.37 (range 58–83).

Feeding, Intake, and Performance

During the first 3 d of life, calves received 6 L/d of transition milk from their dams, provided in bottles. From the fourth day onward, they were fed 6 L/d of whole milk, offered in nipple buckets at 0800 and 1430 h (Milkbar, McInnes Manufacturing Limited, Waipu, New Zealand). Weaning was carried out gradually between d 61 and 67 (wk 9), with 3 L/d of whole milk offered once daily at 0800 h. Complete weaning occurred at 67 d of age.

Water and starter (20% CP; composed of 32.4% soybean meal, 61.6% cornmeal, and 6% mineral premix, Bovigold Prima, Tortuga by DSM-Firmenich, Brazil) were provided ad libitum until d 28 (Table 1). From d 28 onward, calves received a TMR consisting of 95% of the same starter used during the EP, and 5% corn silage (DM basis), offered at a maximum of 2.6 kg/d. After weaning, on d 68, corn silage was also offered ad libitum in addition to the TMR. Daily feed intake was measured by calculating the difference between the feed offered and the leftovers.

Calves were weighed before the first colostrum feeding and weekly using a mechanical scale (ICS 300, Coimma, Dracena, Brazil). To monitor growth, body measurements were taken weekly, with the calves in a standing position, including hip width, withers height, and chest girth.

Physiological Parameters and Health Scores

Heart rate (**HR**), respiration rate (**RR**), and rectal temperature (**RT**) were measured daily at 0600, 1000, 1400, and 1600 h. In the TA, measurements were also collected at 0300 and 1800 h to coincide with programmed THI transitions, whereas in the CON, these extra time points were not included because THI remained constant.

Using an acoustic stethoscope, HR was assessed, and RR was determined by visually counting thoracoabdominal movements per minute. Rectal temperature was measured using a digital thermometer inserted 2 cm into the rectum for 1 min. It was considered hyperthermia when RT exceeded 39.4°C, and severe hyperthermia when temperature exceeded 41°C. Fecal scores were evaluated visually and recorded daily, classified from 0 to 3 according to McGuirk (2008).

Blood Parameters

To evaluate passive immunity transfer efficiency, Brix was assessed 48 h after the first colostrum feeding. Calves showed a good passive transfer with average Brix values of 9.7 (±0.19) in CON and 9.3 (±0.12) in TA. A

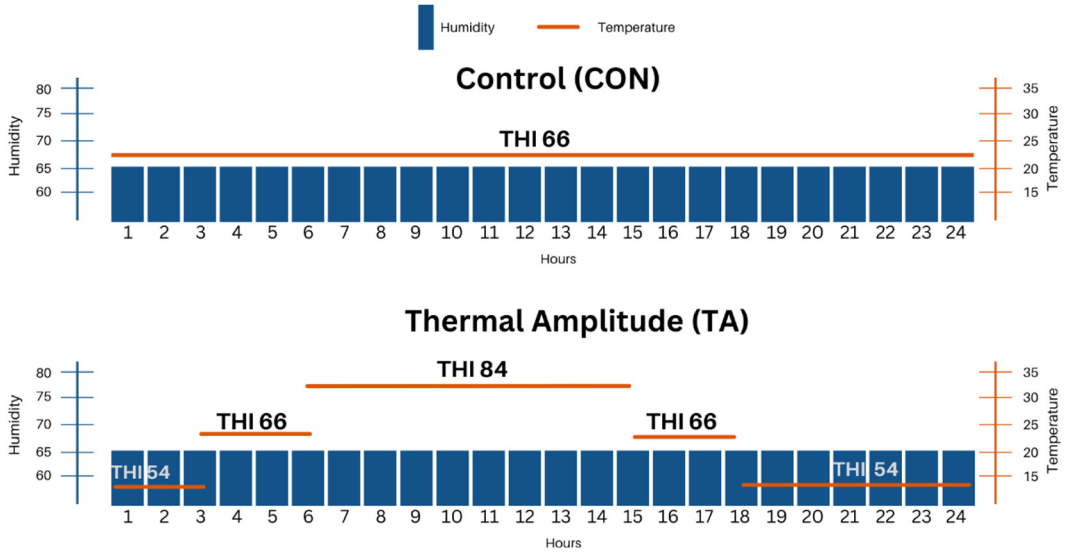

Figure 1. Temperature-humidity index (THI) scheme of the climate chamber for the control (CON) and thermal amplitude (TA) treatments.

Table 1. Average nutritional composition (dry matter basis) of the whole milk, starter, and corn silage

Nutrient[1]	Whole milk	Starter[2]	Corn silage
DM (%)	12.7	86.0	30.7
CP (% DM)	22.2	19.2	5.9
EE (% DM)	28.1	2.5	2.8
NDF (% DM)	—	9.2	50.2
Ash (% DM)	5.6	8.3	4.8
Lactose[3] (% DM)	44.1	—	—
GE (Mcal/kg)	5.8	4.0	4.2

[1]EE = ether extract; GE = gross energy.

[2]Corn, soybean meal, mineral premix.

[3]Lactose (%) = 100 − CP − EE − ash − 2 (Drackley, 2008).

baseline blood sample was collected on d 0 (first day of life), additional samples were collected at wk 1, 2, 4, 6, 8, 12 for the determination of plasma blood glucose (sodium fluoride tube, Labor Import, Osasco, Brazil), total protein, albumin, creatinine, cholesterol, triglycerides, insulin, and cortisol determination (clot activator tube, Labor Import, Osasco, Brazil). All blood samples were obtained 4 h after the morning feeding to standardize collection relative to nutrient intake. Serum for cytokine analysis at 28 d (wk 4) was collected before the morning feeding and used to determine cytokines concentrations, including interferon-gamma (IFN-γ), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-17A (IL-17A), interferon-gamma-induced protein 10 (IP-10), macrophage chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), and vascular endothelial growth factor (VEGF-A). All samples were centrifuged at 2,500 × *g* for 15 min at room temperature (22°C–25°C), and the resulting plasma or serum was stored at −20°C until analysis.

Metabolites were quantified using commercial kits (Biotécnica, Varginha, Brazil; Glucose Plus VET 90.068.00; Total Protein VET 90.019.00; Albumin VET 90.020.00; Creatinine VET 90.012.00; Cholesterol 10.004.00; Triglycerides VET 90.022.00) following the enzymatic colorimetric method on Cobas Mira Plus analyzer (Cobas Mira Plus, Roche Diagnóstica Brazil Ltda., São Paulo, Brazil). Serum insulin and cortisol concentrations were determined by chemiluminescence assays (Immulite2000 Systems 10381455, Insulin 200, and Immulite2000 Systems 10381476, Cortisol 200, Siemens Healthcare Diagnostics Products Ltd., Llanberis, Gwynedd, United Kingdom).

