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Determining the Minimum Time Interval for Completely Eliminating the Carryover Effect of Dehydration in Heat-Stressed Goats: Insights from Physiochemical Mechanisms

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ABSTRACT

Heat stress, coupled with water deprivation, is widely acknowledged as one of the most potent stressors capable of impairing the production performance of small ruminants; therefore, comprehending and mitigating this stressor is of paramount importance. This experiment aimed to investigate the physiochemical adaptability of heat-stressed goats to periods of water deprivation and subsequent rehydration, focusing on determining the minimum time interval required to fully eliminate the carryover effects. Nine healthy Aardi bucks, aged 10 months and weighing 29.14±1.06 kg, underwent three experimental stages: euhydration (EU), dehydration (DE), and rehydration (RE). The EU and DE stages lasted 72 hours each, while the RE stage extended to 10 days. Physiochemical responses, including daily feed intake (DFI), rectal temperature (RT), respiratory rate (RR), hematology, biochemistry, and hemogasometry, were all monitored. Exposure of heat-stressed goats (as evidenced herein by the temperature-humidity index values) to complete water deprivation during the DE stage had manifested significant modulation in their physiological responses (DFI, RT, and RR), which thereby led to notable changes in their hematological, biochemical, and hemogasometric profiles. Surprisingly, blood pH remained unchanged throughout the experiment, underscoring goats' remarkable adaptive mechanisms to tolerate infrequent liquid intake in their natural hot and arid environment. Notably, all measured variables completely returned to EU levels after 10 days of commencing the RE stage. Based on the obtained findings, a minimum recovery period of 10 days post-rehydration is recommended to eliminate carryover impacts of water deprivation for 72 hours before conducting any further experiments on heat-stressed and water-deprived goats. This research contributes to enhancing goat welfare under harsh environmental conditions.

Keywords: acid-base balance; biochemistry; Capra hircus; hematology; hemogasometry; water deprivation

INTRODUCTION

Due to their low input and high output production system, goats (*Capra hircus*) are increasingly recognized as pivotal for sustainable and profitable livestock farming, appealing to both large-scale enterprises and marginal small-scale farmers (Mazinani & Rude, 2020; Reshma *et al.*, 2021; Sejian *et al.*, 2021). Compared to other ruminants and livestock species, goats exhibit superior morphophysiological, thermophysiological, and behavioral traits, making them ideal climate-resilient models capable of coping with diverse stressors and thriving in challenging environments (Gupta & Mondal, 2019). However, their production can be compromised under certain environmental conditions, such as elevated temperatures and water deprivation, which trigger noticeable physiological responses at both organ and cellular levels (Silanikove, 2000a; Samara *et al.*, 2016; Aleena *et al.*, 2018; Cai *et al.*, 2019).

Hematological and biochemical analyses play crucial roles in understanding goats' responses to water restriction or deprivation (Alamer, 2006; Krishnan *et al.*, 2023; Madhavan *et al.*, 2023). Increased blood osmolality, a key factor in modulating homeostatic responses, prompts adaptations such as activation of vasopressin and renin-angiotensin-aldosterone systems, leading to reduced glomerular filtration rates (GFR) and increased water absorption from renal tubules (Sahay, 2024). Consequently, withholding water from goats for extended periods can result in elevated red blood cell counts, packed cell volume, and hemoglobin concentration (Mukherjee *et al.*, 2023). Additionally, plasma/serum concentrations of sodium (Samara *et al.*, 2012), potassium (Samara *et al.*, 2012), chloride and calcium (Madhavan *et al.*, 2023), total protein and albumin (Jaber *et al.*, 2004; Samara *et al.*, 2012), creatinine (Samara *et al.*, 2012), and glucose (Alamer 2006; Samara *et al.*, 2012) will increase in water-deprived small ruminants. Moreover,

under heat stress, goats primarily rely on panting for evaporative cooling, potentially disrupting the acid-base balance (Silanikove 2000a,b; Srikandakumar *et al*., 2003). These physiological responses adversely affect meat, milk, and wool production, impacting goats' overall well-being and welfare (Sejian *et al*., 2017; Sarangi, 2018; Mazinani & Rude, 2020).

Therefore, assessing goat welfare throughout production phases using animal-based or management-based approaches is crucial for ensuring food security and maximizing economic returns (Sarangi, 2018; Sejian *et al.,* 2021). The uniqueness of this experiment lies in its emphasis on unraveling the homeostatic/homeokinetic physiochemical adaptability of buck goats facing both heat stress and water deprivation simultaneously. Specifically, the experiment seeks to establish the shortest time required for these heat-stressed animals to recover from the detrimental effects of water deprivation upon water replenishment. This innovative approach integrates physiological responses to various stressors, providing valuable insights into the adaptive strategies of goats and their welfare implications in challenging environmental circumstances. By addressing these issues, this research contributes to enhancing goat welfare and promoting sustainable livestock production practices, which are vital for ensuring food security and maximizing economic returns.

MATERIALS AND METHODS

The current experiment was conducted during the summer season in adherence to the ethical standards of the Institutional Research Committee of King Saud University, Riyadh, Saudi Arabia, ensuring animal welfare and ethical treatment in scientific research (process number: KSU-SE-21-84).

Nine healthy Aardi bucks, aged 10-12 months, with a mean body weight of 29.14±1.06 kg, were subjected to a pretest/posttest design with three treatments (euhydration [EU]), dehydration [DE], and rehydration [RE] stages). The EU and DE stages lasted 72 hours (h) each, while the RE stage extended to 10 days. The bucks were individually housed in shaded pens (1.50×1.50 m), fed a commercial complete Al-Wafi pelleted diet (Arabian Agricultural Services Co., Riyadh, Saudi Arabia) twice daily at 3% of their body weight, with access to mineral blocks and *ad libitum* water, except during the DE stage. According to the manufacturer's specifications, the pelleted complete diet and contained 13% crude protein, 2% ether extract, 9% crude fiber, 8% ash, 1% calcium, 0.50% phosphorus, 0.70% sodium chloride, and provided 2.95 Mcal/kg of digestible energy on a dry matter basis. All bucks received the same medical care, including deworming, vaccination, and inspection, under veterinarian supervision before the experiment commenced.

