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Effect of ambient temperature and sodium bicarbonate supplementation on water and electrolyte balances in dry and lactating Holstein cows

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ABSTRACT

The aim of this study was to quantify the effect of the interaction between 2 constant ambient temperatures [thermoneutrality (TN; 15°C) and high temperature (HT; 28°C)] and 2 levels of Na bicarbonate supplementation [calculated to provide diet Na contents of 0.20% DM (Na⁻) and 0.50% DM (Na⁺)] on water partitioning in dairy cows. Treatments were compared on 4 dry and 4 mid-lactation Holstein cows according to 2 Latin squares (1 for each physiological stage) over the course of 4 periods of 15 d. Diets consisted of a total mixed ration based on maize silage. Dry cows were restricted to their protein and energy requirements, whereas lactating cows were fed ad libitum. The daily average temperature-humidity index was 59.4 for TN and 73.2 for HT. Lactating and dry cows had higher vaginal temperatures at HT than at TN, but the increase was more pronounced in lactating cows (+1.05 vs. +0.12°C for vaginal temperature, respectively). Dry matter intake (DMI) of lactating cows decreased by 2.3 kg/d at HT. Free water intake (FWI) and estimated volume of water lost to evaporation increased at HT in both lactating and dry cows; no interactions were observed between temperature and physiological stage. When expressed as a proportion of DMI, the increase in evaporation that occurred with increasing temperature was completely compensated for by an increase in FWI for both physiological stages. The urinary water excretion increased slightly at HT in lactating cows but not in dry cows, which may be related to the low chloride content of the offered diet. High Na supplementation increased DMI slightly in lactating cows, but milk yield was not affected. Sodium supplementation did not limit the decrease in DMI observed in lactating cows at HT; this observation is likely due to the high diet electrolyte balance of the offered diets. Sodium supplementation increased FWI in lactating cows and urinary flow in both physiological states. The interaction between

ambient temperature and Na supplementation did not affect either water intake or water evaporation. This study demonstrates that the development of predictive models for water intake that include environmental variables could be based on mechanistic models of evaporation.

Key words: water intake, water excretion, high temperature, dairy cow

INTRODUCTION

Even though dairy herd drinking water represents only 1 to 2% of the total amount of water needed to produce milk on a national scale (Mekonnen and Hoekstra, 2012), its supply is vital for dairy herds. A loss of 20% of body water is fatal to cows (NRC, 2001), and an inadequate supply of drinking water will rapidly cause a marked reduction in intake and milk production (Steiger Burgos et al., 2001). At high ambient temperatures, a lack of drinking water will also impair the ability of cows to adapt to heat (Silanikove, 2000). In intensive systems, drinking water can be supplied by the public water service, which can be very expensive. In this context, it is important to rely on accurate modeling of dairy herd drinking water requirements to ensure that the water supply is not limiting for the herd (especially during warm and dry weather periods) or to build tools that can be used to detect water leaks on farm water distribution networks.

Numerous empirical equations have been developed to predict water intake in dairy cows (Murphy et al., 1983; Holter and Urban, 1992; Khelil-Arfa et al., 2012); however, most of these equations were established in the context of thermoneutrality and, thus, rarely incorporated meteorological measurements as predictors. The increase in water loss by evaporation is the first heat adaptation mechanism of homeothermic animals. For producing dairy cows, homeostatic mechanisms aim to maintain homeothermy by increasing heat dissipation via evaporation at ambient temperatures of 14°C or higher (Maia et al., 2005a,b). When meteorological measurements were included in the abovementioned equations, they were generally introduced in the

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form of multiple regressions from data obtained from a single experimental farm. The risk of this method consists of the possible confounding of meteorological variables, the physiological stage, production level, or diets of the cows throughout the year. An alternative more mechanistic way of including meteorological or environmental variables in predictive equations would be to use published models of the effects of ambient temperature on water evaporation in cows obtained from instantaneous measurements taken in numerous environmental conditions with animals on various feeds and at different physiological stages (Turnpenny et al., 2000; CIGR, 2002; Maia et al., 2005a). This could allow the inclusion of environmental variables in such a way that the predictive equations could be used across geographic contexts. However, this method also assumes that we are cognizant of the effects of an increase in water evaporation on the drinking water requirements of a dairy cow and, more generally, how this increase will affect water partitioning in cows.

Dairy cows lose water through evaporation, urine, feces, and milk production, and the pool of the cow's body water is maintained through water intake from drinking (free water intake, **FWI**), feed, and metabolic oxidation in body tissues. When ambient temperatures increase above 14°C, the increase in evaporated water is generally accompanied by a clear increase in FWI (McDowell et al., 1969; Seif et al., 1973; Kurihara et al., 1984). However, it has also been observed on dry cows that urinary water excretion can increase during heat exposure (El-Nouty et al., 1980; Kurihara et al., 1984). Given that the observed increases in urinary water flow at high ambient temperatures in dry cows was reported on the very first days of heat exposure (El-Nouty et al., 1980; Kurihara et al., 1984) and given that these very first days are characterized by a high and transient increase in the amount of water in the body (Richards, 1985), it is unclear whether the increase in urinary water excretion with increasing temperature is specific to dry cows or to the first days of heat exposure.

High ambient temperatures also strongly modify the electrolyte and acid-base balance of dairy cows for 3 reasons: first, because potassium is the main cation encountered in bovine sweat (Jenkinson and Mabon, 1973); second, because Na is excreted with bicarbonates to compensate for the respiratory alkalosis that can occur during heat stress in cows (West, 2003); and third, because the diurnal excretion of bicarbonates can induce metabolic acidosis during the cooler periods of the day (Schneider et al., 1988). A better adaptation of dairy cows to high temperatures can be achieved by increasing Na and K bicarbonate supplementation in the diet (West, 2003). However, the effects of this practice on water needs and water partitioning in dairy

cows that are exposed to heat stress have not been assessed. Given that Na is the most important electrolyte of the extracellular pool and a major ion involved in the regulation of the renal reabsorption of water and urinary volume (Meyer et al., 2004), Na supplementation may strongly increase urinary water excretion and water requirements of cows.

The overall objective of the present study was to determine the effects of high ambient temperatures on water and electrolyte partitioning (in relation to acid-base regulation) in dry or lactating cows fed diets supplemented with 1 of 2 levels of Na bicarbonates during a second week of heat exposure. The first objective of the study was to determine if the increase in water evaporation at high temperatures was compensated for by an increase in FWI when body water pools were stabilized; if so, thermic models of ruminants could be used to predict the water requirements of cows associated with certain environmental conditions at various geographic sites. The second objective of the study was to determine whether the effect of Na supplementation on urinary water excretion and drinking water was similar at high ambient temperatures and under thermoneutral conditions.

