Water intake and excretion, urinary solute excretion and some stress indicators in mink (*Mustela vison*): effect of ambient temperature and quantitative water supply to lactating females

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Lactation is a physiologically demanding period in mink production, during which kit and dam losses may occur. Ambient temperature and quantitative water supply are thought to affect animal performance and well-being, but conclusive data in the literature are sparse. Therefore, effects of ambient temperature (T_a; low, about 5°; medium, about 15°; high, average 20–25°) and water supply (*ad libitum* (N), or 10 % extra supplementation in the food (E)) were investigated regarding effects on quantitative water intake and excretion, urine osmolality and solute excretion, and urinary cortisol and catecholamines as stress indicators in an experiment with twelve lactating mink with litters of three to seven kits in three consecutive periods, lasting 3, 3 and 2 d respectively. Kit ages ranged from 15 to 20 d at the end of the experiment. Water requirement for milk production (factorial calculations) and water available for evaporation (balance component) were estimated. Period, and hence mainly T_a, had a significant influence on intake of metabolizable energy, quantitative water intake and excretion, but there was less effect of water supply. The total water intake and excretion were very high in relation to the weight of the animals as an effect of lactation. Water intake and excretion, and urinary Na excretion, seemed to be less accurately regulated compared with corresponding functions in non-lactating animals. Rectal temperature increased with increasing T_a, possibly as a means of decreasing evaporative water loss. Water output in milk was estimated to increase from 118 g/d at low T_a to 134 g/d at high T_a. The amounts of water available for evaporation were estimated to be 42, 58 and 69 g/kg$^{0.75}$ at low, medium and high T_a. Cortisol data did not indicate that the animals experienced negative stress. It was concluded that prolonged periods of high T_a may be hazardous for lactating mink because of decreased intake of metabolizable energy resulting in energy deficit and excessive mobilization of body reserves simultaneously as the requirement for intake of water increases considerably.

Water balance: Urinary electrolytes: Stress: Mink

Lactation puts heavy demands on the mechanisms regulating water balance, and water turnover is usually increased to a considerable extent. Furthermore, lactating animals have a restricted ability to regulate fluid losses via milk. The strain of the lactation period is certainly evident for the female mink. Average litter sizes have increased profoundly in the Scandinavian countries during the last two decades, and amount currently to about six kits. The kits are totally dependent on the energy and nutrients provided by their mother's milk until the age of almost 4 weeks, when they start to take some solid food in addition to sucking. Therefore, a sufficient supply of energy, nutrients and water to the lactating dam is necessary to support performance, health and survival of dam and offspring, but even in a situation of adequate nutrition, survival and health may be endangered by internal and external factors.

The kits are born in a very vulnerable state, with a fat content of slightly about 10 g/kg in the body (Tauson, 1994*a*) and since they are blind, nearly hairless, with limited locomotor ability, as well as being devoid of an efficient thermoregulatory capacity, lactation has to be established soon after parturition. Thus, an ample milk supply is

Abbreviations: E, group of mink receiving extra dietary water; EGTA, ethylene glycol-bis-(beta-aminoethylether)-N,N,N′,N′-tetraacetic acid; ME, metabolizable energy; ME_g, ME requirement for growth; ME_m, ME requirement for maintenance; N, group of mink receiving *ad libitum* water supply; T_a, ambient temperature.

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essential for good survival rate and optimal growth performance during the suckling period. However, the kits have the capacity for rapid growth, with a maximum relative growth rate of 23 % in 24 h between days 1 and 2, and an average of 9 % in 24 h over the total 42 d lactation period. Hence, body weight is increased from about 10 g at birth to 300–350 g at weaning (Tauson, 1994*a*).

Quantitative determination of the milk production in mink is, as in most other polytocous animals, difficult to conduct, and therefore reliable information is scarce. A single study with direct measurements made by an isotope dilution technique (Wamberg & Tauson, 1998) and estimates by factorial calculations (Hansen, 1997; Tauson *et al.* 1998) indicate that milk production can be considered high for a small-sized animal, females with a live weight of about 1000 g and with litters of six or seven kits producing about 4000 g milk during the first 4 weeks of lactation. The energy requirement of the lactating dam, therefore, increases steeply as lactation progresses, and after the third week of lactation females with large litters usually are unable to increase their food intake sufficiently to sustain their metabolizable energy (ME) requirement, and consequently have to mobilize fat reserves from the body (Tauson, 1997; Tauson *et al.* 1998). Even in healthy females a weight loss in the order of 15–25 % can occur during the lactation period, most of it taking place after the third week of lactation (Tauson, 1988, 1994*b*; Hansen & Berg, 1998).

