Research Article

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Response of saliva Na/K ratio to changing Na supply of lactating cows under tropical conditions

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Abstract

BACKGROUND: Factorial determination of the sodium (Na) requirement of heat-stressed lactating cows is hindered by accurate estimates of the Na losses through sweat. Direct studies, therefore, may be needed requiring information on the time course of healthy animals to become Na depleted and the subsequent rate of repletion. The rate of Na depletion and subsequent rate of Na repletion with two levels of dietary Na to lactating dairy cows housed under tropical conditions were investigated using the salivary Na/K.

RESULTS: The 12 lactating cows (salivary Na/K ratio 14.6) rapidly developed clinical signs of Na deficiency, including pica, polyuria and polydipsia, reduced body weight and reduced milk yield when fed a low-Na ration (0.33 g kg^{-1} dry matter (DM)) for 3 weeks. Deficiency symptoms were associated with a rapid decrease in salivary Na/K ratio to <4.3 from 7 to 21 days. Subsequent repletion of the cows with NaCl to a ration concentration of 1.1 or 1.6 g Na kg^{-1} DM for 5 weeks did not restore salivary Na/K ratio to values of >6.

CONCLUSION: A daily Na intake of heat-stressed lactating cows to a ration intake of 1.6 g Na kg^{-1} DM was insufficient to restore Na deficiency. One week was sufficient to deplete heat-stressed lactating cows of Na, allowing for rapid dose–response studies utilizing the salivary Na/K ratio as a parameter for Na status of cows under tropical conditions.

Keywords: sodium; saliva Na/K; lactating cows; tropical conditions

INTRODUCTION

Sodium (Na) is essential for dairy cows to maintain water balance: in the event of Na deficiency polyuria and polydipsia occur.[1] Furthermore, Na deficiency is associated with clinical signs such as pica, loss of appetite and a decreased milk yield in dairy cows.[2] Sodium is also a major component of salts in saliva to buffer acid generated during ruminal fermentation.[3] It is generally accepted that in Na-deficient ruminants the salivary Na concentration decreases to a value below 120 mmol L^{-1} with a concomitant increase in salivary potassium (K) concentration. As such, the use of salivary Na/K is a sensitive diagnostic tool to detect Na deficiency in ruminants.[4] Previously, Thiangtum *et al.*[5] showed the value of salivary Na/K in detecting Na deficiency in dairy cows housed under tropical conditions.

The current recommendations regarding an adequate Na supply for dairy cows ranges from 0.7 to 2.2 g kg^{-1} dry matter (DM).[6–10] However, these recommendations were derived from studies with animals housed under temperate conditions and thus do not take into account Na loss through sweating[11] during heat stress. Indeed, it was suggested by Sanchez *et al.*[11] that the Na requirement is increased during heat stress due to the higher Na loss associated with sweating. Furthermore, Schneider *et al.*[12] reported an increased DM intake when the Na content of rations of lactating cows during heat stress was raised from 1.8 to 5.5 g kg^{-1} DM. The issue of Na requirement of dairy cows in tropical countries was addressed by Thiangtum *et al*,[5] who arbitrarily suggested that dairy cows require a dietary Na content of 1.2 g kg^{-1} DM under tropical conditions. However, generalization of this tentative value of 1.2 g Na kg^{-1} DM is currently not warranted owing to a dearth of studies addressing the issue of Na requirements of dairy cattle in the Tropics.

The assessment of Na requirement of heat-stressed dairy cows is hindered by the fact that Na losses through sweat are difficult to

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quantify, and therefore direct studies of the Na requirements under tropical conditions are needed. Important information for such studies is the time course of healthy animals to become deplete of Na and the subsequent rate of repletion. In the current study, the rate of Na depletion and subsequent rate of Na repletion using two levels of dietary Na were investigated using salivary Na/K in heat-stressed lactating dairy cows. Lactating cows were initially fed a Na low ration (0.33 g kg^{-1} DM) for 3 weeks before being provided supplemental NaCl to a dietary concentration of 1.2 g (LNa) or 1.7 g Na kg^{-1} DM (HNa). The aim of the current study was to determine both the rate of depletion and repletion and it was hypothesized that high *versus* low Na repletion would restore the salivary Na/K ratio more rapidly.

