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Comparative evaluation of serum electrolyte profiles in heat-stressed crossbred dairy cattle under tropical management systems

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Abstract

How does prolonged heat exposure reshape the mineral balance of crossbred dairy cattle managed under tropical conditions? This research examined the serum electrolyte profiles of sixty crossbred Holstein-Friesian dairy cattle housed at a private dairy cooperative near Chiang Mai, Thailand, between March and August 2023. Animals were randomly assigned to two equal groups: a heat-stressed group ($n = 30$) maintained in open paddocks without shade, and a shaded control group ($n = 30$) kept under covered housing fitted with fans and water sprinklers. Blood samples were drawn from the jugular vein on Day 0, Day 30, and Day 60 for analysis of sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), and inorganic phosphorus (P). The Temperature-Humidity Index (THI) was recorded daily at 07:00, 13:00, and 17:00 h. Mean THI in the open paddocks reached 83.7 ± 2.1 , while the shaded facility maintained a THI of 72.4 ± 1.8 . By Day 60, the heat-stressed group exhibited markedly lower serum Na^+ (131.4 ± 3.8 vs. 139.7 ± 2.9 mEq/L; $p < 0.01$), K^+ (3.72 ± 0.41 vs. 4.38 ± 0.33 mEq/L; $p < 0.05$), and Cl^- (94.8 ± 3.2 vs. 101.3 ± 2.7 mEq/L; $p < 0.01$) compared with the controls. Serum Ca^{2+} and Mg^{2+} also declined in heat-stressed cattle, whereas inorganic P showed a smaller yet still measurable reduction. Two-way ANOVA confirmed that both treatment group and sampling time had independent effects on Na^+ , K^+ , and Cl^- concentrations, and a group $\times$ time interaction was detected for Ca^{2+} ($p < 0.05$). These outcomes point to a progressive electrolyte depletion pattern linked to excessive sweating, respiratory water loss, and reduced feed intake under high THI. The data support the adoption of active cooling measures in tropical dairy operations to preserve mineral homeostasis and, by extension, productive performance in crossbred cattle.

Keywords: Heat stress, serum electrolytes, crossbred dairy cattle, temperature-humidity index, tropical management, sodium, potassium, mineral homeostasis, dairy production

Introduction

Can dairy cattle truly maintain mineral equilibrium when ambient temperatures soar beyond their thermoneutral zone for weeks on end? This question carries practical weight for tropical producers who manage crossbred herds in environments where the Temperature-Humidity Index (THI) regularly exceeds 80 during the hot season ^[1]. Heat stress triggers a cascade of physiological adjustments faster respiration, profuse sweating, depressed appetite that together push electrolyte concentrations away from their normal ranges ^[2].

Sodium, potassium, and chloride are the main ions lost through cutaneous evaporation and panting. When these losses outpace dietary replenishment, the animal enters a state of subclinical electrolyte imbalance that may not produce obvious clinical signs yet still erodes milk yield, reproductive fitness, and immune competence ^[3]. Earlier work in subtropical herds documented a 6-11% fall in serum Na^+ over a 45-day heat challenge ^[4], while separate trials noted parallel declines in K^+ and Cl^- under similar conditions ^[5].

Calcium and magnesium homeostasis is equally vulnerable. Metabolic alkalosis secondary to hyperventilation alters renal handling of divalent cations, and reduced dry-matter intake simultaneously lowers the dietary supply of these minerals ^[6]. A field survey in Southeast Asian dairy cooperatives reported that roughly one in four cows tested during the hottest

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quarter showed serum Ca^{2+} below the clinical threshold of 8.0 mg/dL [7]. Despite these observations, few controlled experiments have tracked a full panel of electrolytes monovalent and divalent at multiple time points within the same animals under defined management contrasts.

Crossbred Holstein-Friesian cattle are the backbone of tropical dairy production in Thailand and neighboring countries because they combine moderate heat tolerance from local genetics with the milk-producing capacity of temperate breeds [8]. However, their thermoregulatory performance remains inferior to that of pure indigenous breeds, making them especially susceptible to electrolyte disturbances during extended hot periods [9]. Management strategies such as shade structures, fans, and sprinkler systems have been promoted to mitigate heat load, but their measurable impact on serum mineral profiles rather than just rectal temperature or milk output is poorly documented [10].