MATERIALS AND METHODS

Animals and Experimental Design

The 4 treatments consisted of a factorial arrangement of 2 ambient temperatures [thermoneutrality (**TN**; 15°C) or high temperature (**HT**; 28°C)] and 2 levels of feed Na [high (**Na⁺**) or low (**Na⁻**) levels of supplementation]. These treatments were compared in 4 lactating multiparous Holstein cows [98.5 ± 3.32 DIM; 42.1 ± 5.1 kg/d milk yield (**MY**); 653 ± 35.2 kg of BW] and 4 dry Holstein cows (702 ± 95 kg of BW) over the course of 4 periods of 12 d according to two 4 × 4 Latin square designs (1 for each physiological stage). None of the cows was pregnant at the beginning of the experiment. The ambient temperatures were concomitantly produced with 2 climatic chambers housing 2 lactating and 2 dry cows and 2 cows on Na⁺ and 2 cows on Na⁻. The succession of treatments was organized to avoid 2 consecutive HT periods for the same cow. In each chamber, the ambient temperature was maintained constant throughout the day with a thermostatic convective heating system. Hygrometry was not regulated. The dry bulb temperature, relative humidity, and air speed were controlled in both chambers. In the chambers, cows were housed in individual tie-stalls (1.4 × 2.0 m) with individual troughs and individual water bowls providing free access to water. The four 12-d periods comprised 8 d of adaptation to treatments

Table 1. Composition of the offered TMR (DM basis) for the Na⁻ and Na⁺ treatments¹

Item	Treatment	
	Na ⁻	Na ⁺
Ingredient (%)		
Corn silage	74.5	73.0
Dehydrated alfalfa	10.0	10.0
Soybean meal, 48%	13.8	13.8
Mineral-vitamin premix ²	1.2	1.2
NaHCO ₃ ⁻	0.5	1.9
TMR DM (%)	40.4	40.4
Chemical composition (% DM)		
OM	93.4	92.0
CP	14.7	14.0
NDF	34.1	35.0
ADF	18.6	19.1
ADL	2.2	2.3
Ca	0.72	0.72
P	0.31	0.31
Na	0.206	0.489
Cl	0.21	0.21
K	1.22	1.27
Nutritional value		
PDIE ³ (% of DM)	9.2	9.1
PDIN ⁴ (% of DM)	8.9	8.9
NE _L (MJ/kg of DM)	6.32	6.25
EB ⁵ (mEq/kg of DM)	343	479

¹Na⁻ = diet formulated with a low level of Na supplementation; Na⁺ = diet formulated with a high level of Na supplementation.

²Contained 27% calcium, 5.5% phosphorus, 4% magnesium, 600 kIU of vitamin A/kg, 80 kIU of vitamin D₃/kg, 1.5 kIU of vitamin E/kg, 4,000 mg of Zn/kg, 4,000 mg of Mn/kg, 1,000 mg of Cu/kg (sulfate and chelate), 80 mg of I/kg, 20 mg of Co/kg, and 20 mg of Se/kg.

³Protein digestible in the small intestine supplied by microbial protein from rumen-fermented OM (INRA, 2007).

⁴Protein digestible in the small intestine supplied by microbial protein from rumen-degradable protein (INRA, 2007).

⁵Diet electrolyte balance ([Na] + [K] - [Cl], in mEq/kg of dietary DM).

and 4 d of measurements. Two days at 15°C were included between each period. The ambient temperature was increased by 2°C/h during the first day of the HT periods starting at 0900 h. The trial included a 2-wk preexperimental adaptation period at 15°C to monitor voluntary DMI and a 1 wk postexperimental period. The experiment took place at the Institut national de la recherche agronomique (INRA) experimental farm of Méjusseume (Brittany, France; longitude -1.71°, latitude +48.11) from December 26, 2009, to February 19, 2010. Procedures relating to the care and use of animals for the experiment were approved by an animal care committee of the French Ministry of Agriculture in accordance with French regulations (Decree law 2001-464, May 29, 2001).

Diets and Animal Feeding

Two TMR based on maize silage and differing in the level of Na bicarbonate (NaHCO₃⁻) supplementation

were formulated (Table 1). Both diets were formulated to meet the energy and protein requirements of the lactating cows according to INRA (2007), with 14 g of digestible protein in the small intestine/MJ of NE_L (the equivalent of MP in the INRA feeding system), which was equivalent to 22.6 g of CP/MJ of NE_L in this experiment. The quantities of mineral premix included in the diet were also calculated to meet the Ca and P requirements of the lactating cows according to INRA (2007) and the Mg, En, Mn, Cu, I, Co, Se, and vitamin A, D, and E requirements according to Meschy (2010). The quantity of TMR offered to dry cows was limited to 14 kg of DM/d to avoid excessive weight gain. This amount allowed an energy intake equivalent to 2 times the maintenance requirement of dry cows. The Na⁻ diet (2.0 g of Na/kg of DM) was formulated to meet the Na requirement of a 600-kg lactating cow producing 40 kg of milk/d and exposed to thermoneutral conditions (Meschy, 2010). The Na⁺ diet was formulated to provide 5.0 g of Na/kg of DM in the diet, a quantity determined by Schneider et al. (1986) as an optimal dose to improve heat adaptation. The Na content of the diets was adjusted with NaHCO₃⁻ and the NaHCO₃⁻ replaced a portion of maize silage in the Na⁺ diet. To control mineral supply, no straw or mineral blocks were provided. Two equal-sized meals per day were provided at 0830 and 1730 h and orts were recorded daily before the a.m. and p.m. feedings. Lactating cows were fed ad libitum and offered quantities were calculated to allow 10% refusals. Lactating cows were milked twice daily at 0630 and 1700 h in the climatic chambers.

Measurements of Environmental Conditions, Body Temperatures, and Respiratory Rates of Cows

The air temperature, relative humidity, and air velocity were recorded continuously at 15-min intervals in each climatic chamber using temperature and relative humidity probes (HMP 45C; Campbell Scientific Inc., Courtaboeuf, France) and a hot wire anemometer (8450-20M-V-STD-NC-F; TSI Inc., Shoreview, MN) placed at a height of 1.6 m in a central position between the cows and connected to a data acquisition system (CR 1000; Campbell Scientific Inc.).

Vaginal temperatures were continuously measured at 15-min intervals throughout the entire experiment with vaginal indwelling telemetric temperature probes (Vel'Phone; Medria, Châteaugiron, France). Probes were inserted into the cow during the adaptation period. Respiratory rates, heart rates, and skin temperatures were measured at 0800, 1500, and 2100 h on d 8. Respiration rates were determined by counting flank movements for 60 s by 2 observers until the difference between the 2 observers was lower than 15% of the average.

Intake, Milk Production, and Water and Electrolyte Balances of Cows

Intake. Quantities of feed offered and refused were weighed at each feed distribution. Before each distribution, refusals of each cow were removed and weighed. After weighing the amount of feed to be distributed, rations were stored at 4°C before being moved into the climatic chambers about 1 h before distribution. Each day of the measurement period, before the a.m. and p.m. feeding, 2 representative samples (500 g, fresh) of each offered diet were sampled and oven dried (80°C for 48 h) before being pooled by period. Refusals (1 kg, fresh) were also sampled from each cow before the a.m. and p.m. feeding and oven dried for DM determination before being pooled by cow and period. Offered and refused feed samples were further analyzed for OM, fiber (NDF, ADF, and ADL), CP, and Na, K, and Cl contents. The daily volumes of water taken in by cows were recorded daily at 0830 h, using water meters (model P38; Schlumberger Ltd., Paris, France; nominal flow rate: 1.5 m³/h; maximum allowable pressure: 1.2 MPa attached to each drinking bowl. Water was sampled at the end of each period for Na, K, and Cl contents.

Milk. Milk production was measured individually at each milking and 100 mL of milk per cow per milking was sampled from d 9 to 11. Daily samples were pooled by cow and period in proportion with the amount of milk produced at each milking; samples were then frozen at -20°C for further analysis (fat, protein, DM, Na, K, and Cl contents).