MATERIAL AND METHODS

Animals and management

Twelve cross-bred (Holstein Friesian × indigenous) multiparous cows with a mean body weight (BW) of 476 kg (SD = 43) and 61 (SD = 37) days in milk were used. The cows were housed individually in a tie stall for a period of 8 weeks. The experiment was conducted during the summer season from the end of February to April. Ambient temperature and relative humidity inside the stall were recorded every 2 h from 10:00 Friday to 10:00 Saturday during the experiment (Maxim's iButton devices, San Jose, CA, USA).Temperature and relative humidity records were also obtained from the Nakhon Pathom meteorological station, located 500 m from the dairy barn of Kasetsart University (Kamphaengsaen, Nakhon Pathom, Thailand). Rectal temperature of each cow was measured weekly on Friday between 09:00 and 10:00. Cows were fed individually four times daily at 02:00, 07:00, 15:00 and 21:00 and the animals had free access to water. 24 h water intake was recorded between 09:00 and 10:00. The cows were milked twice daily and milk production was recorded at 14:30 Friday and 05:30 Saturday. BW was measured on days 0, 20 and 56 of the experiment.

Experimental design and rations

The experiment consisted of a depletion and a repletion period, with all cows offered the Na-deficient, total mixed ration (TMR, Table 1) *ad libitum* each day throughout the experiment. Orts for each individual cow were collected daily. After the 3-week depletion period, cows were blocked by milk yield and, within each block, cows were randomly allocated to either LNa or HNa. Dry matter intake observed during weeks 2 and 3 was used to determine the amount of supplemental NaCl to achieve 1.2 and 1.7 g Na kg^{-1} DM, resulting in a supplementation of 23.5 and 38.7 g d^{-1} of NaCl, respectively. During the 5-week repletion period, supplemental NaCl was offered daily to each cow in two equal portions fed at 10:00 and 15:00. Throughout the repletion period, all cows consumed all the supplemental salt offered.

Sample collection and chemical analyses

Samples of TMR (~2 kg) were collected weekly, dried at 60 °C, ground and pooled before analysis of dry matter, crude protein and ether extract according to AOAC procedures.[13] Neutral detergent fiber and acid detergent fiber were analyzed according the method described by Van Soest *et al.*[14] During the depletion period and approximately 30 min prior to the supplementation of salt during the repletion period, saliva samples were collected each Friday by placing sponges in the mouths of the cows at the third premolar in the maxilla for a period of 3 min. Subsequently, the liquid in the sponges was collected into a plastic bag, transferred to a tube and stored at −20 °C until mineral analysis. Blood samples (~10 mL) were collected from the jugular vein of each cow on each Friday at 10:00. Serum samples were obtained by centrifuging blood samples at 800 × *g* for 5 min. All serum samples were stored at −20 °C until mineral analysis by means of a blood gas analyzer (Nova Biomedical Corporation, Waltham, MA, USA). A milk sample (~30 mL) of the Friday pm and Saturday am milking of each cow was collected in a tube containing 0.2 g L^{-1} 2-bromo-2-nitro-1,3-propadiol. Preserved milk samples were pooled in proportion to the milk yield of each milking, and stored at 4 °C. The following Monday, fat was removed manually and deproteinated by adding 10% trichloroacetic acid, vortexed and centrifuged at 800 × *g* for 10 min. The collected supernatant was stored at −18 °C until mineral analysis.

During the repletion period, urine was quantitatively collected from each cow for 24 h every Friday. Urine was collected manually either through manual stimulation of the vulva or collection of urine that was spontaneously voided. After 24 h, total urine volume was recorded, thoroughly mixed and a sample (~100 mL) stored at −20 °C until analysis.

