**Research Article** 

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# An attempt to define the sodium requirement of lactating dairy cows in a tropical environment

Wandee Thiangtum,<sup>a,b</sup>\* Adisorn Yawongsa,<sup>a,b</sup> Jan Thomas Schonewille,<sup>b</sup> Theera Rukkwamsuk,<sup>a</sup> Chalermpon Yuangklang,<sup>c</sup> Martin WA Verstegen<sup>d</sup> and Wouter H Hendriks<sup>b,d</sup>

### Abstract

BACKGROUND: Lactating dairy cattle in the tropics may require more sodium (Na) owing to the hot and humid climatic conditions. It is unknown whether the current recommendations on Na for lactating cows can be quantitatively used in tropical countries. This study attempted to define the Na requirement of lactating dairy cows under tropical conditions by measuring Na levels in saliva, milk and faeces.

RESULTS: The concentrations of Na and potassium (K) in milk, faeces and serum were not affected by dietary treatments. The amount of Na absorbed by cows fed the basal (low-Na) diet containing 0.4 g Na kg<sup>-1</sup> dry matter (DM) was equal to the amount of Na lost in the milk, showing that these animals were fed an Na-deficient ration. This observation was corroborated by salivary Na and K levels, with the cows on the low-Na diet having salivary Na concentrations below 120 mmol L<sup>-1</sup> in combination with salivary K concentrations above 20 mmol L<sup>-1</sup> (P < 0.05).

CONCLUSION: Consumption of a daily ration formulated to contain the current Na requirement set by the NRC appears to provide too much Na for lactating cows under tropical conditions. A tentative value of 1.2 g kg<sup>-1</sup> DM is proposed as the Na requirement for dairy cows under tropical conditions. (© 2011 Society of Chemical Industry

Keywords: sodium chloride; sodium requirement; lactating cows; tropical conditions

# INTRODUCTION

Sodium (Na) is a primary extracellular cation in animals and its main functions include maintenance of body fluid balance, osmotic pressure and acid-base balance.<sup>1</sup> Furthermore, Na is a major component of minerals present in saliva and it buffers acids produced during ruminal fermentation.<sup>2</sup> In cows, Na deficiency has been associated with loss of appetite, decreased milk yield and pica.<sup>3</sup> In ruminants the salivary Na concentration drops to below 120 mmol L<sup>-1</sup> with a concomitant increase in salivary K concentration during Na deficiency.<sup>4</sup> Therefore an assessment of salivary Na and K concentrations is highly instrumental in diagnosing Na deficiency. Clearly, a drop in salivary Na concentration in combination with an increased level of salivary K results in a decreased Na/K ratio. Indeed, it was suggested by Schonewille and Beynen<sup>4</sup> that a salivary Na/K ratio lower than 6 is indicative of Na deficiency. The current Na requirements for dairy cows recommended by various authorities range from 0.5 to 2.2 g kg<sup>-1</sup> dry matter (DM) (Table 1). However, it is unclear whether these recommendations can be quantitatively extrapolated to tropical conditions, because all requirements were set under temperate conditions. Consequently, the Na requirements listed in Table 1 do not specifically take into account Na loss through sweating during heat stress. Indeed, it was suggested by Sanchez et al.<sup>5</sup> that the Na requirement is increased during heat stress owing to the higher Na loss associated

with sweating. Furthermore, Schneider *et al.*<sup>6,7</sup> reported that dry matter intake (DMI) was increased during heat stress when the dietary Na content was increased from 1.8 to 5.5 g kg<sup>-1</sup> diet. This increase in DMI can be explained by the possibility that animals fed the low-Na ration were in negative Na balance.

In tropical countries such as Thailand the level of Na in feedstuffs used to formulate dairy rations is much lower than recommended<sup>8</sup> by the various authorities (Table 1) and therefore dairy rations

- a Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen, Nakhon Pathom 73140, Thailand
- b Nutrition Division, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
- c Department of Animal Science, Faculty of Natural Resources, Rajamangala University of Technology Isan, Phangkhon, Sakon Nakhon 47160, Thailand
- d Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

<sup>\*</sup> Correspondence to: Wandee Thiangtum, Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen, Nakhon Pathom 73140, Thailand. E-mail: fvetwdr@yahoo.com