During the late part of lactation, female mink may be affected by nursing sickness, the aetiology of which is still somewhat obscure, but involves energy and water deficit. The prevalence varies, but Clausen *et al.* (1992) reported a morbidity of 14 % and a mortality of 7 %. Affected females are usually emaciated and dehydrated, and during the late stage of illness the animals refuse both food and drinking water. Pathophysiological findings include changes showing a decrease in extracellular fluid volume, electrolyte deprivation and a disturbed acid–base balance (Wamberg *et al.* 1992).

The apparently simple task of supplying sufficient amounts of energy and water may, however, have considerable limitations. During the lactation period ambient temperature (T_a) may vary to a great extent; many locations where mink farming occurs are likely to experience periods of high T_a, and practical experience has shown that the risk for kit and female mortality increases at high T_a. Evaporative water loss from the body of small kits may cause dehydration (Wustenberg & Wustenberg, 1988), and females with high obligatory water loss are also at risk. T_a has been demonstrated to have a great influence on ME intake and water intake and excretion traits of male mink under laboratory conditions. When T_a was high, ME intake was low, resulting in a decreased dietary water intake, whereas the requirement of drinking water increased as well as evaporative water loss (A-H Tauson, unpublished results; Wamberg, 1994). The quantitative water supply also had some influence on water intake and excretion traits. Generally, even a limited restriction in the access to drinking water resulted in a decreased water intake, water excretion in urine was decreased concomitantly and, consequently, urinary osmolality increased, but if extra water was added to the food the opposite occurred (A-H Tauson, unpublished results; Neil, 1988). In addition, mink diets are high in protein, and water will be required for excretion of excess N. Therefore, several factors will make the female mink vulnerable to shortage of water or an augmentation of the demands, for instance induced by high T_a.

The supply of drinking water to lactating mink may, therefore, be crucial for both performance and animal welfare reasons. Both T_a and quantitative water supply may be considered as very important factors for the water intake and excretion and total water balance, and in lactating females their influence may be expected to be strongly determining for animal performance and health.

The objectives of the present study were first, to study how T_a influenced water intake and excretion traits, including urinary osmolality and solute excretion, in lactating dams, and how animal performance was affected by changes in T_a. Second, it was the intention to estimate if facilitation of water intake by addition of extra water in the food influenced water intake and excretion traits and animal performance to any extent. Third, it was the aim to assess if variation in T_a and quantitative water supply imposed stress in the animals as measured by 24 h urinary excretion rates of cortisol and catecholamines.

Materials and methods

Animals and general design of the experiment

The experiment was carried out at Funbo Lövsta Research Station with ten yearling and two 3-year-old female mink of the standard black genotype (Nes *et al*. 1987) originating from the breeding herd of our experimental farm and kept under conventional housing conditions, i.e. exposed to prevailing fluctuations in outdoor temperature. The females had given birth during the period 3–8 May, and the experimental animals were chosen among females with litter sizes of three to seven kits. The animals were free from plasmacytosis according to a counter immunoelectrophoresis test (Hansen, 1974). They were housed in one end of a two-row closed, but non-insulated, mink house with a concrete floor. The cages had a floor area of $0{\cdot}9 \times 0{\cdot}3$ m and a height of 0·4 m. All cages had a nest box especially furnished for lactating animals. The cages were equipped with individual water bottles connected to conventional drinking nipples and jars to collect water spill. The animals were individually fed once daily in cups, placed on trays to permit collection of food waste. The experiment consisted of three periods carried out consecutively, and lasting 3, 3 and 2 d respectively. During the first period (low T_a) the average temperature was about 5°, with a minimum of 0° and a maximum of 10°. During the following two periods, T_a was increased by means of two heaters with fans placed so as to distribute their heat as evenly as possible in the area in which the animals were housed. In the second period (medium T_a) average temperature was about 15°, with a minimum and maximum of 7° and 25° respectively. The third period (high T_a) had an average temperature of between 20° and 25°, with a minimum of 14° and a maximum of 30°. Since the house in which the animals were kept was not insulated, the diurnal fluctuations in temperature mainly reflected outdoor differences between

night and day. In order to keep relative humidity as even as possible, the concrete floor was sprinkled with water several times daily during the periods of medium and high T_a.

The animals were given drinking water *ad libitum* (normal; N) or were given extra water supplementation (E) in the food (water equalling 10 % of the original weight of the food was added before feeding). The general outline of the experiment, litter sizes and kit ages are given in Table 1. The experimental procedures were approved by the Ethical Committee for Experimentation with Animals, Sweden.