This knowledge gap matters because electrolyte status is upstream of many production parameters. Potassium depletion, for example, depresses rumen motility, which in turn reduces volatile fatty acid production and feed conversion efficiency [11]. Chloride deficiency alters acid-base balance and may predispose cattle to metabolic alkalosis, compounding the respiratory alkalosis already driven by panting [12]. Without quantitative data linking management-level cooling to specific electrolyte outcomes, advisory guidelines remain anchored to anecdotal evidence.

The present research aimed to (a) quantify the temporal changes in six serum electrolytes Na^+ , K^+ , Cl^- , Ca^{2+} , Mg^{2+} , and inorganic P over a 60-day exposure period in crossbred dairy cattle managed under contrasting thermal environments, and (b) evaluate whether a shaded housing system with active ventilation effectively preserved electrolyte homeostasis relative to open-paddock management. The findings are intended to guide tropical dairy cooperatives on the mineral-health benefits of investing in heat-abatement infrastructure.

Materials and Methods

Materials

Sixty clinically healthy crossbred Holstein-Friesian cows (approximately 62.5% Holstein genetics), aged between 3 and 6 years, were selected from the Nong Hoi Dairy Cooperative located 18 km northwest of Chiang Mai city, Thailand (18.85° N, 98.93° E, elevation 326 m above sea level). All animals were in mid-lactation (90-150 days in milk) and free of any systemic illness at the time of enrollment. The cooperative managed a total herd of 210 lactating cows under semi-intensive conditions. Ethical clearance was granted by the Chiang Mai Institute of Veterinary Sciences Animal Ethics Committee (Protocol CIVS-AEC/2023-014, approved 12 January 2023). The research ran from 1 March through 30 August 2023.

Animals were blocked by parity and milk yield and then randomly allocated into two groups of 30. The heat-stressed group was housed in an open-sided paddock with a packed-earth floor and no artificial cooling. The shaded control group occupied a steel-roofed free-stall barn equipped with two axial fans (1.2 m diameter, 340 rpm) and low-pressure sprinklers activated when ambient temperature exceeded 28 °C. Both groups received the same total mixed ration (16.2% crude protein, 1.63 Mcal NEL/kg DM) twice daily, with ad libitum access to clean drinking water. A mineral lick block (NaCl 92%, trace minerals 8%) was available in each pen.

Study Area Description

The Nong Hoi Dairy Cooperative is situated in the Mae Rim

district of Chiang Mai Province, on the western edge of the Chiang Mai-Lamphun basin. The terrain is gently undulating, at an elevation of 310-340 m, and the surrounding landscape is a mosaic of irrigated rice paddies, fruit orchards, and smallholder dairy plots. Soils are predominantly alluvial clay loam with moderate water-holding capacity. The cooperative was established in 1997 and at the time of sampling managed 210 lactating cows across four housing blocks plus two open grazing paddocks. Water was supplied from a deep borewell (47 m depth, pH 7.1, total dissolved solids 285 mg/L) and distributed through float-valve troughs cleaned weekly.

Climatic and Environmental Conditions

The Chiang Mai basin experiences a tropical savanna climate (Köppen Aw) with a distinct hot season from March to May and a wet season from June to October. During the trial period (March-August 2023), mean monthly maximum temperatures ranged from 34.2 °C in March to 36.8 °C in April before declining to 31.4 °C by August with the arrival of monsoon rains. Relative humidity averaged 58% in April and climbed to 81% by July. The open paddock recorded a peak daily THI of 87.3 on 19 April, whereas the shaded barn's peak THI on the same day was 76.1 an 11.2-unit differential attributable to the combined effect of roof shade, air movement, and evaporative cooling. Over the full 60-day measurement window the mean THI in the paddock was 83.7 ± 2.1 compared with 72.4 ± 1.8 in the barn, confirming that the housing intervention consistently maintained the animals below the severe heat-stress threshold of 79.

