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Effects of frequent machine milking and suckling in early lactation on blood plasma ion homoeostasis in high-yielding dairy cows

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SUMMARY

Groups of nine or ten cows were assigned, after calving, to treatments in which they were (i) machine milked three times daily (M3), (ii) machine milked six times daily (M6) or (iii) suckled three times daily in addition to being machine milked three times daily (S). Treatments were administered during the first 6 weeks postpartum. On one day, at weeks 1 and 6 postpartum, blood samples were collected from all cows at 30-min intervals between 06.00 and 13.00 h and these were analysed for plasma osmolality and plasma concentrations of Na^+, K^+ and Cl^-. Milk yield was significantly higher in suckled cows than in cows milked six times daily, but significantly lower in cows milked three times daily. In cows milked six times daily, and to a greater extent in suckled cows, there was a reduction in plasma osmolality and monovalent ion concentrations (Na^+, K^+ and Cl^-), which could increase the susceptibility of the cows to water intoxication. Moreover, suckling or milking the cows six times daily was associated with increased fluctuations in plasma osmolality and plasma Cl^- concentrations. The decrease in plasma osmolality and ion concentration and the increased variation in plasma osmolality and Cl^- were probably related to increased water intake and may be indicative of a severe challenge to homoeostasis regulation.

INTRODUCTION

In humans (Phillips *et al*. 1984) and pigs (Anderson & Houpt 1990; Houpt & Anderson 1990) as well as in dry cows in late pregnancy (Maltz *et al*. 1994), drinking may occur in the absence of the classic hypertonic stimuli. Animals of several species, such as rats (Fitzsimons & LeMagnen 1969), pigs (Anderson & Houpt 1990), ponies (Sufit *et al*. 1985), humans (Phillips *et al*. 1984), goats (Rossi & Scharrer 1992) and cattle (Dado & Allen 1994) imbibe a large fraction (*c*. 75%) of their daily water intake before, during and shortly after meals. This feed-related drinking is considered to be the major mechanism responsible for minor fluctuations in plasma Na^+ and osmolality in mammals having free access to feed and water (Kraly 1984; Silanikove 1989). Unlike humans (Phillips *et al*. 1984), rats (Kraly 1984) and pigs (Houpt & Anderson 1990), all of which exhibit a periprandial drinking pattern, cows mainly drink postprandially (Maltz *et al*. 1994).

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Changes of 5 mM in Na^+ and of 10 mosm/kg in osmolality are thought to act as stimuli for drinking in humans (Phillips *et al*. 1984), rats (Fitzsimons & LeMagnen 1969; Kraly 1984), pigs (Anderson & Houpt 1990; Houpt & Anderson 1990) and ponies (Sufit *et al*. 1985). For dairy cows under heat stress in early and established lactation, fluctuations in plasma concentrations of Na^+ and Cl^- have been reported to be larger than these values (Maltz *et al*. 1994). Drinking during lactation under heat load was probably the result of a response to combinations of feed- and hypertonicity-related stimuli (Maltz *et al*. 1994). The reduced homoeostatic efficiency of plasma monovalent ions during early and established lactation in hot weather may consist of an accumulation of their deficiencies because of negative balances, enhanced excretion of K^+ in sweat, and the sequestration of Na^+ and Cl^- in the rumen (Maltz *et al*. 1994).

The increased heat production which is associated with an increase in milk yield may increase sweating even under moderate environmental conditions (Silanikove *et al*. 1995). Increased milk yield may also

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Table 1. *Milk yield (4% FCM), dry matter intake and loss of body weight at weeks 1 and 6 postpartum of cows machine-milked 3 times daily (M3), machine-milked 6 times daily (M6) and machine-milked 3 times daily plus suckled 3 times daily (S). The mean initial BW was 563 kg ± 15 (S.D.) for all three groups*

	Milking treatment			S.E.
	M3	M6	S	(D.F. 28)
Week 1 postpartum				
FCM milk yield (1/day)	25·3	32·0	37·8	1·0
Dry matter intake (kg/day)	12·2	13·8	12·3	0·6
Loss of body weight (kg)	8·8	9·1	9·2	1·3
Week 6 postpartum				
FCM milk yield (1/day)	39·6	48·8	56·5	1·1
Dry matter intake (kg/day)	18·8	22·2	18·9	1·1
Loss of body weight (kg)	25·2	31·5	58·8	4·2

be associated with increased saliva secretion because of the strong interrelationship between feed intake and salivation (Silanikove 1992). Lactating cows produce > 250 litres of saliva per day, and the amount of Na^+ secreted with the saliva is 5–10 times greater than that present in plasma (Cassida & Stokes 1986; Silanikove *et al*. 1987). Reabsorption of Na^+ from the rumen is an active process that accounts for *c*. 50% of the Na^+ reabsorption from the gastrointestinal tract (Dobson 1959). Therefore, the likelihood of transient sequestration of Na^+ and Cl^- (because Cl^- absorption is closely related to Na^+ absorption) in the rumen is higher in high-yielding dairy cows.