In the current experimental setup, the dry-bulb ambient temperature (Ta) and relative humidity (RH) were consistently monitored at 10-minute intervals. This was achieved through the utilization of two high-precision HOBO H-08 Pro Series data loggers (Onset, Bourne, MA, USA) strategically positioned within the pens. One logger was positioned at the level of the animals, while the other was situated approximately 2 meters above ground level. The accompanying software, Box-Car Pro 4 (Onset), was utilized to program these loggers and retrieve data. To estimate the environmental severity on the experimental bucks, the obtained mean T_a and RH data were thereafter used to calculate the temperature-humidity index (THI) using the following formula adopted from Kelly & Bond (1971): [THI = Ta − (0.55 − 0.55 × RH) × (Ta − 58)], where Ta is the ambient temperature in degrees Fahrenheit, and RH is the relative humidity as a fraction of the unit. In addition, the daily feed intake (DFI) of the experimental bucks was determined once every morning before fresh meals were introduced by weighing the remains of the previous day using a single-pan balance that measured to the nearest 20 g (Samara *et al.,* 2016). Meanwhile, rectal temperature (RT) and respiratory rate (RR) were both recorded three times a day (08:00, 12:00, 15:00) throughout the experiment. A calibrated digital rectal thermometer measuring to the nearest 0.10 °C was used to determine RT, while RR was recorded using a stethoscope placed at the 9^{th} and 11^{th} rib intercostal spaces while counting 10 breaths, and then expressing the recorded time as the number of breaths per minute (breath/min) (Samara *et al.,* 2016).

Afterwards, blood samples (approximately 10 mL) were collected daily in the morning from each buck after 72 h of the EU stage, after 48 h and 72 h of the DE stage, at 0, 4, 8, 24, 72 h as well as 4, 6, 8, and 10 days after starting the RE stage. Blood samples were obtained via jugular venipuncture into EDTA tubes for hematological analysis and into plain tubes for serological analysis. On the other hand, urine samples (approximately 50 mL) were collected daily from each animal into a 60 mL sterile urine pot using a self-designed urine collection harness. Once collected, all samples were placed inside an icebox and immediately transferred to the laboratory. Within one hour post-collection, blood samples were analyzed for hematology using a Coulter analyzer (Beckman Coulter, Miami, FL, USA) for hematological parameters (red blood cell count [RBC], white blood cell count [WBC], packed cell volume [PCV], hemoglobin concentration [Hb], and differential WBC count) as well as for hematimetric indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red blood cell distribution width [RDW], and ratio of neutrophils to lymphocytes [N/L]). Meanwhile, sera were separated by centrifuging blood samples at 1500×g for 10 min at 5 °C and were then transferred into Eppendorf tubes and stored at –20 °C until further analysis for total protein (TP), albumin (ALB), sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{++}), glucose (GLU), urea nitrogen (BUN), and creatinine (BCRT) using respective commercial kits and a semi-automated chemistry analyzer (RX monza, Randox Labs, Crumlin, UK). Globulin (GLOB) levels were thereafter calculated as the difference between measured TP and ALB concentrations, while serum osmolality (OSMO) was calculated using the following formula adopted from Khajuria & Krahn (2005): [OSMO (mOs/L) = 1.86 * Na^+ (mmol/L) +

1.15 * GLU (mg/dL) / 18 + BUN (mg/dL) / 2.8]. Regarding the biochemical analysis of urine, samples were lightly centrifuged before being analyzed within 1 hr of collection for pH using a pH meter (Hanna Instruments, Woonsocket, RI, USA). Thereafter, urine subsamples were transferred into Eppendorf tubes and stored at −20 °C until analysis. On the day of analysis, these subsamples were first defrosted, diluted according to the procedure given by the commercial kits, and then spectrophotometrically analyzed for both urine urea nitrogen (UUN) and creatinine (UCRT) using commercial kits.

To determine the influence of a complete water restriction on the hemogasometric profile, blood samples (approximately 2.5 mL) were withdrawn using heparin-coated syringes (Terumo Corp., Tokyo, Japan) and analyzed within 1 hr of collection using a blood gas analyzer (Rapid Systems, Siemens, Malvern, PA, USA) to measure blood pH, partial pressure of carbon dioxide (pCO_2), and partial pressure of oxygen (pO_2). From these variables, the analyzer calculated blood bicarbonate (HCO_3^-), total bicarbonate (tCO_2), base excess (BE), and oxygen saturation (sO_2). Anionic gap (AG) was calculated, however, using the following formula; [AG = $Na^+ + K^+ - (Cl^- + HCO_3^-)$] adopted from Kraut & Madias (2007), while strong ions difference (SID) was calculated according to this formula; [AG = $Na^+ + K^+ - Cl^-$] adopted from Constable (2000).

Statistical analyses were carried out using the statistical analysis system (SAS Institute v9.4, Inc., Cary, NC, USA), and plots were generated using the SigmaPlot software (SigmaPlot v14·0, Systat Software Inc., San Jose CA, USA). Descriptive statistics were obtained using the PROC MEANS procedure. The PROC TTEST procedure was employed to compare differences in variables before and after dehydration using a pretest/posttest design. This design involved assessing the bucks before dehydration (pretest) and after rehydration (posttest). Paired two-tailed Student's t-tests assuming equal variances were used to analyze data pairs, with statistical significance set at $p<0.05$. Unless stated otherwise, means and their pooled standard error of the mean (SEM) were presented. As previously stated, the experimental measurements were conducted on days 4, 6, and 8 of the RE stage. It is important to note that the carryover effect of water deprivation was completely eliminated after 10 days of water restoration; therefore, data for day 10 were merely presented herein.