Feces. To determine the fecal DM excreted over 24 h, gross fecal output was weighed and sampled at 0900 h from d 11 to 13. Two representative samples (500 g of fresh feces for each sample) were oven dried (80°C for 72 h) to determine DM content and the daily amount of fecal DM excreted. To determine the daily amount of fecal water excreted, 5 additional fecal samples (300 g, fresh) were collected at 2-h intervals from 1000 to 1800 h directly from the cow on each collection day and oven dried (80°C for 72 h). The aim of this procedure was to obtain fecal samples that were not affected by losses of water and nitrogen from volatilization due to high temperatures. The amount of fecal water excreted was calculated from the daily amount of fecal DM excreted, and the fecal DM content was determined by sequential sampling. The fecal samples used for the unbiased estimates of DM content were bulked per cow and per period, ground through a 0.8-mm screen, and stored at 4°C for further determination of OM, NDF, Na, K, and Cl contents.

Urine. Daily volumes of excreted urine were measured on d 11 to 13 by equipping cows with harnesses

designed for urine collection that were connected by a Tygon tube to a 25-L container closed with a rubber plug. Sunflower oil (1 L) and 15 g of thymol were added to the container to prevent the evaporation and deterioration of urine, respectively. The urine was weighed and the containers were emptied twice per day before feedings. Subsamples (2 mL) were collected and frozen at -20°C for further analyses of Na, K, and Cl contents.

Water Retention. To assess body water retention, cows were weighed, throughout the entire experiment, every Friday morning (d 5 and 12 of each period) between 0730 and 0800 h, after the morning milking and before the first meal.

Blood Gases

At the beginning of the trial, cows were equipped with a catheter in the left jugular vein and the catheters were filled with a sterile solution of 9% NaCl and 200 IU of heparin/mL. On d 8 of each period, jugular blood was sampled at 0900, 1530, and 2100 h using 2-mL blood gas syringes (S-Monovette; Sarstedt AG & Co., Nümbrecht, Germany). Blood pH, partial pressure of CO₂ (pCO₂), partial pressure of O₂, and HCO₃⁻ concentrations were analyzed immediately by potentiometry (ABL 500 instrument; Radiometer, Copenhagen, Denmark). Blood gases were measured in the 10 min following sampling time.

Chemical Analyses

Samples of offered and refused diets and feces were ground with a 3-blade knife mill through a 0.8-mm screen. Content of OM was determined by calcination at 550°C for 5 h in a muffle furnace. Nitrogen concentration was determined with the Dumas method [NF V 18-120 (AFNOR, 1997); AFNOR, La Plaine Saint-Denis, France] on a Leco apparatus (Leco Corp., St. Joseph, MI). Contents of NDF, ADF, and ADL were determined with a Fibersac extraction unit (Ankom Technology Corp., Fairport, NY) according to the Association Française de Normalisation (AFNOR) methods [NF EN ISO 16472 (AFNOR, 2006) and NF EN ISO 13906 (AFNOR, 2008)]. Minerals (Na and K) were measured by atomic absorption spectrophotometry (Spectra-AA20; Varian SA, Les Ulis, France) of mineralized samples (NF EN ISO 6869; AFNOR, 2002). Content of Cl in urine and milk was measured using a kit for chloride determination (kit 80005; Biolabo S.A., Maizy, France) and content of Cl in feed and feces was determined by titrimetry according to the Volhard method (NF EN ISO 6495; AFNOR, 2011). Milk DM content was determined in an oven at 100°C for 7 h for

milk with sand (NF V 04-367; AFNOR, 1985). Milk fat and protein concentrations were determined by near-infrared spectrophotometry (MilkoScan; Foss Electric A/S, Hillerød, Denmark).

Calculations

The temperature-humidity index (**THI**) was calculated using the formula described by Mader et al. (2006). Water losses due to evaporation were calculated as the difference between input (drinking and feed) and output (milk, feces, and urinary water) water, corrected by the body water retention during the measurement period and the gain of metabolic water. Daily water retention was estimated as the difference between the weight of the cows at d 5 and 12 divided by 7 d. Metabolic water was estimated using the equivalent of 0.002 g/d of water produced per 1 MJ/d of heat production (M. Vermorel, INRA Clermont-Ferrand-Theix, France, personal communication). Heat production was calculated from INRA (2007) as the difference between ME intake in megajoules per day and the sum of the amount of energy lost in milk (MJ/d) and the NE_L balance (difference between the feed NE_L supply and the cow NE_L requirements; MJ/d).

Statistical Analyses

Data related to measurements of animals were analyzed with the PROC MIXED function of SAS (SAS OnlineDoc, version 8; SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ijkl} = \mu + P_i + \text{Temp}_j + \text{Na}_k + \text{Stage}_l + \text{Temp}_j \times \text{Na}_k + P_i \times \text{Stage}_l + \text{Temp}_j \times \text{Stage}_l + \text{Na}_k \times \text{Stage}_l + \text{Temp}_j \times \text{Na}_k \times \text{Stage}_l + \epsilon_{ijkl},$$

where Y_{ijkl} is the analyzed variable, μ is the overall mean, P_i is the effect of period (1 df), Temp_j is the effect of ambient temperature (TN vs. HT; 1 df), Na_k is the effect of the level of Na supplementation (Na^+ vs. Na^- ; 1 df), Stage_l is the effect of lactation stage (lactating vs. dry; 1 df), and ϵ_{ijkl} is the residual error. The effect of cows nested in the lactation stage was used as a random effect. Two sets of 3 orthogonal contrasts were used separately for dry and lactating cows to obtain the P -values associated with the Na, temperature and Na \times temperature effects for each physiological stage.

RESULTS AND DISCUSSION

Environmental Conditions and Effect of TN and HT Treatments on Feed Intake, Milk Production and Composition, and Thermoregulation and Acid-Base Metabolism Responses

The mean daily ambient temperature, relative humidity, and unadjusted THI were 15.5°C, 54.3%, and 59.4, respectively, for TN, and 28.4°C, 28.9%, and 73.2, respectively, for HT. Temperature and relative humidity remained constant throughout the day and throughout the experimental period, with a standard error for hourly ambient temperature throughout the period of 1.44 and 1.01°C for TN and HT, respectively, and a standard error for relative humidity of 8.33 and 4.23% for TN and HT, respectively. The air velocity at a height of 1.6 m was, on average, 0.07 (± 0.055) and 0.41 (± 0.150) m/s for TN and HT, respectively, which corresponds to adjusted THI of 66.0 for TN and 78.7 for HT according to Mader et al. (2006), and assuming that cows did not receive solar radiation.

The average temperature achieved for TN was in the range of thermoneutrality for dairy cows (Berman et al., 1985). This remains true whether thermoneutrality is defined as the range of temperatures within which body temperature is regulated and respiration rate does not increase (Berman et al., 1985) or as the range of temperature within which temperature regulation is achieved by non-evaporative physical processes alone (Silanikove, 2000; Maia et al., 2005a,b). The average daily temperature achieved for HT was above the critical temperature of 25°C at which the thermoregulation of cows is impaired (Berman et al., 1985) and the THI was above the THI threshold defining mild heat stress in dairy cows (Armstrong, 1994). When including air velocity in the calculation of adjusted THI (Mader et al., 2011), adjusted THI was higher than 78, which was considered as the THI threshold defining actual heat stress in dairy cows (Armstrong, 1994). These thresholds may have even been underestimated for modern animal productivity (Berman, 2005).