All samples of milk, saliva, urine and feed were analyzed for Na and K using a flame emission atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan) in accordance with the manufacturer's specifications.

Table 1. Ingredient and nutrient composition of the basal ration

Component	Content
Ingredient composition, (g kg^{-1} as fed)	
Rice straw	200.0
Cassava chips	420.0
Cotton seed	100.0
Soybean meal	120.0
Brewer's grain	50.0
Coconut oil	10.0
Molasses	64.0
Urea	18.0
Calcium phosphate	7.0
MgO	3.0
Sulfur	3.0
Premix[a]	5.0
Analyzed nutrient composition	
Dry matter (DM, g kg^{-1} as fed)	903.0
Crude protein (g kg^{-1} DM)	144.3
Ether extract (g kg^{-1} DM)	62.5
Neutral detergent fiber (g kg^{-1} DM)	275.4
Acid detergent fiber (g kg^{-1} DM)	207.2
Sodium (g kg^{-1} DM)	0.33
Potassium (g kg^{-1} DM)	10.5

[a] The mineral premix consists of 440 000 IU vitamin A, 60 000 IU vitamin D3, 30 000 IU vitamin E, 11.6 g Fe, 0.03 g Mn, 5.6 g Cu, 11.60 g Zn, 0.07 g I, 0.06 g Se, 10.0 g Mg and 15.0 g P kg^{-1}.

Calculations and statistical analysis

The temperature humidity index (THI) was calculated according to the following formula:[15]

$$THI = \left(1.8 \times T_{db} + 32\right) - \left[(0.55 - 0.0055 \times RH) \times \left(1.8 \times T_{db} - 26\right)\right]$$

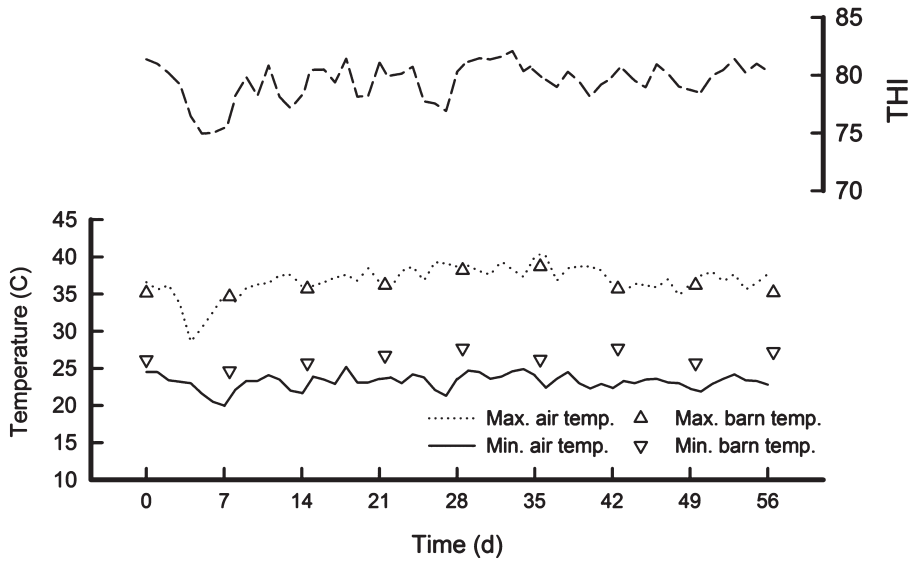

Figure 1. Atmospheric and barn temperatures (maximum and minimum) and temperature humidity index (THI) during the 56-day experiment.

where T_{db} = dry bulb temperature (°C) and RH = relative humidity (%). Data from the depletion period were subjected to repeated-measures analysis of variance (ANOVA). The data from the repletion period were subjected to repeated-measures ANOVA with block and sodium treatment as factors, with the corresponding data of week 2 of the depletion period as a covariate. All statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Time effects were tested by mean of orthogonal polynomials. The level of statistical significance was pre-set at a probability level below 0.05 with a trend considered of $0.10 < P \leq 0.05$.