Cytokine concentrations were measured using the bovine cytokine/chemokine kit (MILLIPLEX; Millipore Corporation, Billerica, MA), with antibodies against IFN-γ, IL-4, IL-8, IL-10, IL-17A, IP-10, MCP-1, MIP-1β, and VEGF-A. Shortly, serum samples were diluted 1:2 in assay buffer, then 25 µL of the standard, the control, and duplicate samples were added to the plate. Metal beads were added, followed by covering the plate and sealing it with aluminum foil. The plates were placed on a plate shaker and left to incubate overnight at 4°C. After incubation, the plates were washed 3 times, and 25 µL of detection antibody was added to each well, followed by 1 h of incubation at room temperature. Subsequently, 25 µL of streptavidin-phycoerythrin was added to each well, and the plates were incubated for an additional 30 min at room temperature. Following this process, the plates were subjected to a series of washes, and 150 µL of sheath fluid was added. The concentrations of the markers were measured using the Luminex MAGPIX instrument and xPONENT software (Luminex Corporation, Austin, TX). All quality control values for each market were consistent with the ranges specified by the manufacturer. Final concentrations (pg/mL) were calculated using Belysa software (Merck KGaA, Darmstadt, Germany) based on the quality control curve.

Ruminal Parameters

Ruminal fluid samples were collected in wk 2, 4, 8, and 12 to determine pH, SCFA, and ammonia nitrogen (NH_3-N) 3 h after the first feeding using an oresophageal tube. Immediately after collection, the ruminal fluid was filtered through gauze, and the pH was measured using a digital pH meter (Phmetro T-1000, Tekna, Araucária, Brazil). Two 10-mL aliquots were stored at −20°C for further analysis. One aliquot was preserved with 1 mL of metaphosphoric acid (20% vol/vol) for SCFA analysis, and the other with 1 mL of sulfuric acid (50% vol/vol) for NH_3-N determination.

The SCFA concentrations were determined using HPLC (Waters Alliance e2695 Chromatograph, Waters Technologies do Brazil Ltda, Barueri, Brazil) after centrifugation at 1,800 × *g* for 10 min at room temperature (22°C–25°C). The NH_3-N was quantified using the colorimetric distillation method proposed by Chaney and Marbach (1962). The absorbance was determined at 630 nm with a spectrophotometer (Thermo Fisher Scientific, Madison, WI) after Kjeldahl distillation with magnesium oxide and calcium chloride according to method 920.03 (AOAC International, 2012).

Digestibility

Two apparent digestibility trials were conducted: the first from 9 to 12 d of age and the second from 23 to 26 d of age. Ten calves were randomly selected to participate in each trial. Total fecal collection was performed over 3 consecutive days, and total urine collection was carried out for 1 d using metabolic cages (Silva et al., 2015). Urine was weighed and total volume measured. Two 50-mL aliquots of urine were collected and stored at −20°C for nitrogen. Milk and starter feed samples were

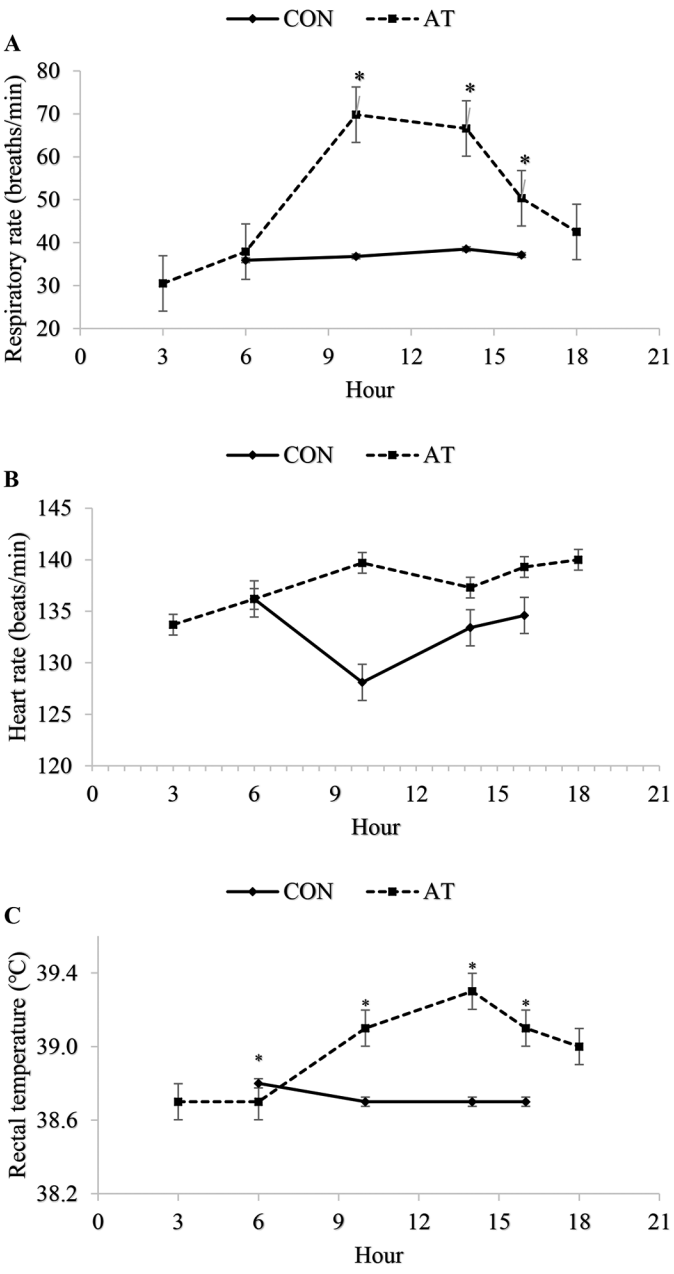

Figure 2. Physiological parameters of calves throughout the day. (A) Respiratory rate (breaths/min); (B) heart rate (beats/min); and (C) rectal temperature (°C) of control (CON) and thermal amplitude (TA) treatments. Measurements were taken at 0600, 1000, 1400, and 1600 h in both treatments, and additionally at 0300 and 1800 h only in the TA to capture changes coinciding with programmed THI transitions. Values are LSM ± SEM. Significant differences between CON and TA at specific time points are indicated by * ($P \leq 0.05$).