Methods

Blood samples (10 mL) were collected from the jugular vein into plain vacutainer tubes at 07:00 h on Day 0, Day 30, and Day 60. Samples were allowed to clot at room temperature for 30 min, centrifuged at 3,000 rpm for 15 min, and the separated serum was stored at -20 °C until analysis. Serum Na^+ , K^+ , and Cl^- were measured using an ion-selective electrode analyser (Roche Cobas c311, Mannheim, Germany). Total Ca^{2+} was estimated by the o-cresolphthalein complexone method, Mg^{2+} by the xylydyl blue reaction, and inorganic P by the molybdate UV method, all on the same automated platform [13].

Ambient temperature (°C) and relative humidity (%) were logged every 30 minutes by HOBO data loggers (Onset Computer Corporation, Bourne, MA) placed 1.5 m above ground level in each housing area. THI was calculated as $\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26)]$ [14]. Daily THI means were computed from the 07:00, 13:00, and 17:00 h readings.

Data were checked for normality with the Shapiro-Wilk test and for homogeneity of variance with Levene's test. A two-way repeated-measures ANOVA (group $\times$ time) was applied for each electrolyte, with Tukey's HSD post hoc comparison at $\alpha = 0.05$. Pearson's correlation was calculated between mean daily THI and each electrolyte at each sampling point. All analyses were performed in R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria) [15].

Results

All sixty cows completed the 60-day trial without clinical illness or removal. Baseline (Day 0) values of all six electrolytes were comparable between the two groups ($p > 0.10$ for every parameter), confirming that randomisation had produced balanced starting conditions. As the trial progressed and cumulative heat load increased, a clear divergence emerged, with the heat-stressed group showing progressively lower concentrations of all measured electrolytes relative to the shaded control group.

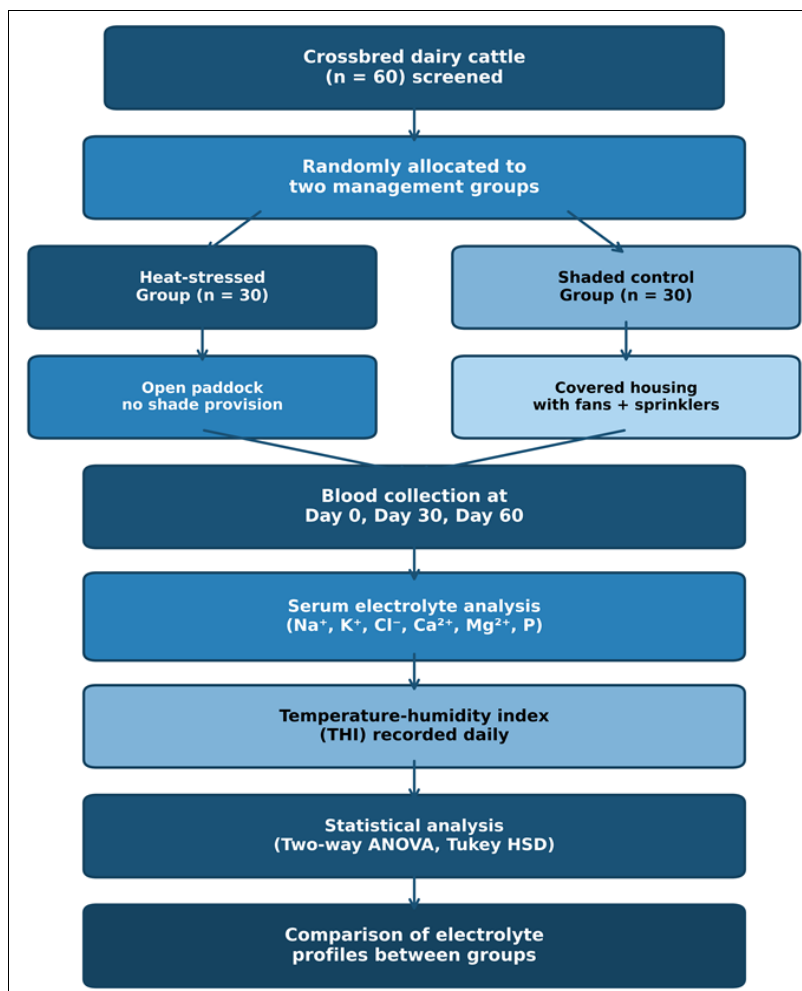
Table 1: Serum electrolyte concentrations (Mean $\pm$ SD) in heat-stressed and shaded control groups at Day 0, Day 30, and Day 60