RESULTS

During the depletion period, the daily THI calculated from the temperature and humidity inside the barn ranged from 79.5 to 83.1 (Fig. 1) with a mean value of 81.6 (SD 1.5). The temperature and humidity recorded in the barn agreed closely with the values recorded by the meteorological station located nearby (Fig. 1). Likewise, a similar range in THI was measured during the repletion period and a mean daily THI value of 82.6 (SD 0.8) was found. Despite the high THI, cows were able to maintain their body temperature within the physiological range during both the depletion and repletion period and, for the two periods combined, the group mean values ranged between 37.9 and 38.9 °C.

Depletion period

BW of the cows decreased ($P < 0.001$) from 476 (SD 43.2, $n = 12$) to 426 kg (SD 47.0, $n = 12$) during the 3-week depletion period. Likewise, DM intake decreased from 12.5 to 10.8 kg d^{-1} (Table 2) during the depletion period but the difference in DM intake over time was not statistically significant. In contrast, milk yield was 15.4% lower ($P < 0.001$) at the end of the depletion period compared to initial yield. Both water intake and urine production significantly increased during the depletion period, and values were found to be 1.7 and 2.5 times greater, respectively, at the end of the depletion period compared to the initial values. In addition, pica and skin licking were observed starting at 7 days. Salivary Na concentration significantly decreased, with a concomitant increase in salivary K concentrations (Table 2), and values of 98.1 and 26.7 mmol L^{-1}, respectively, were observed at the end of the depletion period. Furthermore, the salivary Na/K ratio was found to decrease significantly during the depletion period, with a value at 7 days already significantly lower than the value found at the start of the depletion period. Serum Na and K concentrations responded in a quadratic fashion over time and the lowest values were found 7 days after the start of the depletion period, i.e. 136 and 2.7 mmol L^{-1}, respectively (Table 2). However, in contrast to serum Na concentrations, serum K values at the end of the depletion period were found to be 24.4% lower than the value at day 0.

Repletion period

BW of the cows remained unchanged ($P = 0.121$) and was similar between Na intake ($P = 0.893$) during the repletion period. Across time and Na intake, mean BW was found to be 433 kg (SD 8.6). Dry matter intake was neither influenced by the amount of supplemented salt nor by time × Na intake. However, for DM intake a quadratic time trend was observed during the repletion period (Table 3). Milk yield did not respond to the increase in DM intake and remained constant throughout the repletion period. Furthermore, milk yield was similar for the two levels of Na intakes and across time and, for the two Na intakes combined, it was found to be 14.6 L d^{-1} (SD 2.8). The intake of water was neither affected by Na intake nor by time or by time × Na intake. Average water intake of the two groups over the 5-week repletion period was 120 L d^{-1}. Likewise, urine production was not affected by the amount of supplemented salt, and urine production over time (Table 3) was not significant; however, a time × Na intake trend ($P = 0.063$) was observed.

During the repletion period, interactions between time and Na intake did not occur with respect to the intakes of Na and K and their respective excretions with milk and urine (Table 4). Sodium intake was significantly different between the two levels of salt supplementation. Furthermore, a quadratic trend of Na intake over time was found and this observation is in line with the observed time trend of DM intake during the repletion period. Likewise, for K intake also a quadratic time trend was found but K intakes were similar between the two levels of Na intake (Table 4). Na excretion with urine was not significantly different

Table 2. Dry matter (DM) intake, milk yield, water intake, urine production and concentrations of sodium and potassium in saliva and serum in the course of the depletion period

Item	Days of Na depletion				SEM	Significance of time effect
	0	7	14	21		
DM intake (kg d^{-1})	12.5	12.3	11.8	10.8	0.7	NS
Milk yield (L d^{-1})	16.9	13.7	12.9	14.3	0.6	L,Q
Water intake (L d^{-1})	71	89	94	123	7.9	L
Urine production (L d^{-1})	23	26	39	58	6.1	L,Q
Saliva						
Na (mmol L^{-1})	128.7	97.7	82.1	98.1	4.2	L,Q
K (mmol L^{-1})	10.2	28.8	26.9	26.7	1.8	L,Q
Na/K	14.6	3.8	3.4	4.3	0.6	L,Q
Serum						
Na (mmol L^{-1})	138	136	138	138	0.3	Q
K (mmol L^{-1})	4.5	2.7	3.1	3.4	0.1	L,Q

SEM, standard error of mean; NS, not significant; L, linear; Q, quadratic.