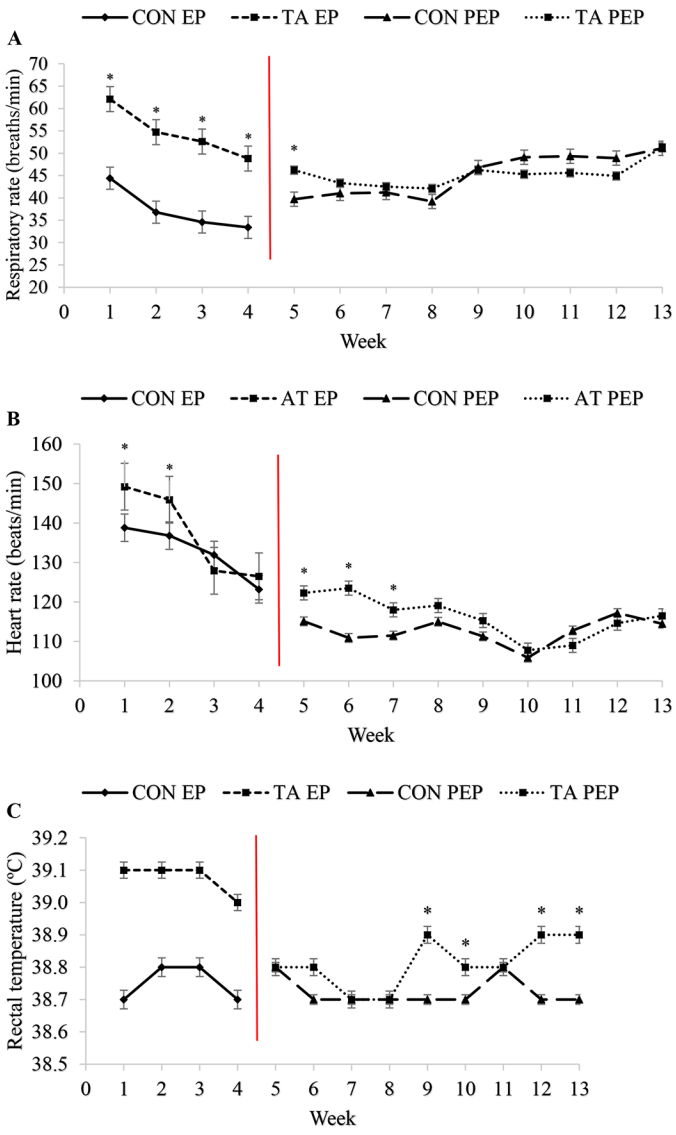

Figure 3. Weekly averages of physiological parameters in Holstein calves during the experimental period. (A) Respiratory rate (breaths/min); (B) heart rate (beats/min); and (C) rectal temperature (°C). Statistical comparison between control (CON) and thermal amplitude (TA) treatments were performed at 0600, 1000, 1400, and 1600 h. Significant when $P \leq 0.05$. *Indicates a significant interaction between treatment and week. The red vertical line separates the exposure period (EP) and the postexposure period (PEP); error bars represent SEM.

collected every 3 d during the digestibility trials. Feces, starter feed, and refusals were weighed and stored at −20°C for proximate composition analysis.

For apparent nutrient digestibility analysis, all samples were dried in a forced-air oven at 55°C for 72 h and ground using a Wiley mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA) with a 1-mm sieve. Analyses included DM (method 934.01), CP (method 988.05), ether extract (method 920.39), and ash (method 942.05) following AOAC procedures (AOAC International, 2012). Gross energy (GE) was determined using an adiabatic bomb calorimeter (Parr 6200, Calorimeter Instrument Company, Moline, IL).

Apparent nutrient digestibility and nitrogen balance were calculated according to the following equations:

Table 2. Milk, starter, water, and silage intake of calves in the control (CON) and thermal amplitude (TA) treatments during the exposure (EP) and postexposure (PEP) periods

	Treatment[1]			*P*-value[2]		
Item	CON	TA	SEM	T	W	T × W
EP[3]						
Milk (g DM/d)	727.7	742.3	4.54	0.03	<0.01	0.17
Starter (g DM/d)	37.2	35.1	6.03	0.75	<0.001	0.65
Total DM (g DM/d)	781.4	788.5	9.46	0.44	<0.001	0.30
Total CP (g/d)	182.7	184.3	1.85	0.41	<0.001	0.13
Total GE (Mcal/d)	4.3	4.3	0.435	0.31	<0.001	0.24
Water (kg/d)	0.7	1.3	0.16	<0.01	<0.001	0.03
PEP[4]						
Milk (g DM/d)	667.8	673.3	—	—	—	—
Starter (g DM/d)	985.9	1038.2	37.01	0.76	<0.001	<0.02
Silage (g DM/d)	519.3	479.0	146.09	0.98	<0.01	0.39
Total DM (g DM/d)	990.6	1070.4	50.02	0.43	<0.001	0.07
Total CP (g/d)	314.2	329.0	5.43	0.33	<0.001	0.05
Total GE[5] (Mcal/d)	7.8	8.3	0.147	0.09	<0.001	0.03
Water (kg/d)	5.1	6.3	0.49	0.05	<0.001	<0.001

[1]Treatments: CON = control; TA = thermal amplitude.

[2]*P*-value: T = treatment effect; S = week effect; T × S = treatment-week interaction. Significant when $P \leq 0.05$.

[3]Exposure period from covers wk 1 to wk 4 of age.

[4]Postexposure period covers wk 5 to wk 13 of age.

[5]GE = gross energy.

$$\textit{Digestibility}\ (\%) = (\textit{Total nutrient intake} - \textit{nutrient excreted in feces}) \times 100 - \textit{Total nutrient intake},\ \text{and}$$

$$\textit{Nitrogen balance}\ (\text{g/d}) = \textit{Total nitrogen intake} - \textit{fecal nitrogen} - \textit{urine nitrogen}.$$

Statistical Analysis

The data collected were organized by week, spanning a total of 13 wk, and analyzed using R software (version 4.3.3; R Core Team, 2024, version 4.3.3). A linear mixed model was applied to evaluate responses, such as intake, physiological parameters, blood variables, ruminal characteristics, and performance (nlme package; version 4.3.3; R Core Team, 2024). The model included the fixed effects of treatment, week, or hour (for physiological parameters) and their interactions as repeated measures. Birth weight, Brix value for passive immunity transfer, and sex were tested as covariates and included in the model when statistically significant ($P < 0.05$). Because calves within the same treatment were housed together in the climatic chamber during the same periods, the housing period for each treatment was included as a random effect to account for potential environmental clustering. Apparent digestibility, nitrogen balance, days with diarrhea or hyperthermia, and cytokine concentrations were analyzed using ANOVA, with treatment as the fixed effect, and animal and period were random effects, implemented through a generalized linear model.