Diet and food supply

The daily food supply was 400 g, which was calculated to support the energy requirement of the females with the largest litters and the oldest kits throughout the experiment (Hansen *et al*. 1991). The diet contained (g/kg): cod offal 300, Baltic herring 100, filleting scrap (heads and backbones) of Baltic herring 100 (both from herring caught during the spring), slaughter house offal 150, poultry wastes 150, a mixture of extruded wheat, oats and barley 50, and potato mash powder 30. Vitamins were added according to standards (Juokslahti, 1987). The analysed chemical composition of the diet (g/kg) was: DM 274, ash 26, crude protein 132, fat 70, carbohydrate calculated by difference 46, and gross energy 1600 kJ/kg. All the food was prepared on one occasion before the start of the experiment and daily portions were weighed and stored deep-frozen until the day before feeding. The food was thawed in a refrigerator overnight. For animals on E water supply, 40 g water was stirred into each portion before it was presented to the animals.

Data collection

Females and individual kits were weighed at the start and at the end of each period, at and the end of each period rectal temperatures of the females were recorded. Daily consumption of drinking water was recorded. Quantitative collection of food refusals, food wastes, faeces and urine was carried out daily. Faeces were collected on netting screens placed under the cages, and urine was collected via funnels into plastic bottles. The collection procedures started at 09.00 hours, and feeding took place when the collection procedure was completed, at approximately 12.00 hours. In order to determine the original water content of the faeces, small samples were taken as soon after voiding as possible, and when calculating water output in faeces, faecal DM was corrected to the level of fresh faeces. The food refusals, wastes and faeces were frozen until analysis. From the urine, two daily samples were taken out and deep-frozen. On the last day of each collection period, urine from every second animal (*n* 6) was collected for catecholamine analyses (see later) in 4 ml 6 M-HCl (pH 1–2). A solution consisting of 90 mg/ml ethylene-glycol-bis-(beta-amino-ethylether)-N,N,N′,N′-tetraacetic acid (EGTA) and 60 mg/ml glutathione was used as a preservative, and 20 μl EGTA–glutathione solution was used per 5 ml urine.

Analyses

Contents of DM were determined in all samples. Before further analyses, diets, faeces and urine were freeze-dried. Diets and faeces were analysed for ash, crude protein (N × 6·25) and fat (official CEC-method; Amtsblatt der Europäischen Gemeinschaften, 1971) contents, and total carbohydrate was calculated by difference.

Osmolality of urine was measured by freezing-point depression (Hermann Roebling Meßtechnik, Berlin, Germany), and Na and K concentrations were measured using ion-selective electrodes (model E2A Electrolyte Analyzer; Beckman Instruments, Stockholm, Sweden). Cortisol excretion in urine was measured by radioimmunoassay using an Amerlex Cortisol RIA kit (Amersham International plc, Amersham, Bucks., UK). For a detailed description of the method, see Madej *et al*. (1992). Catecholamines (adrenaline and nonadrenaline) were analysed with a commercial catecholamines [^{3}H]radioenzymic assay system (Amersham International plc). The detection limit of the assay was below 100 pg/ml, and all samples analysed had far higher concentrations. The interassay CV was 4 % and the intra-assay CV was about 10 %.

Calculations of apparent digestibility and water balance

The apparent digestibilities of crude protein, fat and total carbohydrate (by difference) in the experimental diet were

Table 1. Animals and design of experiments with low, medium or high ambient temperature (T_a; 5, 15 or 20–25°) and water supply (*ad libitum*, N, or extra, E) to lactating female mink

Female no.	Water supply	No. of kits	Kit age (d) at the start of period with T_a			
			Low	Medium	High	End of expt
1	N	6	12	15	18	20
2	N	6	12	15	18	20
3	E	6	12	15	18	20
4	E	6	12	15	18	20
5	N	3	10	13	16	18
6	N	7	10	13	16	18
7	E	5	10	13	16	18
8	E	6	10	13	16	18
9	N	6	8	11	14	16
10	N	5	7	10	13	15
11	E	5	8	11	14	16
12	E	7	7	10	13	15

calculated for individual animals in each period as: (dietary intake of nutrient − faecal output of nutrient)/dietary intake of nutrient. The daily ME consumption (kJ) was calculated as intake of digestible protein (g) × 18·8 + digestible fat (g) × 39·8 + digestible carbohydrate (g) × 17·6) (Hansen *et al.* 1991).