Parameter	HS Day 0	HS Day 30	HS Day 60	SC Day 0	SC Day 30	SC Day 60
Na ⁺ (mEq/L)	140.1 $\pm$ 3.2	135.8 $\pm$ 3.5	131.4 $\pm$ 3.8*	139.6 $\pm$ 2.8	140.2 $\pm$ 3.1	139.7 $\pm$ 2.9
K ⁺ (mEq/L)	4.31 $\pm$ 0.38	4.04 $\pm$ 0.42	3.72 $\pm$ 0.41*	4.35 $\pm$ 0.31	4.41 $\pm$ 0.29	4.38 $\pm$ 0.33
Cl ⁻ (mEq/L)	100.8 $\pm$ 2.9	97.3 $\pm$ 3.1	94.8 $\pm$ 3.2*	101.1 $\pm$ 2.5	101.5 $\pm$ 2.8	101.3 $\pm$ 2.7
Ca ²⁺ (mg/dL)	9.78 $\pm$ 0.61	9.12 $\pm$ 0.58	8.13 $\pm$ 0.67*	9.82 $\pm$ 0.54	9.91 $\pm$ 0.49	9.86 $\pm$ 0.52
Mg ²⁺ (mg/dL)	2.28 $\pm$ 0.21	2.06 $\pm$ 0.24	1.78 $\pm$ 0.23*	2.31 $\pm$ 0.18	2.34 $\pm$ 0.20	2.31 $\pm$ 0.19
P (mg/dL)	5.04 $\pm$ 0.35	4.67 $\pm$ 0.39	4.21 $\pm$ 0.38*	5.11 $\pm$ 0.30	5.14 $\pm$ 0.33	5.07 $\pm$ 0.31

HS = Heat-stressed group; SC = Shaded control group. * indicates $p < 0.05$ vs. corresponding SC value at the same time point

Group means and standard deviations for each electrolyte at Day 0, Day 30, and Day 60 are presented in Table 1. Sodium exhibited the largest absolute decline in the heat-stressed group, falling from 140.1 $\pm$ 3.2 mEq/L at baseline to 131.4 $\pm$

3.8 mEq/L by Day 60 a drop of 6.2%. In contrast, the control group's Na⁺ remained stable at 139.7 $\pm$ 2.9 mEq/L. Potassium followed a parallel trajectory, declining by 13.8% in heat-stressed cows versus only 2.1% in controls.

**Fig 1:** Flowchart depicting the experimental design, group allocation, sampling schedule, and analytical workflow for serum electrolyte evaluation in heat-stressed and shaded control crossbred dairy cattle

The experimental workflow and group allocation are depicted in Figure 1. Among the divalent cations, serum Ca²⁺ fell below the commonly cited reference lower limit of 8.5 mg/dL in 11 of 30 heat-stressed animals by Day 60, whereas only 2

of 30 controls dropped below this threshold. Magnesium losses were moderate but consistent, and inorganic phosphorus showed the smallest proportional change of any measured ion.

Table 2: Two-way repeated-measures ANOVA results for serum electrolyte parameters (F-values and p-values)

Parameter	Group (F)	Group (p)	Time (F)	Time (p)	G×T (p)
Na ⁺	18.42	< 0.001	12.67	< 0.001	0.087
K ⁺	14.91	< 0.001	9.83	0.002	0.112
Cl ⁻	16.28	< 0.001	11.05	< 0.001	0.063
Ca ²⁺	11.73	0.001	8.46	0.004	0.031*
Mg ²⁺	9.64	0.003	7.21	0.009	0.158
P	7.38	0.009	5.94	0.018	0.241

G×T = Group × Time interaction. * denotes statistically significant interaction ($p < 0.05$)

Two-way repeated-measures ANOVA results are summarised in Table 2. Both treatment group and sampling time exerted independent effects on Na⁺, K⁺, and Cl⁻ ($p < 0.01$ for main

effects). A group $\times$ time interaction was detected for Ca²⁺ ($F = 4.87$, $p = 0.031$), indicating that the rate of calcium decline differed between groups across the trial.