For lactating cows, DMI decreased by 2.3 kg/d between TN and HT [SE of the difference (**SED**) = 0.63, $P \leq 0.01$; Table 2], whereas for dry cows, DMI was similar to the fed amounts for both HT and TN. Compared with TN, HT decreased MY (-2.0 kg/d; SED = 1.69; $P \leq 0.10$), milk DM content (-0.3 points; SED = 0.32; $P \leq 0.01$), fat content (-1.4 g/kg; SED = 2.72; $P \leq 0.05$), and protein content (-2.1 g/kg; SED = 0.72; $P \leq 0.01$) of lactating cows. The effects observed on milk composition were in accordance with expected

Table 2. Effects of ambient temperature and Na supplementation on DMI, milk production and composition, and heat production of lactating and dry cows

Item	Stage ¹	Treatment ²				SEM ³	P-value ⁴		
		TN		HT			Temp	Na	Temp × Na
		Na ⁻	Na ⁺	Na ⁻	Na ⁺				
Intake									
DMI (kg/d)	Lact	20.9	21.3	18.5	19.0	0.47	≤0.01	≤0.05	0.94
	Dry	13.8	13.9	13.8	13.9		0.99	0.61	0.99
Offered DM content (%)	Lact	40.4	40.7	40.4	40.9	0.08	0.28	≤0.01	≤0.10
	Dry	40.0	40.4	40.0	40.4		1.00	≤0.01	1.00
NDF orts (%)	Lact	44.5	43.2	40.4	40.8	1.58	≤0.1	0.75	0.56
CP orts (g/kg of DM)	Lact	115.6	115.3	134.7	131.2	4.70	≤0.01	0.59	0.63
Milk									
Yield (kg/d)	Lact	31.3	30.0	28.3	29.1	1.34	≤0.10	0.77	0.27
DM content (%)	Lact	12.10	12.25	11.75	11.98	0.235	≤0.01	≤0.05	0.56
Fat content (g/kg)	Lact	38.8	40.3	37.5	38.7	1.93	≤0.05	≤0.05	0.73
Protein content (g/kg)	Lact	30.0	30.0	28.0	27.8	0.51	≤0.01	0.74	0.71
Lactose content (g/kg)	Lact	46.50	48.72	48.35	46.55	1.275	0.88	0.85	0.11
IHP ⁵ (MJ/d)	Lact	64.6	63.4	57.4	59.9	3.22	≤0.05	0.73	0.34
BW (kg)	Lact	632.5	633.7	622.1	623.7	26.51	≤0.05	0.71	0.95
	Dry	766.7	786.0	763.2	771.7		0.97	0.25	0.38
Variation of BW in wk 2 (kg)	Lact	4.5	7.0	-12.0	-9.0	3.72	≤0.01	0.47	0.94
	Dry	0.2	2.7	-1.0	3.7		0.97	0.34	0.76
Heat production (MJ/d)	Lact	126.5	125.4	118.4	121.1	3.08	≤0.01	0.56	0.2
	Dry	71.4	71.5	71.2	71.7		0.99	0.79	0.84

¹Lact = lactating cows; Dry = dry cows.

²TN = thermoneutrality; HT = high temperature; Na⁻ = diet formulated with a low level of Na supplementation (5.0 g/kg of NaHCO₃⁻); Na⁺ = diet formulated with a high level of Na supplementation (19.0 g/kg of NaHCO₃⁻).

³Standard error of the mean of the effect of the stage × temperature × Na supplementation interaction.

⁴Temp = temperature; Na = Na supplementation; Temp × Na = interaction between both effects.

⁵Incremental heat loss due to milk production.

results in cows under heat stress, with a decrease in milk protein and fat contents that can also be observed in such experiments (Bernabucci et al., 2010). This led, in this present experiment, to a decrease of the milk DM content at HT.

The responses of cows to HT and TN in our experiment illustrated that both lactating and dry cows need to make physiological adjustments to heat and that the thermoregulatory capacities of lactating cows were insufficient at HT. At both physiological stages, daily respiration rate was higher at HT than at TN (+27.0 and +17.4 inspirations/min in lactating and dry cows, respectively; SED = 3.59; $P \leq 0.0001$; Table 3). At HT, daily respiration rates were close or higher than 40 inspirations/min, indicating that cows were panting to increase body cooling (Silanikove, 2000). Compared with TN, HT increased vaginal temperatures of cows at both stages of lactation ($P \leq 0.001$). The increases in vaginal temperatures were more pronounced in lactating cows than in dry cows (+1.05 vs. +0.10°C in lactating and dry cows, respectively). The high increase in the vaginal temperatures of lactating cows indicates their inability to regulate their body temperature. In accordance with Silanikove (2000), this difference in body temperature of 1°C between lactating cows at HT

and TN was associated with a decrease in DMI and MY. On average, the daily heart rate was also lower at HT than at TN in lactating cows (-6.91 pulses/min; SED = 6.10; $P \leq 0.05$), which may have been a strategy to decrease the basal heat production.

Blood pCO₂ was higher in HT than in TN at 0800 and 1500 h in lactating cows ($P \leq 0.01$; Table 3) and at 1500 h in dry cows ($P \leq 0.05$; Table 3); this partial pressure reached values above 45 mm of Hg in lactating cows at TN, especially at 1500 h. This result was in accordance with the increased respiration rate of cows on the HT treatment. Consequently, blood pH was markedly higher in HT than in TN in lactating cows at 0800 and 1500 h ($P \leq 0.001$; Table 3), and in dry cows, the blood pH was higher at 0800 h ($P \leq 0.05$). Blood pH always exceeded 7.45 in lactating cows at HT. Ambient temperature did not affect concentration of HCO₃⁻ or base excess, indicating that cows did not compensate high blood pH by an increased excretion of HCO₃⁻.

Water Intake, Urine and Fecal Excretion, and Water Balance

Free water intake was higher at HT than at TN, with values of 8.3 and 14.4 kg/d for lactating and dry cows,

Table 3. Effects of ambient temperature and Na supplementation on vaginal temperature, respiration and heart rates, and blood gas measurements of lactating and dry cows at 0800 and 1500 h

Item	Stage ¹	Treatment ²				SEM ³	P-value ⁴		
		TN		HT			Temp	Na	Temp × Na
		Na ⁻	Na ⁺	Na ⁻	Na ⁺				
Daily average									
Vaginal temperature (°C)	Lact	38.4	38.3	39.4	39.4	0.09	≤0.001	0.62	≤0.10
	Dry	38.3	38.3	38.4	38.4		≤0.05	0.71	0.21
Respiration rate (/min)	Lact	23.2	22.2	49.0	49.5	2.85	≤0.001	0.89	0.70
	Dry	23.1	23.1	38.7	41.8		≤0.001	0.41	0.40
Heart rate (/min)	Lact	66.9	68.3	59.9	61.5	4.31	≤0.05	0.55	0.96
	Dry	59.9	58.3	55.7	57.6		0.35	0.94	0.50
0800 h									
Blood pH	Lact	7.443	7.447	7.488	7.500	0.0149	≤0.01	0.58	0.78
	Dry	7.372	7.413	7.420	7.426		≤0.05	≤0.1	0.20
pCO ₂ ⁵ (mm of Hg)	Lact	44.9	47.7	40.9	40.6	2.01	≤0.01	0.50	0.39
	Dry	41.6	44.7	41.0	40.3		0.20	0.51	0.32
HCO ₃ ⁻ (mmol/L)	Lact	30.7	33.1	31.2	31.7	1.46	0.63	0.12	0.33
	Dry	23.9	28.0	26.4	26.4		0.63	≤0.05	≤0.05
Base excess (mmol/L)	Lact	6.6	8.7	7.5	8.1	1.41	0.84	0.12	0.38
	Dry	-0.6	3.7	2.3	2.4		0.36	≤0.05	≤0.05
1500 h									
Blood pH	Lact	7.434	7.461	7.480	7.486	0.0120	≤0.01	0.16	0.37
	Dry	7.416	7.420	7.423	7.441		0.25	0.34	0.52
pCO ₂ (mm of Hg)	Lact	46.1	47.8	41.3	41.8	1.73	≤0.01	0.42	0.64
	Dry	43.1	43.7	39.9	39.8		≤0.05	0.84	0.77
HCO ₃ ⁻ (mmol/L)	Lact	30.6	34.3	30.9	31.5	1.17	0.15	≤0.05	0.10
	Dry	27.4	28.0	25.9	26.9		0.15	0.38	0.83
Base excess (mmol/L)	Lact	6.4	10.0	7.1	7.8	1.12	0.38	≤0.05	≤0.10
	Dry	3.2	3.8	2.0	3.1		0.25	0.34	0.74