Water balance was calculated as the difference between water intake in food and drinking-water and water output in urine and faeces. Results are presented both in g/animal per d and as g/kJ ME intake.

Calculation of water requirement for milk production

The calculation of the water requirement for milk production was made by factorial approach based on the following data and assumptions. The daily weight gain of the individual kits was calculated for each period, and the metabolic live weights ($kg^{0·75}$) were calculated from average kit weights within each period. Data for body composition of the kits were taken from Tauson (1994*a*) for 5-d-old kits, and from N Glem-Hansen (unpublished results) for 24-d-old kits. The increases in protein and fat contents in the body were assumed to be linear over the period studied, resulting in increases in protein and fat of 0·1 and 0·25 percentage units respectively, per day increase in age. Daily protein and fat retentions were then calculated from daily weight gain and body composition data. Energy retained in protein was 23·86 kJ/g and in fat 39·76 kJ/g (Brouwer, 1965). The ME requirement for maintenance of the kits (ME_m) was set at 527 $kJ/kg^{0·75}$ (Chwalibog *et al.* 1980), and ME for growth (ME_g) was calculated by use of daily protein and fat accretion and the efficiencies of utilization for ME for protein retention (k_p) and fat retention (k_f) of 0·5 kJ/kJ and 0·8 kJ/kJ respectively, estimated on other single-stomached species (Klein & Hoffman, 1989; Chwalibog, 1991). ME requirement for kits (ME_{kit}) was calculated as the sum of ME_m and ME_g. For milk consumption, data for DM (DM_{milk}) and gross energy (GE_{milk}) contents from Olesen *et al.* (1992) were used, covering days 8–22 in lactation. Metabolizability of gross energy in milk was set at 0·85, and the daily milk requirement per kit (MILK) was calculated as $MILK = ME_{kit}/(GE_{milk} \times 0·85)$. The daily water output in milk per female ($WATER_{MILK}$) was calculated as:

$$WATER_{MILK} = \text{no. of kits per litter} \times (MILK \times (1–DM_{milk})).$$

Statistical analyses

ANOVA were carried out according to the general linear models procedure in Statistical Analysis Systems (1985). The following model (1) was used to evaluate the quantitative water intake and excretion data and the data on urinary osmolality, Na and K:

$$Y_{ijkl} = \mu + a_i + b_j(i) + c_k + (ac)_{ik} + e_{ijkl}, \qquad (1)$$

where Y_{ijkl} is the *ijkl* th observation, μ is the general mean, a_i is the fixed effect of water supply, $b_j(i)$ is the effect of animal within water supply, c_k is the fixed effect of period, $(ac)_{ik}$ is the interaction effect between water supply and period and e_{ijkl} is the random error. Effect of water supply was tested by using random animal within water supply as an error term.

Data on ME consumption, water turnover in relation to ME consumption, 24 h excretion rates of Na, K, cortisol and catecholamines were evaluated with a model comprising the fixed effects of water supply and period and the interaction between these effects. Estimates of water requirement for milk production were tested regarding the fixed effect of period. Results are presented as least squares means

Table 2. Live weights of females and kits in experiments with low, medium or high ambient temperatures (T_a; 5, 15 or 20–25°) and water supply (*ad libitum*, N, and extra, E) to lactating female mink

	Water supply			
	N	E	RMSE	Effect of water supply (P =)
Experimental start/start of period with low T_a				
Litter size	5·50	5·83		
Kit age (d)	9	9		
Female live weight (g)	1183	1135	149	0.59
Kit live weight (g)	41·3	43·8	16·3	0·53
Start of period with medium T_a				
Female live weight (g)	1197	1192	149	0·95
Kit live weight (g)	65·3	69·3	21·9	0·44
Start of period with high T_a				
Female live weight (g)	1179	1204	159	0·79
Kit live weight (g)	86·8	90·2	25·5	0·58
End of experiment				
Litter size	5·50	5·83		
Female live weight (g)	1141	1146	154	0·96
Kit live weight (g)	100·5	102·5	27·8	0·77

RMSE, root mean square error.

achieved according to the described models. The significance level for differences between least squares means was determined by test with comparison-wise error rate 5 %, i.e. protected *t* test after significant *F* test (Fisher, 1935).

Results

Animal performance

Litter sizes averaged 5·5 and 5·8 kits for females given N and E water supply respectively, and all kits survived throughout the experimental period. Weight gain of the kits was not affected by water supply, and the recorded kit weights were normal. For N females live weights were stable until the start of the last period, during which they decreased. For E females, correspondingly, there was an increase in weight until the start of the last period, during which the weights decreased. There was not, however, any significant effect of water supply (Table 2).