RESULTS

Meteorology and Physiology

Meteorological parameters and physiological variables collected during different experimental stages are illustrated in Figure 1. The consistent values of Ta, RH, and THI throughout all stages indicate a uniform distribution of environmental conditions during the experiment (Figure 1A, B, and C). Additionally, data from the shaded pens reveal a diurnal rhythm, with minimum Ta and THI values recorded in the early morning (05:00 to 07:00 h) and maximum values in the afternoon (13:00 to 15:00 h), while RH exhibited an inverse pattern (data not shown). Notably, the calculated average THI suggests that bucks experienced heat stress throughout the experimental period (Figure 1C) (El-Tarabany *et al.*, 2017).

Water deprivation for 72 h resulted in a ($p<0.05$) decrease in the overall mean of the animals' DFI, with dehydrated bucks consuming approximately 80% less than their euhydrated counterparts (Figure 1D). Moreover, both RR and RT showed ($p<0.05$) increases in dehydrated bucks (Figure 1E and F). However, 72 h after water restoration, RT ($p<0.05$) decreased, while DFI and RR ($p<0.05$) increased. As anticipated, all these variables completely returned to their euhydration levels ($p>0.05$) by day 10 of the RE stage (Figure 2).

Hematology

Withholding water for 72 h ($p<0.05$) impacted several hematological variables measured in dehydrated and heat-stressed buck goats (Table 1). The overall means of RBC, PCV, Hb, MCV, and MCH ($p<0.05$) increased from their EU levels to 8.19±0.33 x10^6/μL, 58.13±2.08%, 17.31±0.49 g/dL, 86.40±2.22 fL, and 29.70±0.90 pg in dehydrated bucks, respectively. However, upon access to water in the RE stage, RBC, PCV, Hb, and MCH ($p<0.05$) decreased to 5.41±0.27 x10^6/μL, 39.29±1.55%, 12.10±0.37 g/dL, and 24.69±0.67 pg, respectively, while MCV increased to 88.69±1.65 fL.

Furthermore, the overall mean of WBC ($p<0.05$) increased to its peak (25.50±3.14 x10^3/μL) after 72 h of water deprivation and gradually decreased after rehydration until reaching the normal euhydration level 10 days later. Meanwhile, the overall mean percentage of neutrophils ($p<0.05$) increased, reaching its highest level 8 h after water restoration (Table 1). Conversely, the overall mean percentage of lymphocytes exhibited a ($p<0.05$) decline during dehydration and continued to decrease until 8 h after rehydration. However, the overall mean of the N/L ratio followed a similar trend to neutrophils (Table 1). Despite these fluctuations, all hematological variables completely returned to their euhydration levels ($p>0.05$) 10 days after commencing the RE stage (Table 1 and Figure 2).

Biochemistry

Analysis of serum and urine biochemical variables indicated ($p<0.05$) changes after 72 h of complete water restriction (Table 2). Dehydration of heat-stressed goats remarkably ($p<0.05$) increased the overall mean serum osmolality (OSMO) from the euhydration level to 441.48±37.63 mOsmol/L during dehydration. This elevation was associated with increases ($p<0.05$) in serum concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) to 182.14±1.50 mmol/L, 5.19±0.09 mmol/L, and 111.12±1.70 mmol/L, respectively, in dehydrated bucks. However, serum calcium (Ca^{++}) concentration ($p<0.05$) decreased under water deprivation to its lowest level of 1.04±0.06 mmol/L 4 h after water restoration (Table 2). Once these animals gained access to water, serum osmolality declined