Table 1.         Summary of sodium (Na) requirements set by different authorities					
Reference	Na requirement (g kg <sup>-1</sup> dry matter)				
Underwood and Suttle, 1999 <sup>23</sup> ARC, 1980 <sup>34</sup> CVB, 2005 <sup>35</sup> DLG, 2001 <sup>36</sup> INRA, 1989 <sup>37</sup> NRC, 2001 <sup>3</sup>	0.5-1.0 0.8-1.2 0.7 <sup>a</sup> -1.4 <sup>b</sup> 1.0-1.5 <sup>b</sup> 1.0-1.7 1.9-2.2				
<sup>a</sup> Dry cow. <sup>b</sup> Lactating cow, 40 kg of milk.					

are routinely supplemented with Na. The scientific basis for the amounts of Na addition to the ration, however, is scant. Generally, dairy cattle in tropical countries have lower production and consume poorer-quality feeds while being exposed at certain times to high temperature and relative humidity. In addition, excessive Na intake relative to the animals' requirement should be avoided in tropical countries in the light of responsible water management and water scarcity, as water consumption increases with excessive Na intake to maintain normal physiological Na concentrations in the extracellular fluid. The aim of this study was to attempt to define the Na requirement of lactating dairy cows under tropical conditions by measuring Na levels in saliva, milk and faeces.

# MATERIALS AND METHODS

#### Animals and experimental design

Fifteen crossbred multiparous Holstein Friesian (HF × indigenous) cows with a mean body weight of 485 kg (standard deviation (SD) 74 kg) and 93 days in milk (SD 39 days) were used. The cows were housed individually in a naturally ventilated tied stall for a period of 28 days, whereafter, during the following 9 days, they were housed in individual metabolic cages to facilitate the collection of faeces, saliva and blood. The experiment was conducted during the summer season from March to April with the cows exposed to local climatic conditions. Daily minimum and maximum temperatures directly outside the stall were measured throughout the study. Cows were fed individually and milked twice daily at 06:00 and 17:00. Prior to the start of the experiment, animals were randomly assigned to one of three dietary treatments, i.e. low, medium or high Na.

#### **Experimental rations**

All cows were fed a total mixed ration (TMR). The basal TMR was formulated to be deficient in Na (Table 2) and contained 0.4 g Na kg<sup>-1</sup> DM. The medium- and high-Na rations were formulated by supplementing the basal ration with two levels of salt (NaCl). The Na contents of the medium- and high-Na rations were 1.6 and 4.0 g kg<sup>-1</sup> DM respectively. The ingredient and nutrient compositions of the three dietary treatments are given in Table 2. The TMR was offered *ad libitum* twice daily and leftovers were removed twice daily before feeding. Water was available to all cows at all times.

#### Sample collection and chemical analyses

Feed intake of each cow was recorded daily during the collection period. Samples of the TMR were taken weekly, dried at 60  $^{\circ}$ C,

**Table 2.**Ingredient and nutrient compositions of low-, medium- andhigh-sodium (Na) experimental rations

Component	Low Na	Medium Na	High Na
Ingredient composition			
Constant components <sup>a</sup> (g kg <sup><math>-1</math></sup> as fed)	630.0	630.0	630.0
Cassava chips (g $kg^{-1}$ as fed)	370.0	367.0	361.0
Salt (NaCl) (g $kg^{-1}$ as fed)	-	3.0	9.0
Analysed nutrient composition			
Dry matter (g kg $^{-1}$ as fed)	797.2	768.6	791.7
Crude protein (g $kg^{-1}$ )	173.3	171.2	166.1
Ether extract (g $kg^{-1}$ )	47.7	45.1	46.0
Crude ash (g kg $^{-1}$ )	90.5	86.4	91.7
Neutral detergent fibre (g $kg^{-1}$ )	376.2	385.3	352.6
Acid detergent fibre (g $kg^{-1}$ )	231.8	240.8	212.9
Sodium (g kg <sup>-1</sup> )	0.4	1.6 <sup>b</sup>	4.0 <sup>b</sup>
Potassium (g kg <sup>-1</sup> )	10.2	10.7	10.2

<sup>a</sup> The constant components were 112 g soybean meal, 50 g dried tomato pomace, 100 g cottonseed meal, 51 g dried brewer's grain, 17 g tallow, 62 g molasses, 18 g urea, 1 g dicalcium phosphate, 3 g magnesium oxide, 6 g oystershell, 2 g sulphur, 205 g milled rice straw and 3 g mineral premix kg<sup>-1</sup> diet. The mineral premix consisted of 440 000 IU vitamin A, 60 000 IU vitamin D<sub>3</sub>, 30 000 IU vitamin E, 11.6 g Fe, 0.03 g Mn, 5.6 g Cu, 11.6 g Zn, 0.07 g l, 0.06 g Se, 10 g Mg and 15 g P kg<sup>-1</sup>.