Quantitative water turnover

Period had a significant influence on consumption of drinking water ($P = 0{\cdot}003$), the consumption increasing with increasing T_a, and the intake of water with the food ($P = 0{\cdot}009$), the intake decreasing with increasing T_a, but the total water intake was not affected by period. Period had a strong influence on faecal water, urinary water and total water output ($P < 0{\cdot}001$), the water output decreasing with increasing T_a. The combined effects, therefore, resulted in increased water balance with increasing T_a ($P = 0{\cdot}008$) (Fig. 1).

Water supply had no significant effect on water intake but for water output traits, treatment effects were more evident, with significantly less water voided in urine ($P = 0{\cdot}02$) and in total ($P = 0{\cdot}04$) for N animals. The water balance was independent of water supply ($P = 0{\cdot}16$) (Fig. 1).

Metabolizable energy intake and water intake and excretion in relation to metabolizable energy intake

ME intake decreased with increasing T_a. Period had a strong influence on water intake traits, with increases in drinking, dietary and total water intake per kJ ME as temperature increased. All output traits were affected by period, with the highest water output during the period with the low T_a. Water balance, on the other hand, increased when T_a increased (Table 3).

ME intake of animals given E water supply was close to significantly ($P = 0{\cdot}06$) higher than for N animals. Similar to the quantitative water output, water voided in urine and total water output were significantly affected by water supply, the highest outputs being achieved for animals given E water supply. Because E animals had lower water intake per kJ ME intake, water balance in relation to ME intake was highest for N animals (Table 3).

Estimated water loss in milk and rectal temperature

The calculated water loss in milk increased during the course of the experiment, reflecting the increasing ME requirement of the kits. Daily weight gain of the kits remained stable throughout the experiment, and hence ME_g only increased slightly from the period with low T_a to the two following periods (Table 4).

The rectal temperature of the females was above 40° in all periods, and increased significantly during the two periods with medium and high T_a (Table 4).

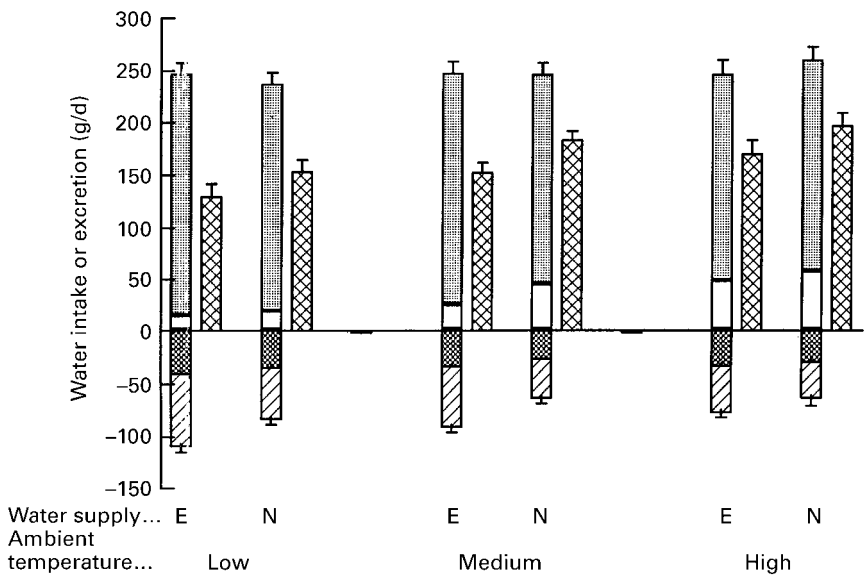

Fig. 1. Quantitative water turnover data for lactating female mink kept at low (about 5°), medium (about 15°) and high (about 20–25°) ambient temperature, and given drinking water *ad libitum* (N) or given an extra supply of water in the food (E). (□), Drinking water; (▤), dietary water; (▪), faecal water; (▨), urinary water; (▩), balance. Values are means for twelve mink. Standard errors of the means, represented by vertical bars, are given for total water intake, total water excretion and water balance. Significant effects of T_a were recorded for intake of drinking water ($P = 0{\cdot}003$), dietary water ($P = 0{\cdot}009$) and the faecal, urinary and total water excretion (all $P < 0{\cdot}001$) as well as water balance ($P = 0{\cdot}008$). Urinary ($P = 0{\cdot}02$) and total ($P = 0{\cdot}04$) water excretion values were lower in N than in E animals.