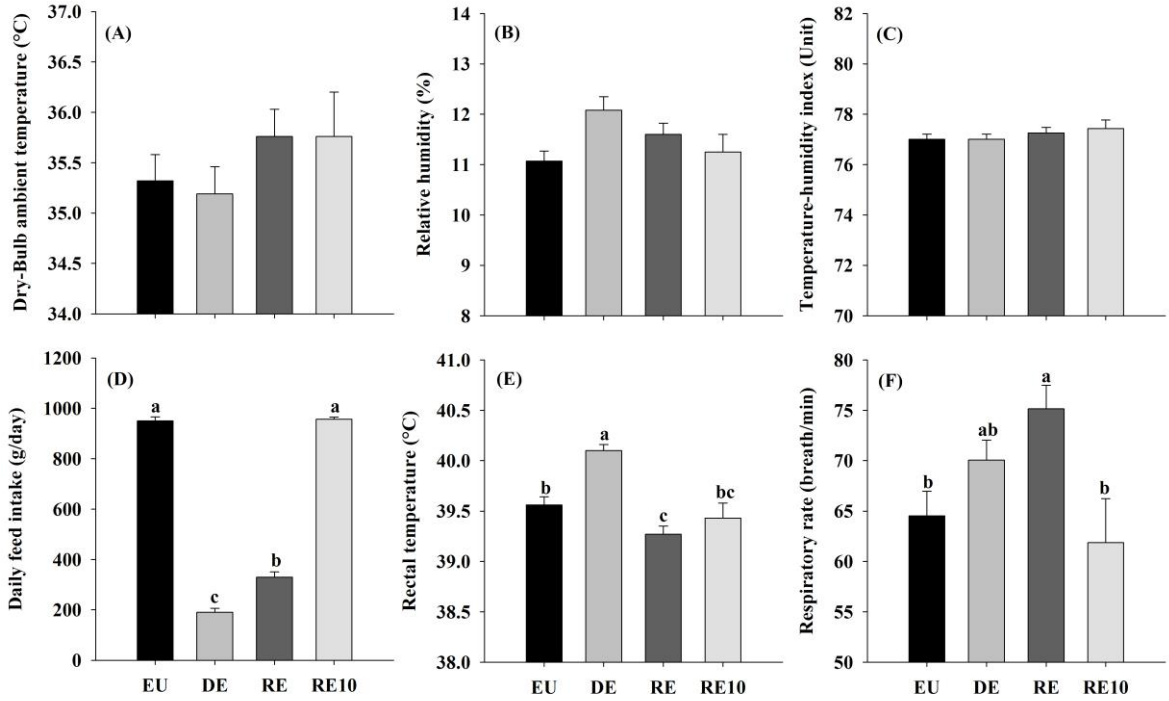

Figure 1. Meteorological parameters and physiological variables assessed across different experimental stages performed herein on heat-stressed goats (Mean±SE). EU denotes the euhydration stage, DE signifies the dehydration stage, RE indicates the rehydration stage, and RE10 represents measurements taken on day 10 of the rehydration stage. It is noteworthy that dry-Bulb ambient temperature, relative humidity, and temperature-humidity index were continuously recorded at 10-minute intervals throughout the day. Additionally, rectal temperature and respiratory rate were assessed three times daily (at 8:00, 12:00, and 15:00), while daily feed intake was measured once daily in the morning prior to feeding fresh meals to these goats. [a-c]Means bearing different superscripts are significantly different at p<0.05.

Table 1. Impact of dehydration and subsequent rehydration on the hematological profile in heat-stressed goats

Variables[1]	Experimental stages								SEM
	Euhydration	Dehydration		Rehydration					
	72 h	48 h	72 h	4 h	8 h	24 h	72 h	day 10	
Hematology									
RBC (x10^6/µL)	5.46 [b]	7.82 [a]	8.55 [a]	5.55 [b]	5.24 [b]	5.57 [b]	5.28 [b]	5.22 [b]	0.31
Hb (g/dL)	13.40 [b]	17.04 [a]	17.58 [a]	12.70 [c]	11.90 [c]	13.30 [bc]	11.70 [c]	13.67 [b]	0.59
PCV (%)	42.00 [b]	57.25 [a]	59.00 [a]	37.63 [b]	37.33 [b]	43.50 [b]	38.73 [b]	39.25 [b]	3.09
WBC (x10^3/µL)	15.44 [c]	15.40 [c]	25.50 [a]	21.40 [a]	19.05 [b]	21.18 [ab]	12.50 [c]	13.98 [c]	2.01
Neutrophil (%)	55.20 [b]	58.40 [b]	62.00 [b]	66.50 [a]	76.00 [a]	60.75 [b]	55.50 [b]	55.25 [b]	4.54
Lymphocyte (%)	39.80 [a]	36.00 [a]	31.25 [ab]	28.25 [bc]	19.75 [c]	33.75 [ab]	37.75 [a]	38.50 [a]	4.43
Monocyte (%)	4.00	4.60	5.00	4.25	3.25	3.75	5.25	4.75	0.83
Eosinophil (%)	1.00	1.20	1.75	1.00	1.25	1.75	1.50	1.50	0.33
Basophil (%)	0	0	0	0	0	0	0	0	-----
Hematimetric									
MCV (fL)	73.80 [c]	83.80 [b]	90.00 [a]	94.75 [a]	92.50[a]	92.25[a]	75.25 [c]	75.75 [c]	3.30
MCH (pg)	27.60 [bc]	29.00 [a]	30.40 [a]	24.50 [c]	24.50 [c]	24.50 [c]	25.25 [c]	28.00 [b]	1.34
MCHC (g/dL)	34.40 [a]	35.40 [a]	35.50 [a]	29.50 [b]	32.75[a]	35.75[a]	33.75 [a]	34.75 [a]	1.16
RDW (%)	17.18	16.46	13.63	16.73	18.83	16.85	16.03	17.75	1.63
N/L ratio	1.45 [c]	1.68 [bc]	2.78 [b]	2.96 [b]	4.26[a]	1.92[b]	1.51 [c]	1.45 [c]	0.60

Note: [1]Refer to text for details. [a-e]Means bearing different superscripts are significantly different at p<0.05. RBC= red blood cell count; Hb= hemoglobin concentration; PCV= packed cell volume; WBC= white blood cell count; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; RDW= red blood cell distribution width; N/L ratio= ratio of neutrophils to lymphocytes.

to 349.98±26.61 mOsmol/L after 72 h and further to 272.84±53.21 mOsmol/L 10 days after rehydration. Serum Na^+, K^+, and Cl^- concentrations returned to their euhydration levels within 24 h, while Ca^{++} concentration normalized 10 days after water restoration (Table 2).

Additionally, the overall means of serum TP, ALB, GLOB, GLU, BUN, BCRT, UUN and UCRT concentrations (p<0.05) increased due to the experimental treatments, reaching 9.02±0.31 g/dL, 4.67±0.16 g/dL, 4.35±0.31 g/dL, 125.61±11.59 mg/dL, 265.10±16.66 mg/dL, 3.69±0.34