¹Lact = lactating cows; Dry = dry cows.

²TN = thermoneutrality; HT = high temperature; Na⁻ = diet formulated with a low level of Na supplementation (5.0 g/kg of NaHCO₃⁻); Na⁺ = diet formulated with a high level of Na supplementation (19.0 g/kg of NaHCO₃⁻).

³Standard error of the mean of the effect of the stage × temperature × Na supplementation interaction.

⁴Temp = temperature; Na = Na supplementation; Temp × Na = interaction between both effects.

⁵pCO₂ = partial pressure of CO₂.

respectively (Table 4). In lactating cows, water excreted in urine increased by 2.62 kg/d ($P \leq 0.01$) at HT and fecal water excretions decreased by 8.6 kg (SED = 1.99; $P \leq 0.01$). The urine and fecal water excretions of dry cows was unaffected by ambient temperature. The calculated volume of evaporated water increased in both dry and lactating cows at HT (from 20 to 35 kg/d for lactating cows and from 15 to 29 kg/d for dry cows; $P \leq 0.01$), with no significant interaction between temperature and physiological stage.

For dry cows, the increase in evaporated water at HT was very similar to the increase in FWI (+13.9 and +14.3 kg/d at HT and TN, respectively). This illustrates that water evaporation became an important mechanism of dissipating body heat as ambient temperature increased above the upper limit of thermoneutrality (Silanikove, 2000). The water evaporation increases we observed were in the range of those reported in experiments performed with lactating cows under similar conditions using similar methodologies (McDowell et al., 1969; Kurihara et al., 1984). We

also observed that the total amount of water lost to evaporation was higher for lactating cows than for dry cows, which is consistent with the notion that lactating cows have more heat to dissipate because of their higher metabolic rate. Water evaporation in our experiment was calculated as the difference between input and output flows of the pool of water contained in the organism. An advantage of this simple method is that it gives an individual and integrated assessment of the water evaporated at the scale of the entire animal during an entire day; however, the main risk of this method is the accumulation of measurement errors for all other flows (urine, feces, intake, and water retention) or of errors associated with calculations of metabolic water. In this experiment, special care was applied to limit water losses during measurement and sampling. Fecal samplings were adapted to the particular aim of this experiment by calculating the amount of daily fecal water excretion as the ratio between the daily amount of fecal DM excreted and the DM content of feces determined by sequential sampling at 2-h intervals. Be-

Table 4. Effects of ambient temperature and Na supplementation on water intake and excretions of dry and lactating cows

Item	Stage ¹	Treatment ²				SEM ³	P-value ⁴		
		TN		HT			Temp	Na	Temp × Na
		Na ⁻	Na ⁺	Na ⁻	Na ⁺				
Measured input (kg/d)									
Free water intake	Lact	74.2	80.0	82.8	88.0	2.57	≤0.01	≤0.05	0.94
	Dry	25.4	29.3	40.8	42.6		≤0.01	0.26	0.67
Water ingested with feed	Lact	30.7	31.1	27.4	27.4	0.63	≤0.01	0.56	0.57
	Dry	20.7	20.5	20.7	20.5		0.99	0.53	0.99
Measured output (kg/d)									
Urine	Lact	15.4	20.1	16.3	24.5	1.26	≤0.01	≤0.01	≤0.05
	Dry	11.8	15.2	11.4	16.2		0.71	≤0.01	0.41
Fecal water	Lact	47.7	47.7	40.0	38.3	1.62	≤0.01	0.46	0.48
	Dry	23.6	23.4	24.4	23.0		0.84	0.50	0.63
Milk water	Lact	27.5	26.3	25.1	25.6	1.19	≤0.10	0.72	0.27
Balance (kg/d)									
Calculated metabolic water ⁵ (input)	Lact	4.5	4.5	4.3	4.4	0.10	0.99	0.16	0.41
	Dry	2.6	2.6	2.6	2.6		0.99	0.97	0.96
Retained water ⁶ (output)	Lact	0.71	1.00	-1.72	-1.29	0.533	≤0.01	0.51	0.89
	Dry	0.04	0.39	-0.14	0.54		0.97	0.35	0.76
Evaporated water ⁷	Lact	18.0	20.3	35.0	33.7	2.58	≤0.01	0.77	0.31
	Dry	13.2	13.4	28.4	26.0		≤0.01	0.51	0.45

¹Lact = lactating cows; Dry = dry cows.

²TN = thermoneutrality; HT = high temperature; Na⁻ = diet formulated with a low level of Na supplementation (5.0 g/kg of NaHCO₃⁻); Na⁺ = diet formulated with a high level of Na supplementation (19.0 g/kg of NaHCO₃⁻).

³Standard error of the mean of the effect of the stage × temperature × Na supplementation interaction.

⁴Temp = temperature; Na = Na supplementation; Temp × Na = interaction between both effects.

⁵Metabolic water was estimated using the equivalent of 0.15 g/d of water produced per kilocalorie of heat production.

⁶Retained water was calculated as the difference between 2 successive BW measurements divided by 7.

⁷Evaporated water was calculated as the difference between water intake (free water intake, ingested with feed and metabolic water) and water excretion (milk, urine, and feces), adjusted with the volume of water exchanged in the animal (retained water).

fore the experiment was conducted, we confirmed that this sampling procedure prevents significant water loss due to evaporation from fecal samples. Urine containers were closed to avoid evaporation, and, during the experiment, we checked that cows did not waste water from their bowls. The estimations of water retention and metabolic water were based on assumptions about the linearity of water retention during the measurement period and the assessment of metabolic water produced from the INRA energy system for ruminants (INRA, 2007), as described in the Materials and Methods section; however, the values of these flows were very small.