Urine osmolality and solute excretion

The concentration of Na in urine was not affected either by water supply or by period. K, on the other hand, was significantly higher in N animals ($P = 0{\cdot}005$), and also increased as T_a increased ($P = 0{\cdot}01$). Also urine osmolality was significantly greater for N animals ($P = 0{\cdot}02$) and increased with temperature ($P < 0{\cdot}001$), the highest values of about 2600 mOsm/kg water being recorded for N animals at medium and high T_a (Fig. 2).

The 24 h excretion of Na was significantly affected both by water supply and period, the highest excretion being recorded for E animals, but the rate of excretion decreased when T_a increased. For K excretion a similar tendency for higher excretion in E animals was found, and excretion decreased when temperature increased (Table 5).

In relation to ME consumption, Na excretion was not affected by period but was influenced by water supply, the excretion being highest for E animals. K excretion per kJ ME consumed was not significantly affected either by period or water supply (Table 3).

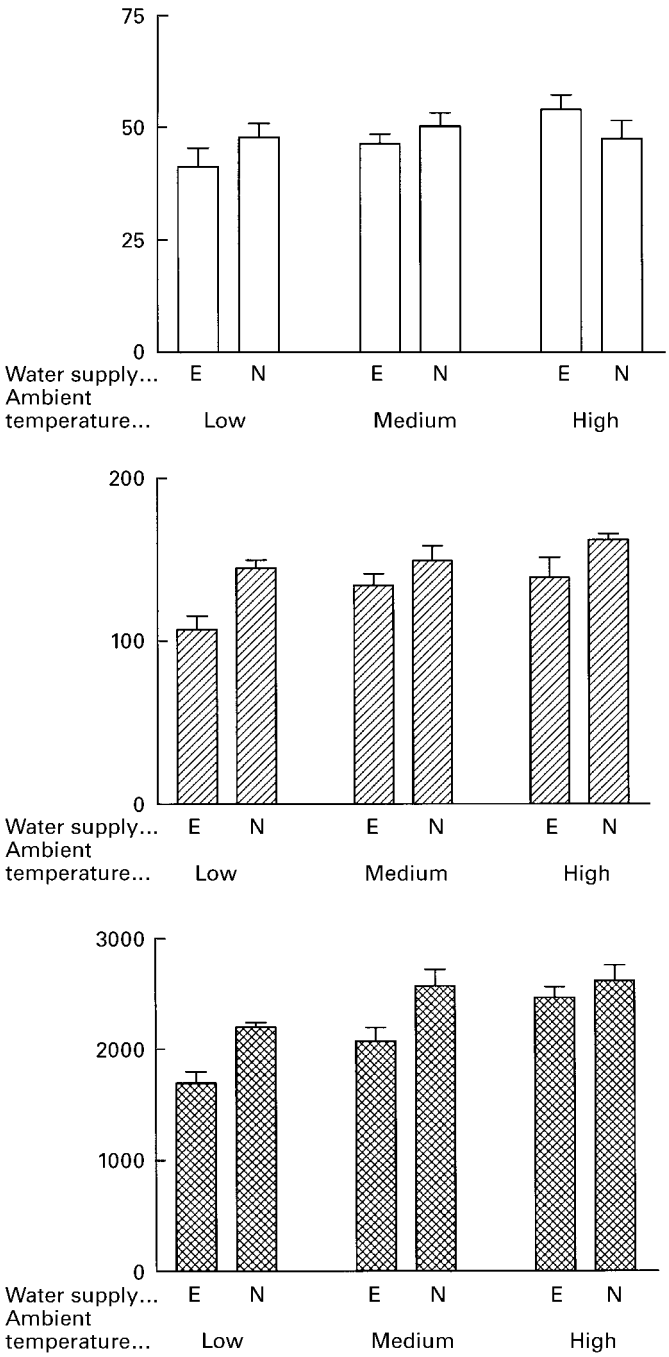

Fig. 2. Urinary excretion of sodium and potassium, and urinary osmolality for lactating female mink kept at low (about 5°), medium (about 15°) and high (about 20–25°) ambient temperature, and given drinking water *ad libitum* (N) or given an extra supply of water in the food (E). Urinary potassium concentration was significantly affected by period ($P = 0{\cdot}01$) and water supply ($P = 0{\cdot}005$). Osmolality was significantly affected both by period ($P < 0{\cdot}001$) and water supply ($P = 0{\cdot}02$).

Urinary excretion of cortisol and catecholamines

Cortisol excretion was independent of water supply and T_a. Catecholamines were not significantly affected by period, but at low and medium temperatures the highest excretion was recorded for E animals (Table 5).