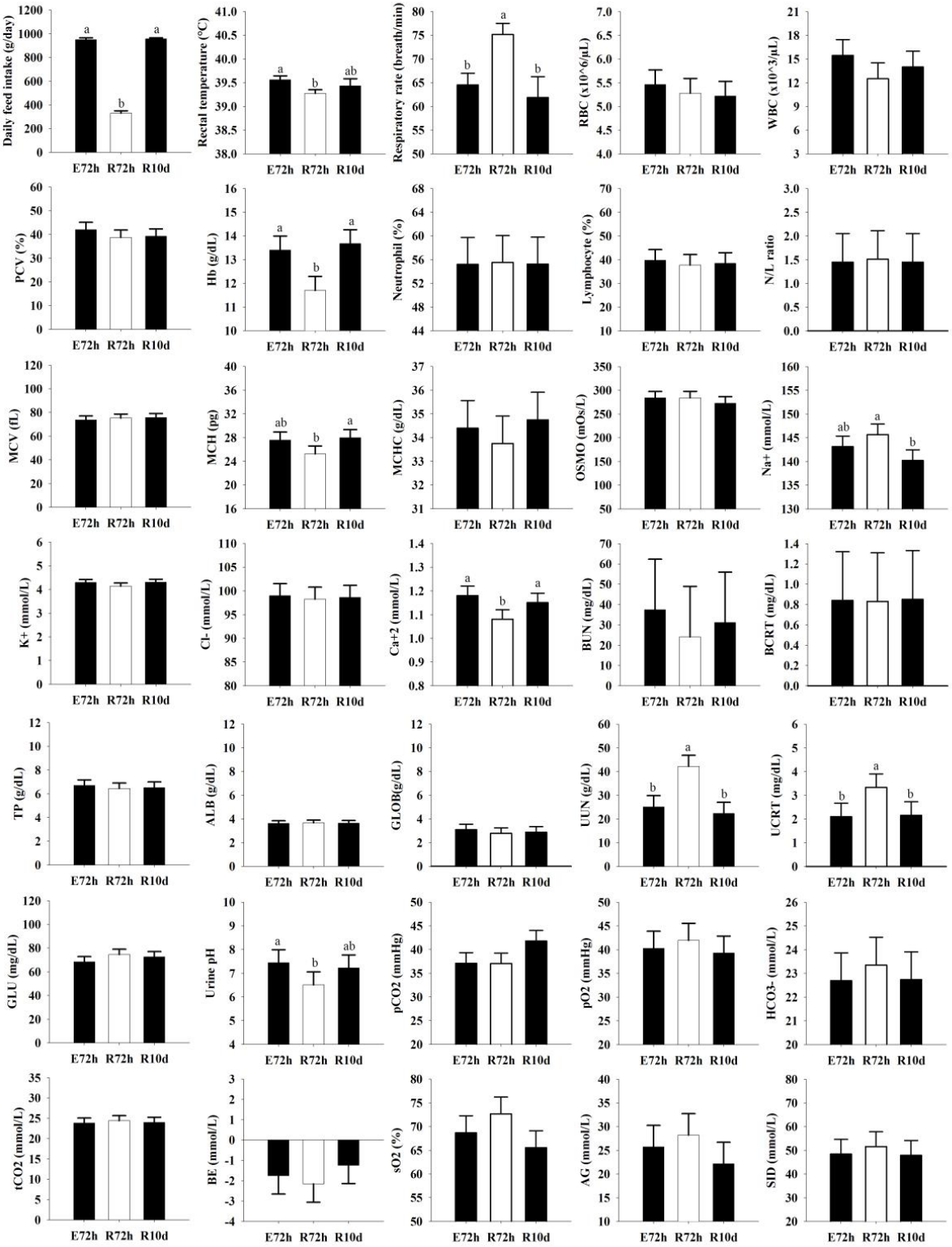

Figure 2. Vertical bar charts depicting the return of some selected measured physiochemical responses to their baseline levels after 10 days of initiating the rehydration phase in heat-stressed goats. E72h represents measurements taken at 72 hours into the euhydration stage, R72h signifies readings at 72 hours into the rehydration stage, and R10d denotes observations made ten days into the rehydration stage. a-bMeans bearing different superscripts are significantly different at $p<0.05$. RBC= red blood cell count; WBC= white blood cell count; PCV= packed cell volume; Hb= hemoglobin concentration; N/L ratio= ratio of neutrophils to lymphocytes; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; OSMO= serum osmolality; Na^+= sodium; K^+= potassium; Cl^-= chloride; Ca^{++}= calcium; TP= total protein; ALB= albumin; GLOB= globulin; GLU= glucose; BUN= urea nitrogen; BCRT= creatinine; pCO_2= carbon dioxide; pO_2= partial pressure of oxygen; HCO^{3-} = blood bicarbonate; tCO_2 = total bicarbonate; BE= base excess; sO_2= oxygen saturation; AG= anionic gap; SID= strong ions difference.

mg/dL, 65.08±6.43 mg/dL, and 8.18±0.47 mg/dL, respectively, during the DE stage. However, serum BUN and BCRT concentrations remained elevated during the first 8 h after water restoration (Table 2 and Figure 2).

Hemogasometry

Imbalances in the acid–base profile were evident in the dehydrated heat-stressed bucks (Table 3). Withholding water for 72 h ($p<0.05$) decreased the overall means of venous blood partial pressure of CO_2, HCO_3^-, tCO_2, and BE from their EU levels to 32.03±1.48 mmHg, 21.29±0.79 mmol/L, 22.27±0.83 mmol/L, and -2.79±0.60, respectively, in the dehydrated animals. Meanwhile, water deprivation ($p<0.05$) increased the overall means of venous blood partial pressure of pO_2, sO_2, AG, and SID to 43.72±2.40 mmHg, 75.09±2.04%, 54.91±6.42 mmol/L, and 76.19±3.20 mmol/L in the dehydrated samples, respectively. However, after water restoration, pCO2, HCO3−, tCO2, BE, AG, and SID continued to ($p<0.05$) decrease, while pO2 and sO2 continued to ($p<0.05$) increase (Table 3). Interestingly, the average daily venous blood pH remained unchanged ($p=0.08$) and within its physiological range (7.40±0.04) throughout the experiment, while urine pH ($p<0.05$) decreased (Table 3). Once again, all variables returned completely to their EU levels ($p>0.05$) 10 days after starting the RE stage (Table 3 and Figure 2).