Notably, the amounts of evaporated water measured in our experiment, at least at HT, were very similar to those predicted by the equations of latent heat loss in lactating Holstein cows established by Maia et al. (2005a,b), assuming that the skin surface of the cows was approximately 6.5 m² and that 1 L of evaporated water resulted in the loss of 2,500 kJ. The equations of Maia et al. (2005a,b) were obtained from the direct measurement of body evaporation using ventilated capsules held for 5 to 10 min on 3 regions of the cow's body and respiratory masks. The equations of Maia et al. (2005a,b) allow prediction of both cutaneous and respi-

ratory latent loss from ambient temperatures; however, several other equations have also been published, and Thompson et al. (2011) found that these equations produced highly variable predictions of cutaneous latent heat loss. For instance, for the same skin temperature of 37°C, the predicted sweating rate from 4 predictive equations can vary 4-fold (Thompson et al., 2011). Assuming that the skin temperature can reflect a same ratio of heat load/liveweight for different physiological stages, it is likely that the variability in the results of these predictive equations largely results from the methodologies used in the measurement of supporting data. Methodologies for measuring sweat rates can range from the direct measurement of sweating rate by sophisticated ventilated capsules or with the measurement of water charges on paper disks placed on the skin (Thompson et al., 2011) to indirect measurements over the course of a few minutes obtained by precisely and continuously weighing an animal (Ehrlemark, 1991). However, it is noteworthy that, in all of the equations reported by Thompson et al. (2011), a surface temperature of 29°C never produced a sweating rate of more than 10 L/d in a 600-kg cow, which is much lower than the predicted daily amount of water lost to evaporation

in dairy cows obtained by calculating the difference between water intake and excretions at thermoneutrality (McDowell et al., 1969; Kurihara et al., 1984; Silanikove et al., 1997). Considering that respiratory loss may represent only a small proportion of total water loss (Maia et al., 2005a,b), it is likely that the difference between the evaporative water losses predicted by the equation based on direct measurements and the amount predicted by daily measurements of evaporation made in the water balance experiment could be explained by the fact that the amount of water lost to evaporation measured by direct measurement may be at the limit of the detection threshold when cows are subjected to ambient temperatures less than 15°C. For instance, the cutaneous evaporation measured by Maia et al. (2005a) was measured with a capsule area of 0.00724 m² placed on the skin of the animal for 10 min; for a dairy cow with a surface area of 6.5 m² and a daily evaporation of 20 L, only 0.2 mL of the water lost to evaporation will be captured in the capsule of 0.2 mL under thermoneutral conditions. The quantity of water lost to evaporation at low temperatures has been qualified as insensible sweating or perspiration (Silanikove, 2000).

Effect of Temperature on the Compensation Between Water Flows and on Water Flows Expressed as a Proportion of DMI

In dry cows, the increase in water evaporation due to HT was completely compensated for by an equivalent increase in water intake (Table 4), likely because DMI of these cows was not affected by ambient temperature. In lactating cows, the increase in water evaporation at HT (+15.2 kg/d; SED = 3.65; $P \leq 0.01$) was equally compensated for by an increase in FWI (+8.3 kg/d; SED = 2.57; $P \leq 0.01$) and by a decrease in the daily amount of water excreted in feces (-8.6 kg/d; SED = 2.29; $P \leq 0.01$). However, when water flows were reported as a proportion of DMI (Table 5), the increase in water evaporation at high ambient temperatures (+0.92 kg/kg of DMI per day; SED = 0.182) was completely compensated for by the increase in FWI (+0.92 kg/kg of DMI per day; SED = 0.170) in both dry and lactating cows. For lactating cows, all water flows reported as a proportion of DMI were highly affected by ambient temperature ($P \leq 0.01$), with the exception of milk water and water ingested with feed. However, the amplitude of the variation in fecal and urinary flows (-0.175 and + 0.245 kg/kg of DMI, respectively) was lower than the amplitude of the variation in FWI and evaporated water (+0.92 kg/kg of DMI).

These observations were consistent with those of McDowell et al. (1969) and Richards (1985), who measured

water balances of dairy cows exposed to various ambient temperatures. Kurihara et al. (1984) also observed a clear association between water evaporation and FWI reported as a proportion of DMI in lactating cows when ambient temperatures increased; however, those authors also observed a huge increase in the volume of water excreted in urine in dry cows with increasing ambient temperature (+5.4 L/d between 18 and 32°C, comparable to the volume of evaporated water of +7.1 L/d), leading to a huge increase in FWI. A possible explanation for this discrepancy in results is that cows in our experiment had more than 10 d to acclimate to the high ambient temperatures, whereas those from the experiment of Kurihara et al. (1984) had less than 5 d. The adaptation of cows to heat stress has been shown to increase the extracellular water pool in these animals (Silanikove, 2000), which likely increases the body heat load capacity and buffers the rise in core body temperature. Richards (1985) suggested that this increase may mainly occur during the first days of heat stress, as illustrated by the important variations in liveweight he observed during the first week of his experiment. By the second week, he observed that the liveweight of cows returned to preexperiment values, likely because other adaptive processes such as the progressive increase in the flow of evaporated water were largely initiated (McDowell et al., 1969). The increase in urinary flow observed by Kurihara et al. (1984) in dry cows after 5 d of heat stress could be due to the decrease in the extracellular water compartment of these cows after the implementation of the first adaptive processes. A similar phenomenon was not observed in lactating cows possibly because their first adaptive mechanism was to decrease milk yield or because the electrolyte status was different in dry and lactating cows, given that the diet composition was not adapted for each physiological stage.

The fact that the increase in water evaporation at high ambient temperatures was completely compensated for by the increase in FWI when water flows were reported as a proportion of DMI provides an interesting new way of predicting the effects of high temperatures on water needs of dairy cows independently of the effect of heat on other variables such as dietary DM, MY, or DMI that may affect FWI at thermoneutral conditions (Khelil-Arfa et al., 2012). A significant amount of data produced equations to predict the effect of the diet or the cow's level of production on her water requirements (Murphy et al., 1983; Holter and Urban, 1992; Khelil-Arfa et al., 2012). However, a small proportion of these data were collected at high ambient temperatures. Moreover, most equations were produced by multiple regressions in which the effect of the ambient temperature, when included, could have

Table 5. Effects of ambient temperature and Na supplementation on water intake and excretions expressed as a proportion of DMI of dry and lactating cows

Item (kg/kg of DMI per day)	Stage ¹	Treatment ²				SEM ³	P-value ⁴		
		TN		HT			Temp	Na	Temp × Na
		Na ⁻	Na ⁺	Na ⁻	Na ⁺				
Measured input									
Free water intake	Lact	3.56	3.75	4.46	4.68	0.120	≤0.01	≤0.10	0.91
	Dry	1.83	2.10	2.95	3.06		≤0.01	≤0.10	0.42
Feed water	Lact	1.47	1.45	1.47	1.44	0.005	0.18	≤0.01	0.18
	Dry	1.50	1.47	1.50	1.47		1.00	≤0.01	1.00
Measured output									
Urine output	Lact	0.73	0.94	0.88	1.28	0.066	≤0.01	≤0.01	≤0.10
	Dry	0.85	1.10	0.82	1.16		0.72	≤0.01	0.37
Fecal water	Lact	2.29	2.24	2.16	2.02	0.116	≤0.01	0.11	0.46
	Dry	1.70	1.68	1.76	1.65		0.81	0.27	0.44
Milk water	Lact	1.3	1.2	1.3	1.3	0.06	0.18	0.43	0.38
Balance									
Metabolic water ⁵	Lact	0.21	0.21	0.23	0.23	0.010	≤0.01	0.65	0.65
	Dry	0.18	0.18	0.18	0.18		0.99	0.99	0.76
Retained water ⁶	Lact	0.03	0.04	-0.09	0.07	0.031	≤0.01	0.64	0.87
	Dry	0.00	0.03	-0.01	0.03		0.96	0.27	0.73
Evaporated water ⁷	Lact	0.86	0.95	1.88	1.77	0.129	≤0.01	0.91	0.25
	Dry	0.95	0.96	2.05	1.86		≤0.01	0.28	0.26

¹Lact = lactating cows; Dry = dry cows.