Discussion

Ambient temperature

The present investigation was performed in a period which corresponded to increasing milk production, but most probably the females had not yet reached peak lactation. Despite this, the daily water intake and excretion values were greater than those found in males (A-H Tauson, unpublished results) of approximately double the body weight.

Most water intake and excretion traits were strongly affected by period, but since it was not technically possible to divide the experimental facilities into separate compartments that could be individually climatized, and the mink whelping season is very concentrated in time, the animals had to be exposed to the different temperature treatments consecutively, and hence effects of period cannot be ascribed unambiguously to the increase in T_a, but may also, to some extent, be explained by increasing metabolic demands on the females to support the energy requirement of the growing kits. On the other hand, previous findings (A-H Tauson, unpublished results) have given clear evidence for a strong influence of temperature on the total water intake and excretion.

The present results demonstrate clearly that ME intake decreased from the first to the last period, a change which was probably caused by the increase in T_a, because lactating mink normally increase their ME intake considerably during the first 3 weeks of lactation (Tauson, 1997). For lactating dams a low ME intake is a serious problem because it will lead to energy deficit and concomitant mobilization of body reserves. Since lactating mink are usually in negative energy balance after the third week of lactation (Tauson, 1997; Tauson *et al.* 1998) even under normal temperature conditions, and weight losses during a 6-week lactation period are in the order of 15–25 % (Tauson, 1988, 1994*b*; Hansen & Berg, 1998), a decrease in ME intake caused by a high T_a may result in excessive weight loss and eventually in

Table 3. Effect of periods with low, medium or high ambient temperature (5, 15 and 20–25°) and different water supply (N, *ad libitum* drinking water supply, or E, extra water supplementation in the food) on metabolizable energy (ME) intake and water intake and excretion, and urinary excretion of Na and K per kJ ME consumed in lactating female mink

	Ambient temperature			Water supply			Effect of ($P =$)	
	Low	Medium	High	E	N	RMSE	Period	Water supply
ME intake (kJ/d)	1543[a]	1417[b]	1212[b]	1467	1315	230	0·005	0·06
Water intake/excretion (mg/kJ ME)								
Drinking	13[a]	29[a]	46[b]	24	35	2	0·003	0·14
Dietary	145[a]	151[a]	165[b]	149	159	10	<0·001	0·003
Total intake	158[a]	180[a]	211[b]	173	194	2	<0·001	0·02
Faecal	25[a]	21[b]	25[ac]	25	23	4	0·02	0·25
Urinary	39[a]	33[b]	32[b]	39	30	7	0·03	<0·001
Total excretion	64[a]	53[b]	57[a]	63	53	9	0·02	0·002
Balance	96[a]	125[b]	154[c]	110	141	27	<0·001	0·002
Na (μmol/kJ ME)	1·9	1·7	1·7	2·0	1·5	0·6	0·31	0·002
K (μmol/kJ ME)	5·1	4·9	4·7	5·1	4·7	1·3	0·63	0·16

RMSE, root mean square error.
[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.

Table 4. Metabolic live weight of mink females and kits, female rectal temperature, water requirement for milk production and energy requirement of kits at low, medium or high ambient temperature (5, 15 or 20–25°)

	Ambient temperature				
	Low	Medium	High	RMSE	Effect of period ($P =$)
Females					
Metabolic weight* ($kg^{0·75}$)	1·11	1·13	1·11	0·10	0·50
Rectal temperature(°)	40·6[a]	41·4[ab]	41·7[b]	0·65	< 0·001
Water requirement for milk production (g/d)	118[a]	129[ab]	134[b]	41	0·05
Kits					
Metabolic weight* ($kg^{0·75}$)	0·112	0·146	0·170	0·03	< 0·001
ME_m (kJ/kit per d)	59[a]	77[b]	89[c]	17	< 0·001
ME(kJ/kit per d)	115[a]	144[b]	151[b]	43	< 0·001

RMSE, root mean square error; ME, metabolizable energy; ME_m, ME requirement for maintenance.
[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* Average of the live weight at the start and end of each period.