Table 3. Dry matter (DM) intake, milk yield, water intake and urine production in the course of the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
DM intake (kg d^{-1})	Low	11.4	11.4	12.3	11.9	13.6	13.3	0.88	Q[a]	NS	NS
	High	10.2	10.6	13.0	12.6	13.1	13.7				
Milk yield (L d^{-1})	Low	14.2	14.5	14.8	15.2	13.4	14.2	0.56	NS	NS	NS
	High	14.4	15.2	15.4	14.9	15.2	14.1				
Water intake (L d^{-1})	Low	97	104	106	117	102	105	16.1	NS	NS	NS
	High	148	122	151	143	121	125				
Urine production (L d^{-1})	Low	40	37	38	49	35	37	14.0	NS	NS	L[a]
	High	75	57	73	68	56	50				

SEM, standard error of the mean; NS, not significant; L, linear; Q, quadratic.
[a] Trend ($0.10 < P \leq 0.05$).

between the two levels of salt supplementation or between the different time points (Table 4). For all time points combined, mean urinary Na excretion was found to be 0.3 and 1.7 g d^{-1} for the LNa and HNa group, respectively. K excretion with urine showed a linear trend ($P = 0.055$) over time, while no effect was found for Na intake or the interaction between time and Na intake. The Na and K concentrations of milk (data not shown) increased linearly ($P < 0.024$) in time, i.e. from 0.33 to 0.35 g L^{-1} and from 1.47 to 1.65 g L^{-1}, respectively. These increases were independent ($P > 0.559$) from the amount of supplemental salt and were not affected ($P > 0.281$) by time × Na intake. Sodium and K excretion with milk was not influenced by Na intake, time or their interaction (Table 4). For the two Na intakes combined, the mean Na and K excretion with milk over the repletion period was found to be 5.1 and 23.1 g d^{-1}, respectively.

There was no effect observed for salivary Na concentrations due to the supplementation of Na (Table 5). Furthermore, salivary Na concentrations were not affected by time × Na intake but a linear trend ($P = 0.050$) in time was observed. Neither the salivary K concentration nor the salivary Na/K ratio was statistically different between the dietary Na intake (Table 5), and no time or interaction was found. The serum concentrations of Na and K were not influenced by the amount of supplemental salt, time or by time × Na intake (Table 5).

DISCUSSION

During the depletion period, cows developed clinical signs of Na deficiency such as polyuria, polydipsia, pica and a decrease in milk yield, 1 to 2 weeks after the start of feeding of the ration containing 0.33 g Na kg DM^{-1}. The occurrence of polyuria and polydipsia during Na deficiency is caused by the inability of the cow to maintain the osmotic pressure of the extracellular fluid (ECF). Briefly, Na is the main cation of the ECF and, together with its associated anions, it accounts for more than 90% of the osmotic pressure of the ECF.[16] A deficit of Na in the ECF hampers the release of antidiuretic hormone (ADH), thereby causing a rapid renal excretion of excess water to counteract the initial decrease of the Na concentration of the ECF.[16] However, at this stage the volume of the ECF is lowered, which triggers activation of angiotensin II, leading to an increased water intake to restore the volume of ECF.[17] Clearly, the latter action leads to lower Na concentrations of the ECF, thereby triggering the aforementioned physiological actions. The clinical observations of Na deficiency were associated with decreased salivary Na and increased salivary K concentrations, leading to a salivary Na/K ratio of <4.3, which is indicative of Na deficiency.[18] The current observations on salivary Na and K concentrations are in line with data reported by Thiangtum *et al.*,[5] who reported that the salivary Na/K ratio decreased to 4.8 when a ration was fed containing 0.4 g Na kg^{-1} DM for 32 days. In the latter study, however,