²TN = thermoneutrality; HT = high temperature; Na⁻ = diet formulated with a low level of Na supplementation (5.0 g/kg of NaHCO₃⁻); Na⁺ = diet formulated with a high level of Na supplementation (19.0 g/kg of NaHCO₃⁻).

³SEM: standard error of means of the effect of the interaction stage × temperature × Na supplementation.

⁴Temp = temperature; Na = Na supplementation; Temp × Na = interaction between both effects.

⁵Metabolic water was estimated using the equivalent of 0.15 g/d of water produced per kilocalorie of heat production.

⁶Retained water was calculated as the difference between 2 successive BW measurements divided by 7.

⁷Evaporated water was calculated as the difference between water intake (free water intake, ingested with feed and metabolic water) and water excretion (milk, urine, and feces), adjusted with the volume of water exchanged in the animal (retained water).

been confounded with the effect of season on feeding and lactation management. A more mechanistic way of including temperature in predictive equations would allow better adaptation of predictive equations to various geographic localizations. One way of adapting the equations developed in thermoneutral conditions to high temperature contexts would be to add the amount of water lost to evaporation at the considered temperature to the water requirement at thermoneutrality. The amount of water lost to evaporation could be obtained from predictive equations of the effects of ambient temperature on latent heat loss in cows, assuming that the vaporization of 1 L of water at 39°C results in the loss of 2,500 kJ of latent heat. The equations of Maia et al. (2005a,b) fit our data well. The equation of the Commission Internationale du Genie Rural (CIGR, 2002) largely underpredicted the water lost to evaporation in our experiment, likely because this equation was not developed specifically for the high heat loss of lactating cows. Several other mechanistic models of heat loss in cattle have been published that also describe the effect of meteorological descriptors other than ambient temperature, such as wind speed or solar radiation that modify latent heat loss (Turnpenny et al., 2000).

Lack of Significant Effect of Na Supplementation on the Adaptability of Cows to Heat

The supplementation of the diet with Na or K bicarbonates has been recommended as a way of improving acid-base regulation and thus enhancing heat adaptation in cows (West, 2003). As observed in the present experiment, high ambient temperatures can induce increased respiration rates (for thermoregulation) that can lead to decreased blood pCO₂ and increased blood pH. Schneider et al. (1988) observed that the variations in blood pCO₂ and pH could be high enough to produce respiratory alkalosis, which would, in turn, produce compensatory increase in the renal excretion of bicarbonates to restore the blood pCO₂/HCO₃⁻ ratio and pH. This compensation can even lead to nocturnal metabolic acidosis (Schneider et al., 1988). We expected Na bicarbonate supplementation to enhance the ability of cows to adapt to the heat by avoiding a nocturnal decrease in plasmatic pH (Schneider et al., 1988), by inducing compensatory excretion of Na along with bicarbonate (West, 2003) and by sparing K for the loss of electrolytes associated with cutaneous water evaporation (El-Nouty et al., 1980; West, 2003).

In the present experiment, compared with the Na⁻ diet, the Na⁺ diet led to slightly improved DMI of lactating cows (+0.5 kg/d; $P \leq 0.05$; Table 2); however, this slight increase in DMI with the Na⁺ diet was similar at TN and HT (interaction between Na supplementation and ambient temperature: $P > 0.15$). Sodium supplementation did not affect milk production and increased milk DM and fat content ($P \leq 0.05$). Milk production and composition were unaffected by the interaction between Na supplementation and ambient temperature ($P > 0.10$). For dry cows, DMI was similar to the fed amounts for both Na⁻ and Na⁺.

Sodium supplementation did not improve indicators of heat adaptation of dairy cows, such as vaginal temperature or blood pH (Table 3). In both lactating and dry cows, daily vaginal temperatures were unaffected by Na supplementation ($P > 0.15$). Sodium supplementation did not decrease vaginal temperature at HT even though the interaction between ambient temperature and Na supplementation tended to be significant in lactating cows ($P \leq 0.10$). Blood pH only tended to be higher on the Na⁺ diet in dry cows ($P \leq 0.10$) and was always unaffected by the interaction between ambient temperature and Na supplementation in both dry and lactating cows ($P > 0.10$). At 0800 h in dry cows and at 1500 h in lactating cows, the concentration of HCO₃⁻ and the base excess were higher in animals fed the Na⁺ diet than in animals fed the Na⁻ diet ($P \leq 0.05$). This difference was more pronounced at TN than at HT (interaction temperature \times Na supplementation: $P \leq 0.05$ for dry cows and $P \leq 0.10$ for lactating cows).

The increase in DMI in lactating cows given the Na⁺ diet in the present experiment was lower than the increases in DMI reported in lactating cows supplemented with Na at high temperatures by other authors (Escobosa et al., 1984; Schneider et al., 1986; West, 2003). The main reason for this observation must be that the low chloride content of the diet offered to cows in this experiment produced high diet electrolyte balances (EB) in cows fed the Na⁻ and Na⁺ diets. The EB of diets in the current experiment was largely over 200 mEq/kg of DM, a threshold value at which increases in EB result in increased DMI, albeit with very low marginal yields (Meschy, 2010). In all previous experiments (Escobosa et al., 1984; Schneider et al., 1986; West, 2003), control treatments consisted of diets with an EB lower than 200 mEq/kg of DMI. Because of the low chloride content of the corn silage used in our experiment, Cl contents of the diets did not meet Cl requirements of cows, as illustrated below by their negative chloride balances and their low concentrations of urinary chloride (Meschy, 2010). In lactating cows, low chloride contents of the diet were likely responsible for the high concentrations of blood bicarbonates, (ex-

ceeding 28 mmol/L) and high base excess (exceeding 8 mEq/L), which are symptomatic of a metabolic alkalosis (Offenstadt and Bernardin, 2009).

A Few Interactions Between Na Supplementation and Ambient Temperature on Water Flows

Sodium supplementation enhanced the FWI of lactating cows (+5.5 kg/d; SED = 2.57; $P \leq 0.05$; Table 4) but did not affect FWI of dry cows ($P > 0.10$). Sodium supplementation also increased the amount of water excreted in urine in both lactating (+6.5 kg/d; SEM = 1.78; $P \leq 0.01$; Table 4) and dry cows (+4.1 kg/d; SEM = 1.78; $P \leq 0.01$). In lactating cows, the increase in the amount of water excreted in urine with the Na⁺ diet was higher at HT compared with TN (+4.7 and +8.2 kg/d at TN and HT, respectively; interaction of ambient temperature \times Na supplementation: $P \leq 0.05$). Neither Na supplementation nor the interaction between Na supplementation and ambient temperature affected the other water flows at either stage of lactation. When water flows were reported as a proportion of DMI (Table 5), the Na⁺ diet also tended to enhance the FWI of lactating (+0.21 kg/kg of DMI; SED = 0.170; $P \leq 0.10$) and dry cows (+0.19 kg/kg of DMI; SED = 0.170; $P \leq 0.10$) and enhanced the urinary water excretion (+0.31 kg/kg of DMI for lactating cows and +0.30 kg/kg of DMI for dry cows; SED = 0.093; $P \leq 0.01$). The effect of the interaction between Na supplementation and ambient temperature tended to be significant in lactating cows ($P \leq 0.10$).