<sup>b</sup> Calculated on the basis of the analysed Na content of the low-Na ration and the amount of Na supplementation.

ground and pooled per treatment before analyses of dry matter, crude protein and crude fibre according to AOAC procedures.<sup>9</sup> Intrinsic Na and K of the TMR were measured using wet ashing and atomic absorption spectrophotometry.

Faeces were quantitatively collected and weighed each day during the 5 day collection period. The faeces collected each day were thoroughly mixed and samples of 10% of the total amount of faeces were taken and stored at -18 °C in plastic bags. At the end of the experiment the faeces samples were pooled for each cow and mixed thoroughly. The pooled samples were dried at 60 °C, ground and stored until analyses for Na and K. Data from feed and faeces were used to derive the amount of Na and K being absorbed.

Milk production was recorded during the collection period and samples of approximately 30 mL per milking time (06:00 and 17:00) were collected for 5 days. Milk samples in proportion to yield on the sampling day were preserved by the addition of 0.2 g L<sup>-1</sup> 2-bromo-2-nitro-1,3-propanediol and stored at 5 °C. Five-day milk samples from each cow were mixed, deproteinised with 100 g L<sup>-1</sup> trichloroacetic acid, vortexed and centrifuged at  $800 \times g$  for 10 min, whereafter the supernatant was stored at -18 °C until mineral analyses.

Blood samples (10 mL) were collected from the jugular vein of each cow on the last day of the collection period at 10:00. Serum samples were obtained by centrifuging the blood samples at 800 × g for 5 min. All serum samples were stored at -20 °C until mineral analyses.

Saliva samples were collected on day 4 of the faeces collection period before morning feeding to prevent Na contamination by the feed. Saliva sampling was done 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 into a tube and stored at

(P < 0.05). SEM, standard error of mean.

 $-18\,^\circ\text{C}.$  Each sponge was washed thoroughly with demineralised water and dried at 40  $^\circ\text{C}$  on a glass plate before being used to collect saliva.

All samples of faeces, milk, serum and saliva were analysed for Na and K using a flame emission atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan) in accordance with the manufacturer's specifications.<sup>10</sup>

#### **Calculations and statistical analysis**

The data were statistically analysed by subjecting them to analysis of variance with treatment as factor. Multiple comparisons between groups were made by Duncan's test. All statistical analyses were performed using SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA) with the level of significance set at 5%.

# **RESULTS AND DISCUSSION**

The mean neutral detergent fibre content of the experimental rations was found to be 371 g kg<sup>-1</sup> (SD 16.9, n = 3), which is lower than recommended by the NRC.<sup>3</sup> However, the animals showed normal rumination, were apparently healthy and remained in good condition during the experiment. DMI was not affected by dietary treatment (P > 0.38). The mean intake for all cows was 16.5 kg DM day<sup>-1</sup> (standard error (SE) 0.33). The observed level of DMI was somewhat lower than anticipated, i.e. 16.5 vs  $18 \text{ kg day}^{-1}$ . Although there were no significant differences in DMI among treatments, DMI tended to increase with Na supplementation  $(low, 15.9 \text{ kg day}^{-1}; medium, 16.3 \text{ kg day}^{-1}; high, 17.1 \text{ kg day}^{-1}).$ In a study by Sanchez et al.,<sup>11</sup> dietary supplementation with NaCl did not alter DMI. In some earlier studies, DMI was increased when cows received a ration with an increased amount of both Na and K.<sup>12,13</sup> We only applied our treatment for a period of 37 days, which was not sufficiently long to induce severe Na deprivation in the low-Na-fed animals. Aines and Smith<sup>14</sup> reported that mild to severe symptoms of Na deficiency were shown after 16 months of feeding a low-Na diet, because the rumen could act as a buffer and contain up to 50% of the available body Na.<sup>15</sup>