In the cytokine analyses, hormones and blood metabolites, d 0 values were included as covariates. All models were tested for normality and homoscedasticity using the Shapiro-Wilk and Bartlett tests ($P < 0.05$). The null hypothesis was evaluated at a 95% CI, with significance determined by $P \leq 0.05$ using a *t*-test. Fecal scores were analyzed using a nonparametric aligned rank transform performed with the ARTool package (version 4.3.3; R Core Team, 2024).

RESULTS

Physiological Parameters

During the EP, significant differences in RR and HR were observed between treatments at 1000, 1400, and 1600 h ($P < 0.01$; Figure 2). This time corresponded to the period of heat exposure in the TA (1000 and 1400), and the immediate postexposure phase (1600 h). At 1600 h, the RR in the TA was 81.3% higher than CON. During the PEP, significant effects of hour were observed ($P < 0.01$). Across this period, RR increased throughout the day, ranging from 33.0 ± 0.94 breaths/min (**bpm**) at 0600 h to 54.1 ± 1.65 bpm at 1600 h (Figure 2).

Regarding the RT during the EP, significant effects of treatment, hour, and treatment × hour interaction were observed (Figure 2). At 1000, 1400, and 1600 h, mean

Table 3. Blood metabolites and hormones concentrations in calves from control (CON) and thermal amplitude (TA) treatments during the exposure (EP) and postexposure (PEP) periods

	Treatment[1]			*P*-value[2]		
Item	CON	TA	SEM	T	W	T × W
EP						
Glucose (mg/dL)	127.7	127.6	3.04	0.98	<0.001	0.98
Total protein (g/dL)	6.4	6.1	0.11	0.09	<0.001	<0.01
Albumin (g/dL)	3.6	3.6	0.03	0.60	<0.001	0.57
Creatinine (µg /dL)	867.0	864.6	28.1	0.93	0.24	0.16
Cholesterol (mg/dL)	75.6	82.0	3.88	0.34	<0.001	<0.01
Triglycerides (mg/dL)	28.8	32.0	2.11	0.29	0.46	0.22
Insulin (ng/dL)	13.6	10.24	1.49	0.11	<0.001	<0.01
Cortisol (µg/dL)	0.6	0.5	0.03	0.19	<0.01	0.68
PEP						
Glucose (mg/dL)	98.7	98.6	2.55	0.60	<0.001	0.13
Total protein (g/dL)	5.8	5.7	0.09	0.19	<0.01	0.16
Albumin (g/dL)	4.0	4.1	0.06	0.60	<0.001	0.96
Creatinine (µg /dL)	654.2	708.9	37.6	0.05	<0.001	<0.01
Cholesterol (mg/dL)	74.8	85.4	5.42	0.02	<0.001	0.04
Triglycerides (mg/dL)	26.2	28.7	2.41	0.22	0.02	0.67

[1]Treatments: CON = control; TA = thermal amplitude.

[2]*P*-value: T = treatment effect; W = week effect; T × W = treatment-week interaction. Significant when $P \leq 0.05$.

RT in the TA (39.1°C, 39.3°C, and 39.1°C ± 0.03°C) was higher than that of the CON (38.7°C ± 0.03°C all 3 times). Conversely, at 0600 h, RT was lower in the TA (38.6°C ± 0.03°C) compared with CON (38.8°C ± 0.03°C). During heat exposure hours (1000 and 1400 h), RT in the TA was, on average, 0.49°C greater than in the CON ($P < 0.01$). Additionally, calves in the TA experienced 10 more days with hyperthermia ($P < 0.001$).

In the PEP, RT was influenced by treatment ($P = 0.01$), hour ($P < 0.01$), as well as treatment × hour ($P < 0.01$). Calves of the CON group had greater RR during EP, but only in the first week during PEP ($P < 0.05$; Figure 3).The barn environment during PEP had a mean THI of 71.2 ± 5.4, with daily values ranging from 58 to 83, and these ranges were similar across overlapping periods when calves from both treatments were housed simultaneously.

Feed Intake

During the EP, no differences in starter intake, total DMI, CP, and GE intakes ($P > 0.05$; Table 2) were observed between the treatments. However, calves in the TA consumed, on average, 14.6 g/d more milk than those in CON ($P = 0.03$). Water intake was significantly greater in the TA during the third and fourth weeks of life. In wk 3, water intake averaged 0.83 ± 0.16 kg/d in the CON and 1.56 ± 0.20 kg/d in the TA; in wk 4, intake was 0.88 ± 0.17 kg/d and 1.83 ± 0.21 kg/d for CON and TA, respectively (Table 2). In the PEP, no differences were observed for nutrient intake, expect for water intake, that remained greater for TA ($P < 0.05$; Table 2).

Blood Parameters

Thermal amplitude did not significantly affect glucose, albumin, creatinine, triglycerides, insulin, or cortisol concentrations ($P > 0.05$; Table 3) during EP. However, a treatment × week interaction was observed for total protein concentration ($P < 0.01$). Calves in TA had a lower total protein level in wk 2 (TA = 6.02 ± 0.13 g/dL vs. CON = 6.58 ± 0.13, $P < 0.01$; Table 3).

In PEP, there were no differences between treatments, but a significant treatment × week interaction was observed. At wk 6, calves in the TA had greater cholesterol (TA = 114.1 ± 7.62 vs. CON = 85.61 ± 5.93 mg/dL; $P = 0.04$) and creatinine concentrations (TA = 0.83 ± 0.04 vs. CON = 0.67 ± 0.03 mg/dL; $P < 0.01$). No significant differences were observed for glucose, albumin, total protein and triglycerides concentrations during this period ($P > 0.05$; Table 3).

Regarding cytokine analysis, the IP-10 and IL-8 concentrations were lower for TA, and IL-4 was higher during EP ($P < 0.01$). No treatment effects were detected for the other cytokines evaluated, including IFN-γ, MIP-1, IL-10, MCP-1, and VEGFA ($P > 0.05$; Table 4).