The purpose of the present experiment was to examine the effect of inducing an increase in milk yield, either by increasing milking frequency or by the inclusion of suckling, on the capacity of cows to regulate the concentrations of Na^+, K^+ and Cl^- in blood plasma within a narrow range.

MATERIALS AND METHODS

Cows

Twenty-nine second lactation Israeli Holstein cows in the dairy herd of Kibbutz Kefar Menahem (Israel), were used concurrently during spring, when the ambient temperature range was 12–23 °C. Cows were penned individually for 10 weeks postpartum, and were in open-air, free-stall barns with free access to an unpaved exercise enclosure for 1 h three times a day.

Cows were allowed *ad libitum* access to a total mixed ration containing (per kg DM) 650 g concentrates, 170 g crude protein and 7·0 MJ net energy for lactation.

Treatments

Cows were assigned to the treatment groups in randomized blocks on the basis of precalving weight, first lactation milk yield and composition, calving date and clinical and fertility history. Treatments were (i) M3 (control; $n = 10$); machine milking three times daily, at 04.00, 12.00 and 20.00 h; (ii) M6 ($n = 9$); machine milking six times daily, first at the routine milking times and again at the end of the routine milking at 07.00, 15.00 and 23.00 h; (iii) S ($n = 10$); machine milking three times daily plus suckling three times daily by two adopted heifer calves. Suckling was allowed for a controlled 15-min period at 07.00, 15.00 and 23.00 h. All treatments were administered during weeks 1–6 postpartum.

Measurements and statistical analysis

During weeks 1 and 6 postpartum, total feed refusals from each cow were collected once daily, dried (105 °C for 24 h), weighed, and individual feed intakes calculated.

Milk yield was recorded at each milking. Milk intake by the calves (treatment S) was measured by the 'weigh-suckle-weigh' method (i.e. the calves were weighed on electronic scales before and after suckling) at each suckling for one day at weeks 1 and 6 postpartum. The weight of suckled milk was multiplied by 7 and added to the total machine milking yield for the week to obtain a total weekly yield. Milk samples were collected at each milking on one day each week for weeks 1 and 6 postpartum. For S cows, milk was sampled only from the machine-milking. Milk composition was determined by the Central Laboratory of the Israel Cattle Breeders' Association and expressed as 4% fat-corrected milk (FCM). Body weight was recorded once weekly, starting 2 weeks before calving. Calving weight and 3 days postpartum weight were averaged and used as initial body weight.

A cannula was placed in a jugular vein 24 h prior to blood sampling. For one day, at weeks 1 and 6 postpartum, heparinized samples (10 ml) were collected at 30-min intervals from 06.00 to 13.00 h. For practical reasons, samples could not be taken during milking; thus, pre- and post-milking samples were taken *c*. 5 min before and 10 min after milking. Plasma osmolality was determined by freezing-point depression, Na^+ and K^+ concentrations by flame photometry, and Cl^- concentration by titration, as described by Shalit *et al*. (1991).

All statistical analyses were carried out using the general linear model of SAS (1989). For each of weeks 1 and 6 postpartum, average daily milk yield at weeks 1 and 6 postpartum (4% FCM), dry matter intake (DMI), and body weight (BW) changes were compared over treatments, number of weeks postpartum and initial BW by analysis of variance, using cows within treatment and BW as the error terms for comparing treatments. The models also included interaction effects between treatment and week postpartum. When the overall effect was significant at $P < 0{\cdot}05$, individual comparisons of treatments were made by the least significant difference (L.S.D.) test.

For each of weeks 1 and 6 postpartum, the values of plasma osmolality, the concentrations of Na^+, K^+ and Cl^-, and the C.V. of each of these values (i.e. the C.V. within cow within treatment within week) were compared over weeks and treatments by two-way analyses of variance, using variance between cows within treatment as the error term for treatment comparisons. When a significant interaction was found, one-way analysis of variance was performed for each week separately.

Correlations between experimental variables were assessed using the complete data set. Significance was at $P < 0{\cdot}05$ unless otherwise stated.

RESULTS

Milk yield, DMI and loss of BW

Milk yields were highest in S cows, lowest in M3 cows and intermediate in M6 at weeks 1 and 6 postpartum; the difference between treatments being significant ($P < 0{\cdot}05$; Table 1). DMI values were similar in M3 and S cows, and both were lower ($P < 0{\cdot}05$) than in M6 cows at weeks 1 and 6 postpartum. Loss in BW was similar for the three treatments at week 1 postpartum, whereas at week 6 postpartum, loss of BW was significantly greater in S cows than in M3 and M6 cows.