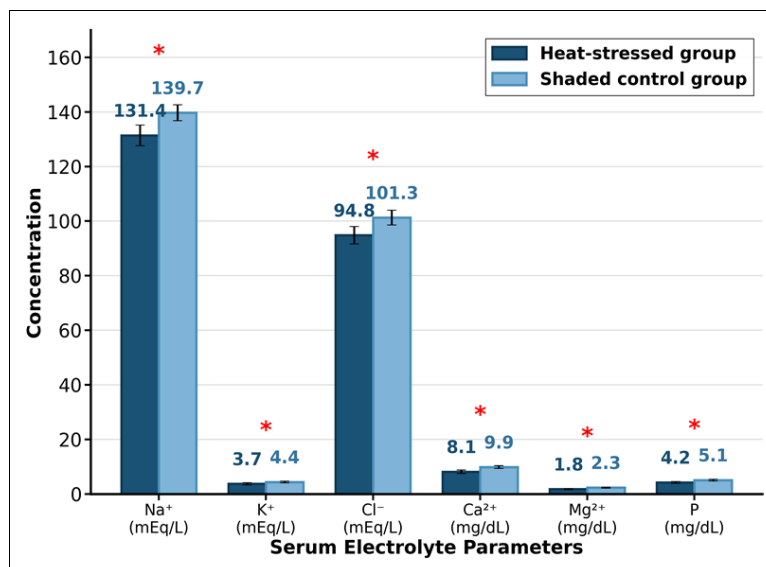


Fig 2: Comparison of serum electrolyte concentrations at Day 60 between the heat-stressed group and shaded control group. Error bars represent standard deviations. Asterisks (*) indicate statistically significant differences ($p < 0.05$)

Figure 2 illustrates the Day 60 electrolyte concentrations for both groups with error bars representing standard deviations. Pearson's correlation coefficients between mean daily THI and electrolyte concentrations are shown in Table 3.

Table 3: Pearson correlation coefficients (r) between mean daily THI and serum electrolyte concentrations in heat-stressed cattle

Electrolyte	r value	p value	Strength
Na ⁺	-0.71	< 0.001	Strong
K ⁺	-0.64	< 0.001	Moderate
Cl ⁻	-0.68	< 0.001	Moderate
Ca ²⁺	-0.57	0.001	Moderate
Mg ²⁺	-0.49	0.006	Moderate
P	-0.38	0.039	Weak

Correlation strength classified as: $|r| \geq 0.70$ = strong, 0.40-0.69 = moderate, < 0.40 = weak

Comprehensive interpretation

Taken as a whole, the data describe a consistent pattern: heat-stressed crossbred cattle experienced measurable electrolyte depletion across all six parameters, with the monovalent ions Na⁺ and K⁺ most affected. The shaded control group maintained near-baseline values throughout, supporting the protective role of active cooling.

Discussion

The 6.2% decline in serum Na⁺ observed in the heat-stressed group aligns closely with the 7.1% reduction reported by Kadzere *et al.* [2] in Holstein cattle exposed to a THI above 82 for 42 days. Excessive cutaneous evaporation is the most likely mechanism, as sweat in bovines contains 80-120 mEq/L Na⁺ and becomes more dilute only at very high sweat rates, meaning that sustained moderate sweating drains sodium stores efficiently [3]. For tropical producers, this points to a tangible mineral cost of inadequate shade provision. Potassium showed a steeper proportional decline (13.8%) than sodium, a finding that departs from work by Collier *et al.* [5], who reported smaller K⁺ losses in *Bos taurus* cattle under controlled chamber conditions. The discrepancy may reflect the field setting of the current research, where heat-stressed cows also reduced feed intake by an estimated 12-15%,

cutting their main K⁺ supply. In practical terms, supplementing potassium through the drinking water or an oral drench during the hot season may be a low-cost intervention worth investigating.