Table 4. Intake and excretions by means of milk and urine of sodium and potassium during the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
Sodium (g d^{-1})											
Intake	Low	13.0	13.0	13.3	13.2	13.8	13.7	0.29	Q[a]	<0.001	NS
	High	18.6	18.8	19.5	19.4	19.6	19.8				
Milk	Low	4.7	4.5	4.9	5.7	5.0	4.9	0.51	NS	NS	NS
	High	4.9	5.0	5.6	5.2	5.6	5.0				
Urine	Low	0.3	0.3	0.2	0.2	0.2	0.7	0.38	NS	NS	NS
	High	1.0	2.4	1.7	1.1	1.2	2.9				
Potassium (g d^{-1})											
Intake	Low	119	119	128	124	142	139	9.25	Q[a]	NS	NS
	High	107	111	136	132	136	143				
Milk	Low	21.1	21.9	24.3	26.4	21.6	23.5	0.73	NS	NS	NS
	High	20.9	22.5	23.4	24.8	23.9	23.2				
Urine	Low	65.8	73.5	56.2	67.8	69.0	85.5	6.69	L[a]	NS	NS
	High	68.7	72.4	67.0	63.0	96.0	83.7				

SEM, standard error of the mean; NS, not significant; L, linear; Q, quadratic.
[a] Trend ($0.10 < P \leq 0.05$).

Table 5. The concentrations of sodium and potassium in saliva and serum during the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
Saliva											
Na (mmol L^{-1})	Low	88	104	88	85	91	100	3.10	L[a]	NS	NS
	High	108	108	104	95	100	103				
K (mmol L^{-1})	Low	31.8	44.2	35.1	39.2	36.7	31.2	2.20	NS	NS	NS
	High	21.5	26.1	27.6	26.3	28.6	25.5				
Na/K	Low	3.4	2.4	2.6	2.3	2.9	3.4	0.29	NS	NS	NS
	High	5.3	4.3	3.8	3.8	3.6	4.7				
Serum											
Na (mmol L^{-1})	Low	138	138	137	139	138	140	0.37	NS	NS	NS
	High	138	139	138	139	139	140				
K (mmol L^{-1})	Low	3.5	3.4	3.5	3.5	3.4	3.4	0.09	NS	NS	NS
	High	3.4	4.0	3.8	3.6	3.5	3.7				

SEM, standard error of the mean; NS, not significant; L, linear.
[a] Trend ($0.10 < P \leq 0.05$).

no clinical signs such as pica were observed. The observed changes in salivary electrolyte concentrations are most likely explained by the action of aldosterone, because it has been shown by Riad *et al.*[18,19] that during Na deficiency this hormone responds to a decline in serum Na concentration and inhibits the salivary secretion of Na and concomitantly stimulates salivary K secretion.

The low intakes of Na during the depletion period caused transient changes in the plasma concentrations of Na and K. It is unknown whether this observation can be generalized because, to the authors' knowledge, time-related changes in plasma Na and K concentrations during a diet-induced Na deficiency have not been previously reported in dairy cattle. These changes are difficult to explain but the data indicate that initial responses of plasma Na and K concentrations to Na deficiency are alleviated over time. It can be speculated that the initial decrease in plasma Na concentration is counteracted by an aldosterone-mediated action on the renal conservation of Na.[20] Furthermore, a severe decrease in plasma Na concentration can be prevented for several months because the rumen can act as a buffer containing up to 50% of the available body Na.[21] Moreover, high levels of circulating aldosterone are known to shift K from the extracellular to the intracellular pool[22] and enhance K secretion into the tubular lumen, thereby increasing the urinary excretion of K.[23] Plasma K concentrations initially may have over-responded to the action of aldosterone, causing plasma K concentrations to decrease to critically low levels (~2.5 mmol L^{-1}), as previously reported.[23] Since aldosterone is the key regulator of extracellular K concentration,[24] such low plasma K concentrations may have alleviated the initial aldosterone responses on the plasma K concentration.