DISCUSSION

Understanding and mitigating the effects of heat stress accompanied by water deprivation is crucial due to its widely recognized impact on the production

Table 2. Impact of dehydration and subsequent rehydration on the biochemical profile in heat-stressed goats

Variables[1]	Experimental stages								SEM
	Euhydration	Dehydration		Rehydration					
	72 h	48 h	72 h	4 h	8 h	24 h	72 h	day 10	
Blood									
OSMO (mOs/L)	283.92 ^{d}	404.05 ^{b}	478.90 ^{a}	400.68 ^{b}	385.08 ^{b}	329.90 ^{c}	284.24 ^{d}	272.84 ^{d}	13.61
Na^+ (mmol/L)	143.11 ^{de}	174.42 ^{b}	189.85 ^{a}	153.86 ^{c}	141.65 ^{d}	136.54 ^{f}	145.64 ^{d}	140.20 ^{e}	2.24
K^+ (mmol/L)	4.29 ^{c}	4.93 ^{b}	5.45 ^{a}	4.80 ^{b}	3.98 ^{d}	2.68 ^{e}	4.14 ^{cd}	4.30 ^{c}	0.13
Cl^- (mmol/L)	98.98 ^{c}	108.96 ^{b}	113.30 ^{a}	95.53 ^{cd}	93.55 ^{d}	88.43 ^{e}	98.24 ^{c}	98.63 ^{c}	2.52
Ca^{++} (mmol/L)	1.18 ^{a}	1.13 ^{b}	1.10 ^{bc}	1.04 ^{c}	1.10 ^{bc}	1.10 ^{bc}	1.08 ^{bc}	1.15 ^{a}	0.04
TP (g/dL)	6.70^{c}	8.85 ^{ab}	9.20 ^{a}	8.21 ^{b}	7.04 ^{c}	6.95 ^{c}	6.44^{c}	6.52 ^{c}	0.47
ALB (g/dL)	3.61^{b}	4.47 ^{a}	4.37 ^{ab}	4.71 ^{a}	4.37 ^{ab}	3.86 ^{b}	3.67 ^{b}	3.64 ^{b}	0.23
GLOB(g/dL)	3.09 ^{b}	4.37 ^{a}	4.84 ^{a}	3.50 ^{b}	2.67 ^{c}	3.09 ^{b}	2.78 ^{bc}	2.88 ^{bc}	0.46
GLU (mg/dL)	68.35 ^{de}	119.60 ^{b}	131.62 ^{a}	86.8 ^{c}	69.09 ^{d}	60.40 ^{e}	74.54 ^{d}	72.63 ^{d}	4.46
BUN (mg/dL)	37.44 ^{c}	201.58 ^{b}	328.63 ^{a}	305.06 ^{a}	328.15 ^{a}	201.84 ^{b}	24.06 ^{c}	31.14 ^{c}	24.84
BCRT (mg/dL)	0.84 ^{c}	2.07 ^{b}	5.30 ^{a}	5.52 ^{a}	4.73 ^{a}	2.47 ^{b}	0.83 ^{c}	0.85 ^{c}	0.48
Urine									
UUN (g/dL)	25.15 ^{d}	59.26 ^{b}	70.88 ^{a}	*	47.36 ^{c}	46.45 ^{c}	42.19 ^{c}	22.38 ^{d}	4.7
UCRT (mg/dL)	2.10 ^{e}	7.57 ^{b}	8.65 ^{a}	*	5.21 ^{c}	2.69 ^{de}	3.33 ^{d}	2.16 ^{e}	0.57

Note: [1]Refer to text for details. $^{a-e}$Means bearing different superscripts are significantly different at $p<0.05$. *Indicates that no urine samples were obtained at that time. OSMO= serum osmolality; Na^+= sodium; K^+= potassium; Cl−= chloride; Ca^{++}= calcium; TP= total protein; ALB= albumin; GLOB= globulin; GLU= glucose; BUN= urea nitrogen; BCRT= creatinine.

Table 3. Impact of dehydration and subsequent rehydration on the hemogasometric profile in heat-stressed goats

Variables[1]	Experimental stages								SEM
	Euhydration	Dehydration		Rehydration					
	72 h	48 h	72 h	4 h	8 h	24 h	72 h	day 10	
Blood									
pH	7.40	7.46	7.43	7.41	7.38	7.39	7.42	7.41	0.02
pCO_2 (mmHg)	37.11 ^{a}	33.15 ^{b}	30.90 ^{b}	23.95 ^{c}	20.45 ^{c}	24.03 ^{c}	37.04 ^{a}	41.79 ^{a}	2.21
pO_2 (mmHg)	40.29 ^{bc}	36.89 ^{c}	50.56 ^{ab}	55.85 ^{a}	51.08 ^{a}	44.76 ^{b}	41.96 ^{bc}	39.29 ^{bc}	3.58
HCO_3^- (mmol/L)	22.69 ^{a}	23.05 ^{a}	19.53 ^{b}	14.54 ^{c}	11.70 ^{c}	14.31 ^{c}	23.35 ^{a}	22.74 ^{a}	1.17
tCO_2 (mmol/L)	23.84 ^{a}	24.06 ^{a}	20.49 ^{b}	15.26 ^{c}	12.30 ^{c}	15.08 ^{c}	24.39 ^{a}	24.01 ^{a}	1.24
BE (mmol/L)	-1.76 ^{a}	-1.85 ^{ab}	-3.73 ^{b}	-8.46 ^{c}	-11.59 ^{d}	-9.06 ^{cd}	-2.16 ^{ab}	-1.24 ^{a}	0.90
sO_2 (%)	68.67 ^{d}	71.22 ^{d}	78.96 ^{bc}	88.23 ^{a}	83.44 ^{ab}	80.23 ^{b}	72.64 ^{cd}	65.54 ^{d}	3.57
AG (mmol/L)	25.72 ^{d}	47.34 ^{b}	62.47 ^{a}	48.59 ^{b}	40.38 ^{bc}	36.48 ^{c}	28.19 ^{cd}	22.15 ^{d}	4.54
SID (mmol/L)	48.41 ^{c}	70.39 ^{ab}	82.00 ^{a}	63.13 ^{b}	51.01 ^{c}	50.79 ^{c}	51.54 ^{c}	47.88 ^{c}	6.25
Urine									
pH	7.44 ^{a}	7.35 ^{a}	*	*	6.27 ^{ab}	5.78 ^{b}	6.51 ^{ab}	7.22 ^{a}	0.55

Note: [1]Refer to text for details. $^{a-e}$Means bearing different superscripts are significantly different at $p<0.05$. *Urine samples were at low volume for pH measurement. pCO_2= carbon dioxide; pO_2= partial pressure of oxygen; HCO_3− = blood bicarbonate; tCO_2 = total bicarbonate; BE= base excess; sO_2= oxygen saturation; AG= anionic gap; SID= strong ions difference.

performance of small ruminants (Silanikove 2000a,b; Samara *et al.*, 2012; Okoruwa, 2014; Aleena *et al.*, 2018; Cai *et al.*, 2019; Al-Badwi *et al.*, 2021). The current study aimed to investigate the physiological mechanisms employed by goats to cope with dehydration and subsequent rehydration, ultimately determining the minimum time required for these animals to eliminate the carryover effects of water deprivation.