Ruminal Fermentation

During EP, no significant differences were observed between treatments for most ruminal fermentation parameters, except for acetate:propionate ratio, which was greater in the TA compared with the CON ($P = 0.04$; Table 5).

In PEP, ruminal pH was greater in the TA ($P = 0.01$). Additionally, total SCFA ($P = 0.04$) and acetate ($P = 0.02$)

Table 4. Blood cytokine concentrations in calves from control (CON) and thermal amplitude (TA) treatments at 28 d of age

	Treatment[1]			
Item (pg/mL)	CON	TA	SEM	*P*-value[2]
IFN-γ	0.99	1.53	0.40	0.76
MIP-1	28.8	48.2	12.17	0.11
IL-4	27.5	39.8	1.57	<0.001
IL-8	124.6	99.9	3.04	<0.01
IL-10	12.55	11.46	1.85	0.69
IL-17A	1.7	0.4	0.56	0.25
IP-10	6722.4	2703.3	710.07	<0.01
MCP-1	280.5	227.1	33.8	0.29
VEGFA	18.4	19.0	3.97	0.95

[1]Treatments: CON = control; TA = thermal amplitude.
[2]Significant when $P \leq 0.05$.

concentrations were lower in the TA. There was no treatment effect for NH_3-N concentration ($P > 0.05$; Table 5).

Digestibility

The apparent DM digestibility, nutrient digestibility and body nitrogen metabolism were not affected by TA in the first digestibility trial ($P > 0.05$; Table 6). However, in the second digestibility trial, although no differences were observed in nutrient digestibility between treatments, fecal nitrogen excretion was significantly greater in the TA ($P = 0.02$; Table 6).

Health Parameters

During EP and PEP, no differences were observed between treatments for fecal scores, days with diarrhea, or days with severe diarrhea ($P > 0.05$; Table 7).

Performance

Observed BW at birth, 28 d, and 90 d did not differ between treatments (Table 8). Likewise, LSM of ADG estimated from the mixed model were not different between CON and TA calves during either EP or PEP ($P > 0.05$; Table 8).

DISCUSSION

This study aimed to mimic daily thermal amplitude and evaluate its impact on physiological parameters, intake, ruminal fermentation, apparent nutrient digestibility, blood metabolites, hormone markers, health status, and performance of Holstein calves during the EP, as well as the residual effects until 90 d of age.

Because the EP was conducted in sequential periods, we prioritized treatment main effects in interpreting physiological and metabolic responses; in this light, TA consistently increased RR, RT, and water intake, with modest carryover into PEP, whereas performance and health remained unaffected. We hypothesized that neonatal daily thermal amplitude would increase the RR, HR, and RT; reduce nutrient intake and digestibility; impair growth and body development; and negatively affect inflammatory status and health, with carryover effects after exposure. However, the results only partially confirmed this hypothesis. Under adequate colostrum management, nutrition (~17% of BW in milk), and hygiene, daily thermal amplitude elicited physiological adjustments (RR, RT, water intake) and modest rumen shifts without compromising health, digestibility, or growth.

The main findings were as follows: calves exposed to thermal amplitude (1) exhibited higher RR and RT during both (EP)and PEP; (2) consumed more water but maintained nutrient intake and metabolic balance across both periods; (3) showed altered ruminal fermentation patterns during EP, with carryover effects persisting into PEP; (4) experienced more days with hyperthermia during EP, although this did not negatively affect health status or growth performance during either EP or PEP; and (5) demonstrated lower concentrations of cytokines IP-10, IL-8, and higher IL-4.

Thermal amplitude markedly affected the calves' physiological responses, especially RR, RT, and HR. During heat exposure, RR increased by over 80% in the TA compared with CON, consistent with an effort to dissipate excess body heat through panting. These findings are aligned with previous studies demonstrating that heat stress stimulates thermoregulatory mechanisms such as increased RR and peripheral vasodilation (Purwanto et al., 1993; Roland et al., 2016). Despite this physiological response, it was not sufficient to fully prevent the rise in RT during the hottest periods. Notably, elevated RR persisted beyond the EP, suggesting prolonged physiological adaptation or delayed recovery after heat exposure. The HR also increased during EP in the TA, particularly in the first 2 wk, which aligns with the physiological response expected during peripheral vasodilation induced by heat stress (Purwanto et al., 1993). As blood shifts toward the skin surface to dissipate heat, cardiac output increases to maintain systemic blood pressure. The observed HR responses suggest that the calves experienced sustained cardiovascular adjustments beyond the immediate EP. This pattern is consistent with the understanding that HR reflects both basal metabolic demands and acute responses to thermal stress (Roland et al., 2016). Looking at the treatments at each time point during the EP, RT mirrored fluctuations in environmental conditions. It rose sharply during periods of heat and dropped during the cold phases, indicating that the calves' thermoregulatory mechanisms were continuously challenged during heat (Koga et al.,

Table 5. pH, ruminal ammonia nitrogen (NH_3-N) and ruminal short-chain fatty acids (SCFA) concentrations in calves from control (CON) and thermal amplitude (TA) treatments during the exposure (EP) and postexposure periods (PEP)

	Treatment[1]			*P*-value[2]		
Item	CON	TA	SEM	T	W	T × W
EP						
pH	6.4	6.3	0.13	0.93	0.58	0.53
NH_3-N (%)	13.3	14.2	1.54	0.79	0.05	0.21
Total SCFA (µmol/L)	37.1	35.4	4.16	0.77	<0.01	0.50
Acetate (µmol/L)	21.4	21.6	2.08	0.92	<0.01	0.73
Propionate (µmol/L)	9.4	7.5	1.27	0.19	<0.001	0.20
Butyrate (µmol/L)	3.7	2.9	0.56	0.20	<0.01	0.75
Acetate:propionate ratio	2.3	2.7	0.61	0.05	0.04	0.53
PEP						
pH	6.3	6.8	0.10	0.01	0.01	0.76
NH_3-N (%)	5.4	5.6	1.02	0.80	0.02	0.52
Total SCFA (µmol/L)	61.3	44.2	5.7	0.04	0.51	0.20
Acetate (µmol/L)	32.7	21.7	3.18	0.02	0.19	0.24
Propionate (µmol/L)	18.6	13.1	2.04	0.09	0.17	0.22
Butyrate (µmol/L)	8.1	8.6	1.07	0.64	0.62	0.29
Acetate:propionate ratio	1.8	1.7	0.17	0.81	0.81	0.74

[1]Treatments: CON = control; TA = thermal amplitude.