The data clearly show that supplemental Na did not restore the salivary Na/K to values of >6.[4] This was unexpected as the highest level of supplementation was calculated to exceed (1.7 g kg^{-1} DM) the dietary requirement (1.2 g kg^{-1} DM)[5] of lactating dairy cows in a tropical environment. The actual Na consumed was found

to be 1.6 g kg^{-1} DM for the HNa group. It thus appears that the amount of Na that was withdrawn from the mobilizable Na pool during the 3 weeks of Na depletion was not replenished by the supplemental Na during the 5-week repletion period. The average amount of Na not accounted for in milk and urine for the LNa and HNa groups during the repletion period was 8.1 and 12.4 g d^{-1}. The losses due to sweat, as well as unabsorbed dietary and endogenous fecal Na, were not measured in the present study. Using the dietary Na absorption data of Thiangtum *et al.*,[5] it can be estimated that the apparent Na absorption of the LNa and HNa cows should have been approximately 85% and 87%, respectively. Using these values, 2.0 and 2.4 g d^{-1} Na would have been excreted via the feces of the cows in the LNa and HNa groups, leaving 6.1 and 9.9 g d^{-1} unaccounted for, respectively. These amounts of Na would have been used by the cow to replenish the mobilizable Na pool of the rumen and sweat production. During the 3 weeks of Na depletion, a total amount of 44 g Na was minimally withdrawn from the animal's mobilizable Na pool.

Throughout the 56-day experiment, the cows experienced, on average, 4 h of mild (THI 72–79), 19 h of moderate (THI 79–89) and 1 h of severe (THI > 89) heat stress[25,26] each 24 h. Jenkinson and Mabon[27] reported that Na losses with sweat ranged from 7.1 to 10 mg $(m^2)^{-1}$ h^{-1} when THI ranged from 79 to 81. Gebremedhin *et al.*[28] reported sweating rates in Holstein cows of up to 660 g $(m^2)^{-1}$ h^{-1} at a THI of 79.6. When a sweating rate of 600 g $(m^2)^{-1}$ h^{-1} is used and an estimated surface area of 4.5 m^2 (BW 433 kg),[29] the Na concentration of sweat under the current conditions were maximally 6.6 mmol Na kg^{-1}, assuming the aforementioned apparent Na absorption and no replenishment of the mobilizable Na pool of the rumen. In the case of replenishment of the rumen pool, the Na concentration of sweat was 5.8 mmol Na kg^{-1}. The latter two values are much higher than the value of 2 mmol Na kg^{-1} sweat reported by Johnson.[30] Clearly, there is a considerable discrepancy between the Na losses with sweat calculated here and the data provided by Jenkinson and Mabon[27] which cannot easily be explained. The issue on Na losses with sweat in cattle remains and requires further research to obtain accurate values. The lack of unequivocal estimates on the Na losses with sweat, in combination with the fact that the Na-supplemented cows did not restore their salivary Na and K concentrations, hinders potential refinement of the previous recommendation regarding the Na requirement of lactating cows under tropical conditions.[5]

Across dietary Na intake and time, cows ingested 128.0 g K d^{-1}. Using the average dietary K absorption data (93.0%) of Thiangtum *et al.*,[5] it can be estimated that the apparent K excretion with feces was 9.0 g d^{-1}. The associated K excretions with milk and urine were found to be 23.1 and 72.4 g d^{-1}. Consequently, 23.5 g d^{-1} K was available for excretion with sweat. Using the data on K excretion with sweat reported by Jenkinson and Mabon[27] and the aforementioned values of surface area and time of exposure to heat stress, K excretion with sweat is estimated to be ~2 g d^{-1}. Therefore, it can be excluded that the animals suffered from K deficiency in the current experiment, corroborated by the lack of clinical signs of K deficiency such as muscular weakness, stiffness and paralysis.[24]

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