Exposing heat-stressed goats (as indicated by THI values; Figure 1C) to complete water restriction elicited considerable alterations in their physiological responses (DMI, RT, and RR; Figure 1D-F), resulting in notable changes in their hematological, biochemical, and hemogasometric profiles. Our findings clearly demonstrate that withholding water for 72 h increased most of the measured hematological variables in heat-stressed goats (Table 1). Similar result was documented earlier due to blood hemoconcentration (Alamer, 2006). However, Abdelatif & Ahmed (1994) have reported a decrease in these variables after three to four days of water deprivation, attributing the declines to hemodilution, where more water might have shifted to the circulatory system for effective evaporative cooling. This discrepancy could be related to the prevailing high Ta during our experiment, resulting in adequate loss of body water content and consequent increase in hematological variables. Likewise, the observed elevation in the percentage of neutrophil cells in dehydrated goats' blood samples might be attributed to the elevated Ta, inducing an acute left-shift in neutrophil production even after water restoration (El-Tarabany *et al.*, 2017; Al-Badwi *et al.*, 2021). The noted hyperosmolality or hypertonicity observed herein further supports this notion.

From a physiological standpoint, the elevation of serum OSMO largely contributes to maintaining plasma volume by inducing continuous water movement into the vascular system (Silanikove, 1992; Alamer, 2006), thereby regulating goats' homeostatic responses to water restriction or deprivation. Plasma proteins are well-recognized as the major determinant of plasma volume (Silanikove, 1992). Calculating the percentage change in plasma volume (PVC) using the plasma TP concentrations obtained at each stage, as outlined by Boyd (1981), reveals a ($p<0.05$) alteration compared to the EU status. Specifically, the calculated PVC value for the DE stage decreased to -2.38±0.14% compared to the EU level. This reduction in PVC, coupled with hyperalbuminemia, may represent an adaptive mechanism in desert goats during water deprivation to sustain their blood hydrostatic pressure. Consistent with these observations, hyperproteinemia has been documented in small ruminants subjected to varying durations of water restriction (Jaber *et al.*, 2004; Samara *et al.*, 2012).

Moreover, one of the major determinants of serum OSMO is the concentration of serum Na^+ (Silanikove, 1992). In the current study, serum hyperosmolality was observed during the DE stage, coinciding with hypernatremia. Such hyperosmolality is considered a signal that triggers the secretion of ADH, which could account for the observed hypernatremia (Silanikove, 1992). These findings align with previous reports on goats (Alamer, 2006; Samara *et al*., 2012), which have documented states of hyperosmolality, hypernatremia, hyperkalemia, and hyperchloremia following several days of water restriction. In contrast, Jaber *et al*. (2004) reported hypokalemia in goats and sheep, respectively, subjected to various periods of water restriction, attributing the cause to deprivation-induced low DFI. Given that our experimental bucks exhibited a decrease in DFI alongside hyperkalemia, this explanation may not be directly applicable to these heat-stressed animals. According to Madhavan *et al.* (2023), such a response could be actually attributed to hyperosmolality, where hypertonicity induces a shift in K+ status from intracellular to extracellular fluids. Moreover, the observed state of hyperglycemia during the DE stage cannot be explained solely by the reduction in DFI. Instead, it might be attributed to the high utilization of GLU during the muscular movements of the respiratory system under heat-stress conditions (Srikandakumar *et al*., 2003). Interestingly, this response was deactivated after the animals were rehydrated, which contrasts with the observed values of bucks' RR (Figure 1F). Nonetheless, deprivation-induced low DFI seems to be associated with hypocalcemia during the DE stage, which returned to its EU level 10 days after water restoration. This came in concordance with the findings of Samara *et al.* (2012) in Aardi goats.

Furthermore, the experimental bucks displayed a notable decrease in their urine volume, as evidenced by data presented in Tables 2 and 3. This reduction in urine output may elucidate the concurrent elevation in blood BUN and BCRT concentrations observed in water-deprived goats, along with the decrease in UUN and UCRT concentrations, given that urinary excretion rates are influenced by GFR. Notably, goats possess kidneys with a prolonged loop of Henle compared to other species (Olsson *et al.*, 1997), which enables these organs to establish a substantial osmotic gradient within the medullary papilla. The action of ADH further facilitates the excretion of maximally concentrated urine during dehydration, as noted by Krishnan *et al.* (2023). Consequently, only the requisite amount of water necessary for waste product excretion is eliminated from the body.

Notably, our findings unveiled a correlation between tachypnea and the observed hemogasometric imbalances in heat-stressed and water-deprived bucks, as illustrated in Table 3 and Figure 1F. Surprisingly, the average daily venous blood pH remained steadfast during the DE stage, as depicted in Table 3 and Figure 2, suggesting an adaptive mechanism employed by these animals. Amidst conditions of heat stress, small ruminants primarily rely on evaporative cooling to regulate their body temperatures within the thermoneutral range, as documented by Silanikove (2000a,b). Nonetheless, this homeokinetic mechanism poses a potential risk to the acid–base balance system, as discussed by Olsson *et al.* (1997) and Srikandakumar *et al.* (2003). Fundamentally, acid–base balance hinges on the equilibrium between anions and cations, with extracellular hydrogen ions (H^+) being the primary determinant of body fluid pH. Normally, acids and