As expected, Na intake was significantly affected by treatment (Table 3). Faecal Na excretion was significantly higher in the medium- and high-Na groups than in the low-Na group. Apparent Na absorption expressed as a percentage of intake was significantly higher with the high-Na diet. Apparent Na absorption ranged between 80 and 96% of intake, which is in agreement with Kemp<sup>16</sup> and Martz et al.,17 who reported a similar range of apparent Na absorption in dairy cows. The relatively low level of apparent Na absorption in cows fed the low-Na ration can be explained by the inevitable faecal Na loss. Based on specific assumptions for estimating the endogenous faecal Na loss, a value of 1.6 g day<sup>-1</sup> was calculated by Schonewille and Beynen.<sup>4</sup> This value is similar to the faecal Na loss of 1.4 g day<sup>-1</sup> in the low-Na fed cows. The cows fed the low-Na diet also had a very low Na concentration in the saliva (Table 4). Apparent K absorption was not significantly affected by dietary Na concentration and was 93% of intake (Table 3). This value is in agreement with the results of Hemken<sup>18</sup> and Greene et al., <sup>19,20</sup> who reported an apparent faecal digestibility of 95% or higher for most feedstuffs.

Concentrations of salivary Na and K are given in Table 4. When the dietary supply of Na is sufficient, the salivary Na concentration is higher than 120 mmol  $L^{-1}$ .<sup>21</sup> In the present study the cows fed the low-Na ration had a salivary Na concentration of 95.6 mmol  $L^{-1}$ , indicating that these animals were fed below their Na requirement. **Table 3.** Sodium and potassium intake, faecal excretion, apparent absorption and milk content of dairy cows fed low-, medium- and high-sodium (Na) rations

		Treatmer			
Parameter	Low Na	Medium Na	High Na	Pooled SEM	P value
Sodium					
Intake (g day <sup>-1</sup> )	6.9a	26.3b	67.8c	6.8	< 0.001
Faeces (g day <sup>-1</sup> )	1.4a	3.0b	2.6ab	0.3	0.041
Absorption (g day <sup>-1</sup> )	5.5a	23.3b	65.2c	6.7	< 0.001
Absorption (% of intake)	80.2a	88.7ab	96.1b	2.4	0.013
Milk (g day <sup>-1</sup> )	5.5	6.5	6.9	0.4	0.446
Potassium					
Intake (g day <sup>-1</sup> )	162.7	173.8	173.7	3.4	0.328
Faeces (g day <sup>-1</sup> )	12.1	11.6	12.1	0.8	0.972
Absorption (g day <sup>-1</sup> )	150.5	162.2	161.6	2.9	0.758
Absorption (% of intake)	92.6	93.3	93.2	0.4	0.198
Milk (g day <sup>-1</sup> )	23.6	25.4	29.3	1.7	0.422
Means with different letters in a row are significantly different					

 Table 4.
 Concentrations of sodium (Na) and potassium (K) in saliva and serum of dairy cows fed low-, medium- and high-Na rations

		Treatment				
Parameter	Low Na	Medium Na	High Na	Pooled SEM	P value	
Saliva						
Na (mmol $L^{-1}$ )	95.6a	121.0ab	132.0b	6.2	0.033	
K (mmol $L^{-1}$ )	21.4a	11.8b	11.7b	1.7	0.020	
Na/K ratio	4.82a	10.27b	12.46b	1.2	0.009	
Serum						
Na (mmol $L^{-1}$ )	139	141	142	1.1	0.641	
K (mmol $L^{-1}$ )	4.0	4.0	4.1	0.1	0.999	
Means with different letters in a row are significantly different $(P < 0.05)$ . SEM, standard error of mean.						

The salivary K concentration was approximately 12 mmol L<sup>-1</sup> after feeding the medium- and high-Na rations. This level is similar to the value set by Silanikove *et al.*<sup>13</sup> as a tentative criterion for a sufficient supply of dietary Na. In Na-deficient ruminants there is a replacement of Na<sup>+</sup> with K<sup>+</sup> in the saliva, causing a reduction in the Na/K ratio.<sup>22,23</sup> The use of the salivary Na/K ratio as a diagnostic tool to detect Na deficiency has been extensively reviewed by Schonewille and Beynen<sup>4</sup> and Suttle,<sup>24</sup> and it was concluded that an Na/K ratio<sup>4</sup> lower than 6 is associated with Na deficiency in ruminants. In the current study the salivary Na/K ratio was 4.8 when the cows were fed the low-Na ration. Such a low Na/K ratio corroborates our conclusion that Na deficiency occurred when the cows were fed the low-Na ration.