[2]*P*-value: T = treatment effect; W = week effect; T × W = treatment-week interaction. Significant when $P \leq 0.05$.

2002; Bhan et al., 2013) and cold stress (Lim et al., 2021; Wang et al., 2023). These variations in RT indicate that the calves exhausted their thermoregulatory mechanisms, expressing changes in RT compared with CON. During PEP, RT continued to be greater in the TA despite uniform environmental conditions for both treatments, indicating a residual physiological effect of the earlier exposure to thermal fluctuation. This trend likely reflects both a carryover effect from the EP and increased metabolic heat production related to dietary changes as calves transitioned from a liquid to a solid diet, with a substantial increase in starter and silage intake contributing to greater ruminal fermentation and internal heat load. In wk 8, the average DM intake was 0.428 kg/d, and in wk 9, this intake increased to 2.92 kg/d. These dietary changes contributed to a higher fermentation rate in the rumen and increased heat production, explaining the rise in physiological parameters until the end of the experiment.

Among the physiological parameters, RR was the most sensitive to cold exposure. Values measured at 0300 and 1800 h reflected the calves' rapid physiological adjustment to nighttime cooling, demonstrating that the thermoregulatory burden imposed by cold was transient and less intense than that caused by daytime heat exposure. Even so, the magnitude of the changes during cold periods was less than that observed during heat exposure, suggesting that short periods of cold had a milder physiological effect under the conditions of this study (Lim et al., 2021; Wang et al., 2023). Water intake increased substantially in the TA, particularly during the hottest parts of the day and continuing into PEP. This increase is a well-established response to elevated respiratory water loss and the thermoregulatory need to facilitate evaporative cooling (Broucek et al., 2009; Bakony and Jurkovich, 2020). Despite the increased water demand, milk intake was not negatively affected; in fact, the TA consumed slightly more milk. These results contrast with the common observation that heat stress typically reduces feed intake in dairy calves (Roland et al., 2016; Fontoura et al., 2023), indicating that the moderate level of nutritional support provided in this study was sufficient to buffer the adverse effects of thermal stress on feeding behavior. In PEP, the TA maintained greater water intake until wk 9. This difference likely stems from ingestive behavior and rumen-filling capacity established in previous weeks in the TA.

The alterations in ruminal fermentation observed in the TA provide further evidence of the systemic impact of thermal stress. The greater acetate:propionate ratio during EP and the elevated ruminal pH during PEP indicate shifts in microbial fermentation patterns. The increase in pH during PEP likely reflects a combination of greater water intake by TA calves, which may have diluted ruminal contents, and resulted in lower total SCFA and acetate concentrations, indicating reduced acid production from fermentation. However, these residual shifts did not translate into differences in digestibility, nitrogen balance or performance, persisting even after calves were co-housed under the same barn conditions. Previous studies have shown that thermal stress, both heat and cold, can influence gut microbiota composition, fermentation end products, and rumen efficiency (Liu et al., 2022; Zhang et al., 2022), which supports the find-

Table 6. Apparent nutrient digestibility and nitrogen balance of calves from control (CON) and thermal amplitude (TA) treatments during the exposure period (EP)

	Treatment[1]			
Item	CON	TA	SEM	*P*-value[2]
First digestibility: 9–12 d of age				
Nutrients[3] (%)				
DM	96.5	96.0	0.05	0.42
OM	99.6	99.5	0.06	0.85
CP	92.3	90.8	1.17	0.30
EE	98.6	97.8	0.50	0.29
GE	96.5	96.1	0.05	0.47
Nitrogen intake (g/d)	29.0	30.9	0.87	0.17
Fecal nitrogen (g/d)	2.2	2.8	0.33	0.26
Urinary nitrogen (g/d)	4.2	4.2	0.65	0.96
Retained nitrogen (g/d)	23.1	23.5	1.27	0.78
Second digestibility: 23–26 d of age				
Nutrients (%)				
DM	96.7	94.8	0.85	0.20
OM	99.6	99.3	0.12	0.13
CP	92.8	88.4	1.86	0.16
EE	97.8	96.2	0.70	0.20
GE	96.8	95.8	0.75	0.39
Nitrogen intake (g/d)	30.9	29.5	0.96	0.39
Fecal nitrogen (g/d)	2.1	4.1	0.44	0.02
Urinary nitrogen (g/d)	6.0	7.5	1.09	0.09
Retained nitrogen (g/d)	23.4	20.2	1.99	0.31

[1]Treatments: CON = control; TA = thermal amplitude.

[2]Significant when $P \leq 0.05$.

[3]EE = ether extract; GE = gross energy.

Table 7. Health parameters of calves from control (CON) and thermal amplitude (TA) treatments during the exposure (EP) and postexposure (PEP) periods

	Treatment[1]			
Item	CON	TA	SEM	*P*-value[2]
EP				
Fecal score	0.58	0.34	0.094	0.87
Days in diarrhea	5.76	3.65	0.17	0.34
Days with severe diarrhea	2.88	1.71	0.19	0.56
PEP				
Fecal score	0.39	0.35	0.077	0.90
Days in diarrhea	7.74	6.9	0.15	0.80
Days with severe diarrhea	3.00	2.31	0.15	0.32

[1]Treatments: CON = control; TA = thermal amplitude.

[2]Significant when $P \leq 0.05$.

ings observed here. Despite alterations, apparent nutrient digestibility was unaffected during the first digestibility trial (Christopherson and Kennedy, 1983; Mathers et al., 1989). In the second trial, although nutrient digestibility remained unchanged, calves in the TA excreted more fecal nitrogen, suggesting a subtle reduction in nitrogen utilization efficiency. However, this was not sufficient to affect overall nitrogen balance or compromise calf performance, which remained consistent between treatments despite the changes in ruminal environment.

Blood metabolites and hormone concentrations were remarkably stable, with no significant treatment effects for glucose, albumin, creatinine, triglycerides, insulin, and cortisol. This stability suggests that the calves maintained metabolic homeostasis despite the physiological challenges imposed by daily thermal amplitude. Exceptions included transient increases in cholesterol and creatinine in the TA during specific weeks of PEP. The elevation in cholesterol may be attributed to the greater milk intake observed during EP, as well as an 84% increase in starter consumption compared with CON during PEP. The rise in creatinine is directly related to protein metabolism, and although animals under heat stress tend to have greater creatinine concentrations, the TA did not show changes in this metabolite. The difference in PEP is not explained by increased starter intake but may likely reflect increased muscle mass development (van Niekerk et al., 1963).