bases are continuously introduced into body fluids through ingestion or cellular metabolism (Krishnan *et al*., 2023; Madhavan *et al*., 2023). To counteract shifts in the hemogasometric profile, mammals employ three fundamental mechanisms: the blood buffer system, exhalation of CO_2 via the respiratory system, and excretion of H and/or HCO_3^- via the urinary system (Krishnan *et al*., 2023). The respiratory system plays a pivotal role in regulating blood pCO_2, a key factor in acid–base balance. As heat stress intensifies, RR increases, albeit with alterations in its depth, known as panting. Small ruminants experience two phases of panting. The first phase (FPP) entails rapid and shallow respiration, primarily confined to the respiratory dead space, thus unlikely to affect the acid–base profile (Srikandakumar *et al.,* 2003). Conversely, second phase panting (SPP) is characterized by slower and deeper respiration, leading to divergences in the acid–base profile. During SPP, increased alveolar ventilation accelerates pCO_2 elimination, potentially resulting in an elevated blood pH or respiratory alkalosis (Srikandakumar *et al.,* 2003). Compensatory mechanisms, including renal elimination of HCO_3^-, help restore the balance, although excessive loss of HCO_3^- can precipitate metabolic acidosis (Srikandakumar *et al.,* 2003). Evidence from our experiment indicated that withholding water for 72 h decreased blood pCO_2, HCO_3^-, tCO_2, and BE, while increasing pO_2 and sO_2, are consistent with SPP-type respiration observed under water deprivation accompanied by heat stress.

In a similar fashion, it was clearly evident based on the obtained findings herein that AG and SID increased in the heat-stressed and water-deprived bucks. In a healthy animal, serum typically contains more unmeasured anions than unmeasured cations (Srikandakumar *et al.,* 2003; Kraut & Madias, 2007). Measured cations in serum include Na^+, K^+, Ca^{++} and magnesium (Mg^{++}), while unmeasured cations encompass serum proteins and other pathological proteins. Likewise, measured anions comprise Cl^-, HCO_3^- and phosphate (PO_3^-), with unmeasured anions including lactate, urate, sulphates (SO_4^{-2}), and ALB. Consequently, changes in serum concentrations of these components can influence the virtual and calculated values of AG and SID, potentially reflecting in blood pH values. The observed hypernatremia in our study could explain the increase in SID but not AG, as serum concentrations of Na^+, K^+, and Cl^- all increased (Table 3). According to Winter *et al.* (1990), such a scenario typically indicates an increase in unmeasured anions. One significant unmeasured anion is ALB, which increased due to the experimental treatments (Table 2). Therefore, the elevation of AG in heat-stressed and water-deprived bucks could be attributed to hyperalbuminemia; however, further research is necessary to pinpoint the exact cause.

Following rehydration, our data revealed a return of all measured variables to their EU levels once the goats regained access to water in the RE stage (Table 1-3 and Figure 2). Notably, the gradual restoration of water loss from the vascular system during water deprivation was reflected in the calculated average PVC values, which were -0.58±0.16% for the RE stage and 0.07±0.19% 10 days after water restoration. This restoration process is primarily regulated by the rumen, where all ingested water is initially retained before being slowly released into the more permeable sections of the gastrointestinal tract (Silanikove, 1992), thereby maintaining blood constituents at normal levels and preventing sudden plasma dilution, thus averting hemolysis. Alamer (2006) noted a slow recovery in OSMO and PCV in goats due to the delayed ruminal absorption of water, which could explain the gradual return of plasma volume to its baseline level. Furthermore, serum BUN and BCRE concentrations remained elevated during the initial 8 h post-rehydration in the current experiment. These findings may be attributed to the delayed return of renal functions, specifically GFR to EU levels, suggesting another adaptive mechanism of these goats to maintain renal function at a lower level. Similar results have been reported in goats (Alamer, 2006) and sheep (Abdelatif & Ahmed, 1994). Conversely, serum ALB concentration decreased in the EU stage, explaining the subsequent return of serum AG 72 h after rehydration.

Additionally, pCO_2, HCO_3^-, tCO_2, and BE continued to decrease, while pO_2 and sO_2 continued to increase after rehydration. In response to the sudden reduction in stressful conditions (e.g., rehydration after dehydration or reduced heat stress), animals typically decrease their RR, leading to a decrease in CO_2 loss and an abrupt rise in blood pCO_2, resulting in mixed acidosis via both respiratory and metabolic pathways, by increasing the blood pCO_2 via the respiratory system in addition to the urinary loss of HCO_3^-. Although this scenario has been observed in cattle (Schneider *et al.,* 1988), it was not evident in sheep (Srikandakumar *et al.,* 2003). Unexpectedly, evidence from the present experiment indicated that the average daily blood pH of heat-stressed and water-deprived goats remained unchanged and within its physiological range after water reintroduction, suggesting a robust adaptive mechanism to prevent mixed acidosis in these animals (Table 3 and Figure 2). Further studies are warranted to validate these findings, particularly in larger samples of free-ranging goats, allowing for a fuller expression of their physiochemical adaptations. Additionally, incorporating essential variables reflecting hydration status, such as total body water, extracellular and intracellular fluid volumes, plasma and blood volumes, as well as interstitial fluid volume and urinary specific gravity, alongside monitoring cortisol and aldosterone hormone levels throughout all experimental periods, could significantly enrich our understanding of water physiology in heat-stressed and water-deprived bucks.

CONCLUSION

The exposure of heat-stressed goats to water deprivation led to noticeable alterations in their physiological responses, affecting hematological, biochemical, and hemogasometric profiles. The stable blood pH observed highlights their robust water-conserving mechanisms. Notably, all variables returned to baseline levels after 10 days of rehydration,

emphasizing the importance of allowing a recovery period of at least 10 days post-water restoration to mitigate the lingering effects of water deprivation before conducting experiments on heat-stressed goats. These findings underscore the adaptability of goats to arid conditions and suggest that further research can improve their welfare in challenging environments.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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