The apparent K absorption was greater than 150 g day<sup>-1</sup>, while 23.6–29.3 g day<sup>-1</sup> of K was excreted in the milk. The inevitable urinary loss of K is estimated to be 38 mg kg<sup>-1</sup> body weight.<sup>25</sup> Thus in cows with a body weight of 485 kg the inevitable urinary K loss can be expected to be around 18.4 g day<sup>-1</sup>. Based on this

calculation, approximately 100 g of K was available for excretion in sweat, at least in adult, non-pregnant cows. Quantitative data on K loss through sweating are, at least to our knowledge, not available. The clinical signs of K deficiency are not well documented, but reduced appetite, poor growth, muscular weakness, stiffness, paralysis and intracellular acidosis have been reported.<sup>23</sup> In the current study the animals appeared healthy, indicating that K deficiency did not occur during the trial.

The increase in Na intake did not induce any changes in serum concentrations of Na and K. These results are consistent with previous studies that reported an increased intake of Na via supplementation of the diet with NaCl and NaHCO3 without any effect on plasma Na concentration.<sup>7,26-28</sup> Also, homeostatic regulation, which is controlled by several hormones, can maintain serum Na levels during Na deficiency.<sup>29</sup> Aldosterone responds to a decline in serum Na concentration or to systemic low blood pressure. As a result, there is an increase in renal conservation of Na, which increases renal K excretion.<sup>29</sup> It was reported by Schneider et al.<sup>6</sup> that plasma Na concentration increased when NaHCO<sub>3</sub> was used to increase dietary Na from 1.8 to 8.8 g kg<sup>-1</sup> DM. However, no increase in the level of Na was seen when NaCl was utilised as Na source. It should be noted that the present study only used the different diets for a period of 37 days, so there is still sufficient Na present in the body to maintain plasma Na levels within the normal physiological range even if the cow is fed a low dietary Na concentration.

Milk Na concentration and milk yield (P < 0.292) were not affected by dietary Na content. The mean milk production for all cows was  $15.0 \text{ kg day}^{-1}$  (SE 0.68), which was lower than anticipated. The discrepancy between the predicted and the observed milk yield was at least partially explained by the observed level of DMI. The dietary NaCl content had no effect on the level of milk production in the current study, which agrees with earlier studies.<sup>30,31</sup> The Na excretion via milk was numerically lower after feeding the low-Na ration because of the lower milk yield (low, 13.7 kg day<sup>-1</sup>; medium, 14.9 kg day<sup>-1</sup>; high, 16.4 kg day<sup>-1</sup>). The apparent faecal Na absorption in cows fed the low-Na diet was equal to the amount of Na lost in milk. In addition to the losses in milk and faeces, there are some inevitable Na losses in urine<sup>32</sup> as well as losses due to sweating caused by the warm humid conditions.<sup>23</sup> Mean minimum and maximum daily temperatures were 24.6 and 34.8 °C respectively and mean minimum and maximum daily relative humidities were 50.0 and 88.1% respectively. Because the thermal neutral zone for cows has a temperature humidity index lower than 72, it is likely that the animals in the present study experienced heat stress during some parts of the day.<sup>33</sup> Therefore it is speculated that the cows fed the low-Na diet were in negative Na balance owing to the inevitable Na loss with both urine and sweat. Because cows maintain Na balance by Na excretion with urine, it is anticipated that the excess of Na absorbed by cows fed the medium- and high-Na diets was excreted with urine. The outcome of the current study clearly showed that an Na content of 1.6 g kg<sup>-1</sup> DM was sufficient to meet the Na requirement of dairy cows under tropical conditions. Clearly, the minimum Na requirement could not be derived from this study, but the current estimate of the Na requirement for dairy cows set by the NRC<sup>3</sup> is too high. On the basis of the data provided in Table 1, a mean Na requirement of 1.2 g kg<sup>-1</sup> DM can be calculated. This value is in line with the conclusion of Suttle,<sup>24</sup> who suggested that 1.2 g Na kg<sup>-1</sup> DM is sufficient for cows with a DMI and milk yield similar to those observed in the current study. Therefore we propose a tentative value of  $1.2 \text{ g kg}^{-1}$  DM as the Na requirement for dairy cows under tropical conditions.

# CONCLUSIONS

A large increase in Na intake did not induce any changes in serum, faecal and milk Na concentrations but did affect the Na concentration in saliva. The salivary Na/K ratio was a good indicator of Na intake by dairy cattle. Consumption of a diet containing Na at the level recommended by the NRC appears to provide too much Na for lactating cows under tropical conditions.

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