Total protein was transiently lower in the TA during the second week of EP. This reduction may not be directly linked to thermal stress but could reflect hydration status differences or responses to minor health disturbances, such as peaks in diarrhea episodes observed at that time. The greater water intake in the TA likely mitigated the degree of dehydration, contrasting with the CON, where dehydration could have contributed to a relative increase in total protein concentration.

The immune response was affected by thermal amplitude. The significant change was a reduction in the concentration of IP-10 and IL-8 and an increase in IL-4 in the TA. The chemokines IP-10 and IL-8 play a critical role in inflammatory responses and the recruitment and activation of immune cells (Widdison and Coffey, 2011). The observed reduction could suggest a dampened inflammatory response, although there were no corresponding changes in other cytokines such as IFN-γ, IL-10, or MCP-1. The absence of treatment effects on cortisol supports the interpretation that the calves did not experience prolonged or severe stress capable of suppressing or overstimulating immune function (Bagath et al., 2019). The thermal amplitude did not increase the incidence or severity of health disorders, such as diarrhea or respiratory disease. Although the TA experienced significantly more days with hyperthermia, these elevations in RT were physiological responses to environmental conditions rather than indicators of clinical disease. Proper management, including effective colostrum intake, hygiene practices, and adequate nutrition, likely contributed to the maintenance of good health in both treatments, even under thermal stress conditions (Barrington et al., 2002; Gorden and Plummer, 2010). Calf growth and body development were not negatively affected by exposure to thermal amplitudes. Body measurements and ADG remained consistent between treatments, with only minor,

Table 8. Performance parameters of calves from control (CON) and thermal amplitude (TA) treatments during the exposure (EP) and postexposure (PEP) periods

	Treatment[1]			
Parameter[2]	CON	TA	SEM	*P*-value[3]
Weight				
Initial weight (kg)	35.1	34.7	1.14	0.89
Final weight 28 d, EP (kg)	51.1	51.5	0.74	0.45
Final weight 90 d, PEP (kg)	109.1	109.0	1.53	0.50
ADG				
ADG, EP (g/d)	521.9	573.2	23.88	0.24
ADG, PEP (g/d)	953.4	927.8	25.92	0.28
Body measurement				
Withers height at 28 d (cm)	82.2	82.1	0.51	0.87
Hip width at 28 d (cm)	24.2	24.2	0.29	0.93
Chest girth at 28 d (cm)	86.2	85.6	0.59	0.53
Withers height at 90 d (cm)	96.9	98.2	0.56	0.15
Hip width at 90 d (cm)	29.8	29.3	0.41	0.45
Chest girth at 90 d (cm)	109.4	108.4	0.61	0.28

[1]Treatments: CON = control; TA = thermal amplitude.

[2]Observed BW are raw means,whereas ADG values are LSM estimated by the mixed model; therefore, ADG may not exactly equal arithmetic weight changes over time.

[3]Significant when $P \leq 0.05$.

transient differences observed at specific weeks. These findings indicate that the calves' energy intake was sufficient to meet both maintenance and growth requirements, even with the increased energetic cost of thermoregulation. The calculation based on NASEM (2021) indicates that the metabolizable energy provided by milk alone was adequate to support the energy demands induced by both cold and heat exposure, preventing negative effects on growth performance. There is an increase of 2.01 kcal/kg$^{0.75}$ for every 1.0°C drop below the thermoneutral zone. Calves were offered milk equal to approximately 17% of their BW, and considering the average weight of the animals during the period was 43.8 kg, the average milk solid intake was 0.734 kg/d, and the ME of milk was 5.37 Mcal/kg of DM, the milk alone provided 3.90 Mcal/d, sufficient for an approximate gain of 0.600 kg/d (NASEM, 2021), a value consistent with that found in this study during the EP.

This comprehensive evaluation demonstrates that although thermal amplitude imposes measurable physiological and metabolic adjustments, in a controlled environment, these do not translate into detrimental outcomes for calf growth or health under adequate management and nutritional conditions. An important limitation is that treatments were imposed sequentially during EP, and calves entered the barn at different calendar weeks during PEP. Although not all calves were present simultaneously throughout the entire PEP, there was a prolonged overlap in which animals from both treatments were co-housed under the same barn conditions. For this reason, we emphasize treatment main effects and report the range of barn THI, acknowledging that mean values alone may not capture the full variability in exposure.

CONCLUSIONS

Daily thermal amplitudes challenged thermoregulation in neonatal calves, increasing RR, HR, RT, and water intake. Despite physiological and metabolic adjustments, including altered ruminal fermentation and reduced IP-10 and IL-8, and increased IL-4 concentrations, no negative effects on health, nutrient intake, digestibility, or performance were observed. Residual effects on thermoregulation and rumen parameters persisted but did not impair growth. These results indicate conditional resilience: Neonatal calves managed under robust husbandry can adapt to daily thermal amplitudes, though thermoregulatory carryover and rumen fermentation differences may persist. Further studies are needed to explore long-term effects under different environmental and nutritional conditions.

NOTES

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Nonstandard abbreviations used: bpm = breaths/min; CON = control; EE = ether extract; EP = exposure period; GE = gross energy; HR = heart rate; PEP = postexposure period; RH = relative humidity; RR = respiration rate; RT = rectal temperature; SCFA = short-chain fatty acids; TA = thermal amplitude; THI = temperature and humidity index.

REFERENCES

AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International, Gaithersburg, MD.

Bagath, M., G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, and V. Sejian. 2019. The impact of heat stress on the immune system in dairy cattle: A review. Res. Vet. Sci. 126:94–102. https://doi.org/10.1016/j.rvsc.2019.08.011.

Bakony, M., and V. Jurkovich. 2020. Heat stress in dairy calves from birth to weaning. J. Dairy Res. 87(S1):53–59. https://doi.org/10.1017/S0022029920000618.

Barrington, G. M., J. M. Gay, and J. F. Evermann. 2002. Biosecurity for neonatal gastrointestinal diseases. Vet. Clin. North Am. Food Anim. Pract. 18:7–34. https://doi.org/10.1016/S0749-0720(02)00005-1.

Bhan, C., S. V. Singh, O. K. Hooda, R. C. Upadhyay, and B. Beenam. 2013. Influence of temperature variability on physiological, hematological and biochemical profiles of growing and adult karan fries cattle. Indian J. Anim. Sci. 83:1090–1096.