Mean values of plasma values

Osmolality and plasma Cl^- concentrations ranked in the order highest to lowest, M3 > M6 > S, both at week 1 and at week 6 postpartum; the differences between treatments being significant ($P < 0{\cdot}05$; Table 2). Plasma Na^+ and K^+ concentrations of cows on treatments M6 and S were similar at week 1 postpartum and at week 6 postpartum; both were significantly lower ($P < 0{\cdot}05$, Table 2) than in M3, except for K^+ at week 6 postpartum, for which the differences were not significant.

Variation of plasma values

The C.V. for osmolality was greatest for S, lowest for M3 and intermediate for M6 at week 1 postpartum; and greatest for M6, lowest for M3 and intermediate for S at week 6 postpartum; the differences between treatments were significant ($P < 0{\cdot}05$; Table 3). The C.V.s for Cl^- concentration for treatments M6 and S were similar at week 1 postpartum and, for both treatments, significantly higher than for treatment M3 ($P < 0{\cdot}05$). The C.V.s for Cl^- at week 6 postpartum ranked in the order S > M6 > M3, the differences between treatments being significant ($P < 0{\cdot}05$; Table 3). The Na^+ and K^+ C.V.s did not differ between treatments at week 1 postpartum, which was also the case for K^+ C.V. at week 6 postpartum (Table 3). The

Table 2. *Concentration of Na^+, Cl^-, K^+ and osmolality at weeks 1 and 6 postpartum in blood plasma of cows machine-milked 3 times daily (M3), machine-milked 6 times daily (M6) and machine-milked 3 times daily plus suckled 3 times daily (S)*

	Milking treatment					
	M3		M6		S	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Week 1 postpartum						
Na^+ (mM)	144	1·0	138	1·0	139	0·9
Cl^- (mM)	108	0·8	105	1·3	100	0·7
K^+ (mM)	3·90	0·08	3·66	0·1	3·74	0·08
Osmolality (mosm/kg)	274	1·3	265	4·6	257	2·3
Week 6 postpartum						
Na^+ (mM)	142	0·9	138	1·2	139	1·0
Cl^- (mM)	107	1·3	102	2·0	98	1·1
K^+ (mM)	3·90	0·03	3·80	0·04	3·70	0·07
Osmolality (mosm/kg)	267	1·1	259	2·4	254	1·3

D.F. = 28.

Table 3. *Coefficient of variation (C.V.) of Na^+, Cl^-, K^+ and osmolality at weeks 1 and 6 postpartum in blood plasma of cows machine-milked 3 times daily (M3), machine-milked 6 times daily (M6) and machine-milked 3 times daily plus suckled 3 times daily (S)*

	Milking treatment					
	M3		M6		S	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Week 1 postpartum						
Na^+ (mM)	4·5	0·3	4·3	0·9	4·3	0·3
Cl^- (mM)	3·7	0·1	6·6	0·5	6·0	0·5
K^+ (mM)	6·4	0·5	7·2	0·5	7·1	0·3
Osmolality (mosm/kg)	2·9	0·1	3·7	0·3	4·8	0·05
Week 6 postpartum						
Na^+ (mM)	2·8	0·05	3·4	0·2	4·9	0·07
Cl^- (mM)	3·2	0·05	7·4	0·4	9·2	0·5
K^+ (mM)	6·6	0·50	7·2	0·5	7·3	0·3
Osmolality (mosm/kg)	3·3	0·02	6·3	0·6	3·9	0·02

D.F. = 28.

Na^+ C.V. at week 6 postpartum ranked in the order S > M6 > M3, the differences between treatments were significant ($P < 0·05$; Table 3).

Interrelationships among variables

The interrelationships between variables were examined for the entire data set (i.e. all the cows at weeks 1 and 6 postpartum, $n = 58$). Mean plasma osmolality and mean plasma concentration of Cl^-, Na^+ and K^+ were negatively related to milk yield. The C.V.s of plasma osmolality, Cl^-, Na^+ and K^+ were positively related to milk yield; the linear correlations were highly significant for osmolality ($P < 0·003$) and Cl^- ($P < 0·0001$) (Table 4). The C.V.s of plasma osmolality, and plasma concentration of Cl^-, Na^+ and K^+ were negatively related to mean plasma osmolality and to mean plasma concentration of Cl^-, Na^+ and K^+; the linear correlations were highly significant for osmolality ($P < 0·0004$), K^+ ($P < 0·001$) and Cl^- ($P < 0·0001$) (Table 4).