The increase in water intake was consistent with the increase predicted by the equations of Murphy et al. (1983). This increase was most likely related to the stimulation of the thirst center by the increased plasma osmolarity produced by increased Na ingestion (Offenstadt and Bernardin, 2009). The increase in urinary excretion was also consistent with the increase predicted by the equations of Nennich et al. (2006) and was likely related to the inhibition of the renin-angiotensin-aldosterone system and the reduction in the renal reabsorption of Na and water in response to the increased extracellular fluid volume produced by the increased amount of ingested Na (Offenstadt and Bernardin, 2009). The increase in urinary excretion in highly supplemented cows is associated with the fact that urine excretion is the main homeostatic way of regulating Na and K (Offenstadt and Bernardin, 2009). The current study illustrates an additive effect of Na and heat on FWI in both lactating and dry cows.

Electrolyte Balances

In the present experiment, Na balances were not different from zero ($P > 0.10$) except for in lactating cows

Table 6. Effects of ambient temperature and Na supplementation on the mineral balance of dry and lactating cows

Item	Stage ¹	Treatment ²				SEM ³	P-value ⁴		
		TN		HT			Temp	Na	Temp × Na
		Na ⁻	Na ⁺	Na ⁻	Na ⁺				
Na (g/d)									
Free water intake	Lact	2.1	2.3	2.4	2.6	0.07	≤0.01	≤0.05	0.95
	Dry	0.7	0.8	1.2	1.2		≤0.01	0.25	0.68
Feed	Lact	43.4	104.6	38.0	93.3	3.40	≤0.01	≤0.0001	0.28
	Dry	28.5	68.2	28.5	68.2		1.00	≤0.0001	1.00
Urinary	Lact	15.5	57.4	17.5	73.7	4.27	≤0.05	≤0.0001	0.09
	Dry	13.7	46.0	14.1	52.1		0.42	≤0.0001	0.48
Fecal	Lact	13.2	30.7	10.9	16.0	2.73	≤0.001	≤0.0001	≤0.01
	Dry	11.7	15.7	9.1	14.0		0.23	≤0.05	0.77
Milk	Lact	13.2	10.8	10.7	10.7	1.07	0.23	0.26	0.27
Balance ⁵	Lact	3.8	7.9*	1.3	-4.5	3.48	≤0.05	0.80	0.13
	Dry	3.7	7.3	6.4	3.3		0.83	0.95	0.29
K (g/d)									
Free water intake	Lact	0.41	0.44	0.46	0.5	0.011	≤0.01	≤0.05	0.86
	Dry	0.14	0.16	0.22	0.23		≤0.01	0.26	0.66
Feed	Lact	255.0	271.3	224.1	243.6	7.66	≤0.01	≤0.05	0.81
	Dry	168.8	177.0	168.8	177.0		1.00	0.24	1.00
Urinary	Lact	158.2	150.4	153.5	170.7	8.62	0.31	0.54	0.12
	Dry	134.4	134.1	130.7	140.1		0.88	0.55	0.52
Fecal	Lact	39.3	35.9	29.2	20.0	3.12	≤0.01	≤0.05	0.31
	Dry	20.6	14.2	18.1	12.2		0.43	≤0.05	0.92
Milk	Lact	54.0	50.4	46.5	48.0	1.75	≤0.05	0.52	0.16
Balance ⁵	Lact	3.9	35.1**	-4.7	5.4	3.21	≤0.05	≤0.05	0.22
	Dry	13.9	28.9**	20.2*	25.0*		0.88	0.24	0.54
Cl (g/d)									
Free water intake	Lact	4.9	5.3	5.5	5.9	0.17	≤0.01	≤0.05	0.94
	Dry	1.7	1.9	2.7	2.8		≤0.01	0.27	0.67
Feed	Lact	26.7	27.4	23.8	24.4	0.9	≤0.001	0.36	0.93
	Dry	17.7	17.8	17.7	17.8		1.00	0.84	1.00
Urinary	Lact	6.9	6.7	6.1	7.6	1.20	0.99	0.58	0.49
	Dry	19.3	22.2	18.5	22.7		0.90	≤0.05	0.58
Fecal	Lact	13.5	14.8	11.0	10.9	1.79	≤0.01	0.48	0.39
	Dry	8.5	7.04	8.6	7.1		0.90	≤0.1	0.99
Milk	Lact	38.6	33.8	32.8	33.7	3.86	≤0.05	0.12	≤0.05
Balance ⁵	Lact	-27.4***	-22.7***	-20.6***	-21.8***		≤0.05	0.27	≤0.1
	Dry	-8.5*	-9.5**	-6.7*	-9.2**	3.21	0.53	0.27	0.64

¹Lact = lactating cows; Dry = dry cows.

²TN = thermoneutrality; HT = high temperature; Na⁻ = diet formulated with a low level of Na supplementation (5.0 g/kg of NaHCO₃⁻); Na⁺ = diet formulated with a high level of Na supplementation (19.0 g/kg of NaHCO₃⁻).

³Standard error of the mean of the effect of the stage × temperature × Na supplementation interaction.

⁴Temp = temperature; Na = Na supplementation; Temp × Na = interaction between both effects.

⁵Balance = (free water intake + ingested with feed) - (urine + feces + milk).

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ (for the test of null balance).

fed the Na⁺ diet at TN ($P \leq 0.05$; Table 6). Potassium balances were positive for lactating cows fed the Na⁺ diet at TN ($P \leq 0.01$), for dry cows fed the Na⁺ diet at TN ($P \leq 0.01$), and for dry cows at HT regardless of the level of Na supplementation ($P \leq 0.05$). Potassium balances were not different from 0 for the other modalities. Chloride balances were always negative in lactating and dry cows ($P > 0.01$), showing that the amount of Cl provided by the diet did not cover cow requirements.

Even though HT increased the amount of water lost to evaporation in both lactating and dry cows, we did

not observe increases in the K and Na balances at HT that would account for the electrolytes lost in the sweat or retained in the organism. This observation is likely related to the fact that bovine sweat has lower concentrations of electrolytes than plasma or urine, as long as the temperature did not exceed extreme values of 40 to 45°C (Jenkinson and Mabon, 1973). In lactating cows, HT even decreased the Na and K balances ($P \leq 0.05$) and increased the Cl balance ($P \leq 0.05$) in the current study. This is most likely because the alkalosis experienced by the cows could induced a systematic regulation favoring Na and K excretion and Cl reten-

tion to decrease the plasmatic strong ion difference that can be estimated as the difference between the Na, K, and Cl plasma concentrations. The plasma strong ion difference is positively correlated with the plasma pH according to the strong ions theory (Constable, 1999).

Sodium supplementation had no effect on Na and Cl balances ($P > 0.15$), confirming the fact that the amount of Na in the organism is tightly regulated and that Cl is very dependent on Na regulation; indeed, Cl and Na are the major contributors to the anion-cation balance of the extracellular fluid. Sodium supplementation increased K retention in lactating cows ($P \leq 0.05$), most likely because alkalosis favors intracellular K retention (Offenstadt and Bernardin, 2009).

CONCLUSIONS

This study demonstrated that, when body water evaporation and free water intake were expressed as a proportion of DMI, the increase in evaporation associated with increasing ambient temperatures was mainly compensated for by an increase in FWI in both dry and lactating cows fed diets with different Na contents. Although the urinary flow increased with ambient temperature in lactating cows, the variation in urinary output was lower than the variation in other water flows. These results provide information to better predict the effect of ambient temperature on water requirements of dairy cows independently of the effect of diets or animals with the use of models of latent heat loss.

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