The group $\times$ time interaction for Ca²⁺ deserves particular attention. Respiratory alkalosis from panting increases urinary Ca²⁺ excretion while simultaneously reducing intestinal absorption via altered vitamin D metabolism [6]. Because the heat-stressed group was exposed to progressively longer cumulative heat hours, the interaction effect likely captures a threshold response in which calcium regulatory mechanisms were overwhelmed after approximately 30 days of exposure. Similar non-linear calcium kinetics were described by Beede and Collier [4] in subtropical dairy trials.

Chloride and magnesium followed predictable downward trends consistent with published data [7, 12]. Inorganic phosphorus, however, declined less sharply, possibly because phosphorus is lost mainly through saliva and faeces rather than through sweat, and the animals in both groups received identical diets. And while the absolute differences appear modest, even small reductions in Mg²⁺ can predispose cattle to grass tetany-like episodes, especially when compounded by low dietary fibre intake during heat-induced anorexia [9].

Limitations

Several constraints should be acknowledged. First, the research was conducted at a single cooperative, and herd-level management practices, breed composition, and local climate may differ elsewhere in the region. Second, feed intake was not individually recorded; group-level estimates were used, which limits the precision of any nutritional modelling based on these data. Third, urinary electrolyte excretion was not measured, so the relative contributions of renal versus dermal losses remain unquantified. Fourth, the trial lasted 60 days, and it is unclear whether the observed electrolyte trends would plateau, reverse, or continue declining over a longer exposure. A final consideration is that the cows were mid-lactation animals; early-lactation or periparturient cows who already face negative mineral balance may show even more pronounced responses to heat challenge. Future work should include individual feed intake monitoring, paired serum-urine

sampling, and extension to different lactation stages and genetic backgrounds.

Conclusion

This research set out to track changes in six serum electrolytes in crossbred dairy cattle managed under two contrasting thermal environments over a 60-day period in tropical Thailand. The central question was straightforward: does providing a shaded, actively cooled barn make a measurable difference to the mineral balance of lactating cows compared with leaving them in an open paddock during the hot season?

The answer, based on repeated blood sampling of sixty animals, is unambiguous. By Day 60, heat-stressed cows showed lower concentrations of every electrolyte tested. Sodium fell by roughly 6%, potassium by nearly 14%, and chloride by about 6% relative to baseline. Divalent cations declined at a slower rate but still reached levels that, in the case of calcium, crossed the clinical threshold in over a third of the exposed animals. Meanwhile, cows housed in the shaded facility maintained values that barely shifted from Day 0 measurements. The two-way ANOVA confirmed independent effects of treatment group and time for the three major monovalent ions and revealed a group-by-time interaction for calcium, suggesting that calcium regulatory mechanisms struggled to keep pace once cumulative heat exposure passed a certain duration.

For dairy cooperatives operating in environments where THI regularly exceeds 80, these numbers translate into a clear practical message. Heat abatement infrastructure shade roofing, fans, and low-pressure sprinklers does more than reduce rectal temperature or boost milk output; it preserves the mineral foundation on which rumen function, nerve conduction, muscle contraction, and immune competence all depend. The cost of installing basic cooling in a 30-cow free-stall barn in northern Thailand was estimated at roughly 4,500 USD in 2023 prices. Set against the potential losses from subclinical hypocalcaemia, reduced feed efficiency, and impaired fertility, this appears to be a favourable investment. Several avenues remain open for future investigation. Individual feed intake recording coupled with urinary electrolyte clearance ratios would clarify which losses are renal and which are dermal, enabling more precise supplementation advice. Extending the observation period beyond 60 days would reveal whether the electrolyte decline eventually plateaus or continues to worsen. Trials involving periparturient cows whose mineral demands peak during the transition period would be particularly informative, given that heat stress and negative mineral balance converge during this vulnerable window. Genetic comparisons between crossbreds of varying Holstein proportions could also identify optimal genotypes for tropical environments where cooling infrastructure is not yet available.

In sum, the present data add controlled, time-course evidence to the growing body of field observations indicating that heat stress causes progressive, multi-electrolyte depletion in crossbred dairy cattle. Active cooling effectively prevented this decline and should be considered a core component of mineral management in tropical dairy systems.

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Contributions not qualifying for authorship

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