Table 4. *Interrelationships among experimental variables for the entire data set ($n = 58$) for cows machine-milked 3 times daily (M3), machine-milked 6 times daily (M6) and machine-milked 3 times daily plus suckled 3 times daily (S)*

Independent variable	Dependent variable	Correlation coefficient
Osmolality	Milk yield	−0·48
Na^+	Milk yield	−0·29
Cl^-	Milk yield	−0·57
K^+	Milk yield	−0·005
C.V. osmolality	Milk yield	0·38
C.V. Na^+	Milk yield	0·09
C.V. Cl^-	Milk yield	0·52
C.V. K^+	Milk yield	0·11
Osmolality	C.V. osmolality	−0·45
Na^+	C.V. Na^+	−0·18
Cl^-	C.V. Cl^-	−0·57
K^+	C.V. K^+	−0·49

D.F. = 56.

DISCUSSION

The substantial reductions in plasma osmolality and monovalent ion concentrations found in the present experiment were most likely to be due to an increase in plasma volume. The expansion of plasma volume in lactating ruminants possibly relates to the increased supply of metabolites to the udder, which entails an increased total mammary blood supply (Maltz & Shkolnik 1984). Thus, the positive interrelationship between the apparent increase in plasma volume and milk yield suggests that this was a response to the increase in metabolic demands. Relative to other mammalian species, bovine red blood cells are very fragile in hypotonic salt solution, with haemolysis beginning when medium osmolality drops to 200 mosm/kg (Perk *et al.* 1964). The reduction of *c.* 20 mosm/kg in plasma osmolality of S cows to values as low as 220 mosm/kg may have increased their susceptibility to water intoxication (i.e. haemolysis as a result of hypotonicity), particularly after sudden dehydration.

Plasma osmolality and ion homoeostatic efficiency

The M6 and S cows yielded 6·7 and 12·5 litres/day more milk, respectively, than M3 cows at week 1 postpartum, and 9·2 and 16·9 litres/day more at week 6 postpartum. This increase in milk yield was reflected by an increase in the fluctuations in plasma osmolality, as found in cows yielding 30 litres/day under heat load (Maltz *et al*. 1994). The fluctuations in plasma osmolality above the average were sometimes > 10 mosm/kg, which has been found to act as a stimulant for drinking in various mammals (Phillips *et al*. 1984; Anderson & Houpt 1990; Houpt & Anderson 1990); part of the drinking of the cows on the M6 and S treatments was probably in response to osmotic thirst. The M6 cows consumed more dry matter than did the M3 cows, which compensated for their increased energy demands, and, as a result, M6 cows did not lose significantly more BW than M3 cows (Table 1). Surprisingly, the cows on treatment S did not have a greater DMI than M3 cows and ate significantly less than M6 cows (Table 1). Because osmotic thirst also depressed appetite (Silanikove & Tadmor 1989), this may have accounted for the rather small increase in DMI by M6 cows and for depression in DMI by the cows on the S treatment. The lower DMI in the S cows is consistent with larger fluctuations is osmolality at 1 week postpartum and plasma Na^+ and Cl^- at 6 weeks postpartum compared with that for the M6 cows.

For lactating cows under summer conditions, the variation in plasma monovalent ions was positively related to the water turnover rate and negatively related to drinking frequency (Maltz *et al*. 1994). As discussed by Maltz *et al*. (1994), water turnover rate may indicate a strain on the regulatory system. Water turnover rate was not measured in the present experiment but is positively related to milk yield (Silanikove 1989; Maltz *et al*. 1994; Silanikove *et al*. 1995). The positive interrelationship between milk yield and the C.V. of plasma osmolality and plasma Cl^- is therefore consistent with this model. As proposed, the apparent increase in plasma volume is proportional to the increase in metabolic demands. Because water turnover rate is closely related to energy intake and to milk yield (Silanikove 1989), the inverse relationship between plasma osmolality or plasma Cl^- concentration and their respective C.V.s is also consistent with the model proposed by Maltz *et al*. (1994).

In conclusion, this study provides evidence suggesting that very frequent milking, or a combination of milking and suckling in early lactation, may be associated with reductions in plasma osmolality and plasma monovalent ion concentrations and with an impaired capacity of the cows to regulate their plasma osmolality and plasma ions within a narrow range. The latter response was similar to that observed for dairy cows producing 30 litres/day under heat stress. Consequently, if a similar treatment were carried out during the summer, cows might be driven to the limits of their physiological capacities. This could either endanger the cows or cause a collapse of their lactation curves. In fact, the latter response was frequently recorded for dairy cows in early lactation approaching a yield of 50 litres/day in the summer (E. Maltz & N. Silanikove, unpublished).

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