Broucek, J., P. Kisac, and M. Uhrincat. 2009. Effect of hot temperatures on the hematological parameters, health and performance of calves. Int. J. Biometeorol. 53:201–208. https://doi.org/10.1007/s00484-008-0204-1.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130–132. https://doi.org/10.1093/clinchem/8.2.130.

Christopherson, R. J., and P. M. Kennedy. 1983. Effect of the thermal environment on digestion in ruminants. Can. J. Anim. Sci. 63:477–496. https://doi.org/10.4141/cjas83-058.

Collier, R. J., L. H. Baumgard, R. B. Zimbelman, and Y. Xiao. 2018. Heat stress: Physiology of acclimation and adaptation. Anim. Front. 9:12–19. https://doi.org/10.1093/af/vfy031.

Dikmen, S., and P. J. Hansen. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. 92:109–116. https://doi.org/10.3168/jds.2008-1370.

Drackley, J. K. 2008. Calf nutrition from birth to breeding. Vet. Clin. North Am. Food Anim. Pract. 24:55–86. https://doi.org/10.1016/j.cvfa.2008.01.001.

Fontoura, A. B. P., V. Sáinz de la Maza-Escolà, A. T. Richards, B. N. Tate, M. E. Van Amburgh, E. Grilli, and J. W. McFadden. 2023. 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. J. Dairy Sci. 106:2904–2918. https://doi.org/10.3168/jds.2021-21724.

Gonzalez-Jimenez, E., and K. L. Blaxter. 1962. The metabolism and thermal regulation of calves in the first month of life. Br. J. Nutr. 16:199–212. https://doi.org/10.1079/bjn19620021.

Gorden, P. J., and P. Plummer. 2010. Control, management, and prevention of bovine respiratory disease in dairy calves and cows. Vet. Clin. North Am. Food Anim. Pract. 26:243–259. https://doi.org/10.1016/j.cvfa.2010.03.004.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978. https://doi.org/10.1002/joc.1276.

Koga, A., T. Kuhara, and Y. Kanai. 2002. Comparison of body water retention during water deprivation between swamp buffaloes and Friesian cattle. J. Agric. Sci. 138:435–440. https://doi.org/10.1017/S0021859602002174.

Lees, A. M., V. Sejian, A. L. Wallage, C. C. Steel, T. L. Mader, J. C. Lees, and J. B. Gaughan. 2019. The impact of heat load on cattle. Animals (Basel) 9:322. https://doi.org/10.3390/ani9060322.

Lim, D. H., T. I. Kim, S. M. Park, K. S. Ki, and Y. Kim. 2021. Evaluation of heat stress responses in Holstein and Jersey cows by analyzing physiological characteristics and milk production in Korea. J. Anim. Sci. Technol. 63:872–883. https://doi.org/10.5187/jast.2021.e62.

Liu, X., Y. Sha, W. Lv, G. Cao, X. Guo, X. Pu, J. Wang, S. Li, J. Hu, and Y. Luo. 2022. Multi-omics reveals that the rumen transcriptome, microbiome, and its metabolome co-regulate cold season adaptability of tibetan sheep. Front. Microbiol. 13:859601. https://doi.org/10.3389/fmicb.2022.859601.

Mathers, J. C., R. P. Baber, and R. F. Archibald. 1989. Intake, digestion and gastro-intestinal mean retention time in Asiatic buffaloes and Ayrshire cattle given two contrasting diets and housed at 20° and 33°C. J. Agric. Sci. 113:211–222. https://doi.org/10.1017/S0021859600086792.

McGuirk, S. M. 2008. Disease management of dairy calves and heifers. Vet. Clin. North Am. Food Anim. Pract. 24:139–153. https://doi.org/10.1016/j.cvfa.2007.10.003.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. National Academies Press, Washington, DC.

Neves, L. F. M., M. B. Gomes, J. P. Campolina, M. M. Campos, E. M. B. Souza, J. Diavao, A. S. Silva, T. R. Tomich, W. A. Carvalho, H. F. Lage, and S. G. Coelho. 2025. Impact of heat stress on intake, performance, digestibility, and health of neonatal dairy calves. Animals (Basel) 15:1876. https://doi.org/10.3390/ani15131876.

Purwanto, B. P., F. Nakamasu, and S. Yamamoto. 1993. Effect of environmental temperatures on heat production in dairy heifers differing in feed intake level. Asian-Australas. J. Anim. Sci. 6:275–279. https://doi.org/10.5713/ajas.1993.275.

R Core Team. 2024. R: A language and environment for statistical computing. Accessed Jan. 2, 2026. https://www.r-project.org/.

Rauba, J., B. J. Heins, H. Chester-Jones, H. L. Diaz, D. Ziegler, J. Linn, and N. Broadwater. 2019. Relationships between protein and energy consumed from milk replacer and starter and calf growth and first-lactation production of Holstein dairy cows. J. Dairy Sci. 102:301–310. https://doi.org/10.3168/jds.2018-15074.

Roland, L., M. Drillich, D. Klein-Jöbstl, and M. Iwersen. 2016. Invited review: Influence of climatic conditions on the development, performance, and health of calves. J. Dairy Sci. 99:2438–2452. https://doi.org/10.3168/jds.2015-9901.

Silva, A. L., M. I. Marcondes, E. Detmann, F. S. Machado, S. C. Valadares Filho, A. S. Trece, and J. Dijkstra. 2015. Effects of raw milk and starter feed on intake and body composition of Holstein × Gyr male calves up to 64 days of age. J. Dairy Sci. 98:2641–2649. https://doi.org/10.3168/jds.2014-8833.

van Niekerk, B. D. H., J. T. Reid, A. Bensadoun, and O. L. Paladines. 1963. Urinary Creatinine as an Index of Body Composition. J. Nutr. 79:463–473. https://doi.org/10.1093/jn/79.4.463.

Wang, S., Q. Li, J. Peng, and H. Niu. 2023. Effects of long-term cold stress on growth performance, behavior, physiological parameters, and energy metabolism in growing beef cattle. Animals (Basel) 13:1619. https://doi.org/10.3390/ani13101619.

Widdison, S., and T. J. Coffey. 2011. Cattle and chemokines: Evidence for species-specific evolution of the bovine chemokine system. Anim. Genet. 42:341–353. https://doi.org/10.1111/j.1365-2052.2011.02200.x.

Zhang, X., K. Cui, X. Wen, L. Li, X. Yu, B. Li, H. Lin, H. He, and F. Wang. 2022. The Association between gut microbiome diversity and composition and heat tolerance in cattle. Microorganisms 10:1672. https://doi.org/10.3390/microorganisms10081672.