Table 5. Effect of periods with low, medium or high ambient temperature (5, 15 and 20–25°) and different water supply (N, *ad libitum* drinking water supply, or E, extra water supplementation in the food) on total 24 h urinary excretion of sodium and potassium, cortisol and catecholamines in lactating female mink

	Ambient temperature			Water supply			Effect of ($P =$)	
	Low	Medium	High	E	N	RMSE	Period	Water supply
Na (mmol)	3·1[a]	2·6[b]	2·3[b]	3·1	2·3	0·75	0·002	0·01
K (mmol)	8·3[a]	7·8[a]	6·6[b]	8·2	6·9	2·0	0·01	0·07
Cortisol (nmol)	2·44	2·64	2·47	2·54	2·50	0·65	0·46	0·92
Catecholamines (ng)	184	185	159	201	151	59·3	0·45	0·01

RMSE: Root mean square error
[a,b] Mean values within a row with unlike superscript letters were significantly different, $P < 0·05$.

nursing sickness and death (Clausen *et al.* 1992; Wamberg *et al.* 1992). Moreover, a low ME intake will decrease the dietary water intake, and hence increase the requirement for drinking water. Addition of extra water to the food could be a means of alleviating the increased demand for drinking water, but these data, similar to those of Neil (1992), failed to demonstrate any positive effects on water balance, or dam or kit performance, of extra water supplementation in the food. On the other hand, E animals showed a clear tendency to increase their ME intake, which is a positive effect as such.

The animals kept at high T_a did not show any apparent signs of distress, but the rectal temperature of the females during periods of moderate and high temperature increased significantly, which indicated that some heat stress might have occurred, since it is known from other species that animals under heat stress conditions can allow body temperature to rise, thereby saving water. This has, for instance, been demonstrated in goats (Olsson & Dahlborn, 1989; Olsson *et al.* 1995). The need for this was probably aggravated by the higher metabolic rate caused by increasing milk production, and Olsson & Dahlborn (1989) found a more pronounced increase in rectal temperature in lactating than in dry goats.

Water supply

From the results it was evident that water supply has a strong regulatory effect on water output as well as the urinary osmolality and concentration of K. Hence, extra supplementation with water in the food resulted in increased water excretion with the urine, which was correspondingly diluted. However, in contrast to the findings of Neil (1988), these results failed to demonstrate a significant increase in total water intake despite a close to significant ($P = 0·06$) increase in ME intake.

Water metabolism in relation to metabolizable energy intake and to urinary osmolality

The intake of water is normally strongly related to energy intake as shown by Farrell & Wood (1968) and Maksimov (1973), but T_a may be a stronger determinant of water intake than ME consumption. Such a trend was found in the present investigation, where the highest total water intake per kJ ME was found during the period of high T_a, in which ME consumption was lowest. The level found here agrees fairly well with those found by Farrell & Wood (1968) and Maskimov (1973). The urinary water excretion per kJ ME was affected both by water supply and T_a. This may indicate that lactating females are less able to regulate the excretion accurately than non-lactating animals, which is in agreement with the observations by Olsson & Dahlborn (1989) that pregnant and lactating goats are unable to regulate their water turnover as precisely as dry (non-lactating) animals. The urinary osmolality was below the maximum levels found for males (A-H Tauson, unpublished results) also at the high T_a. This is reasonable, owing to the reduced regulatory ability discussed earlier. In other species, pregnant and lactating animals are less able to concentrate their urine, and unpublished observations from our own laboratory indicate that urinary osmolality decreases during the last part of gestation in mink.

Water excretion in milk and estimated evaporative water loss

The data from the factorial calculations of the requirement of milk to support ME_m and ME_g for the kits must be taken with some caution since, owing to lack of data, the ME_m values had to be taken from experiments with adults (Chwalibog *et al.* 1980), and therefore probably are not correct for juveniles, and the k_p and k_f values were taken from other single-stomached species (Klein & Hoffman, 1989; Chwalibog, 1991). The calculations indicated that daily water loss in milk increased from 118 to 134 g over the experimental period; these values are in good agreement with those calculated by Tauson *et al.* (1998), or found by direct measurements of milk production by the isotope dilution technique (Wamberg & Tauson, 1998). The production of metabolic water is a contribution to the total water balance which is not usually measured experimentally, but is necessary for estimation of evaporative water loss. Tauson *et al.* (1998) based calculations of metabolic water on gas exchange measurements in respiration experiments, and found that in lactating mink metabolic water contributed 10–12 % of the total water intake. Given a similar contribution in this experiment the water available for evaporation would have been 42, 58, and 69 g water/$kg^{0·75}$ at low, medium and high T_a respectively. These values are below those of Wamberg (1994) who reported evaporative losses of about 75 and 120 g/kg over 24 h in female mink kept in a direct calorimeter at 18° and 24°

respectively, but in fair agreement with those of Sørensen (1995) who found evaporative water loss in lactating mink in the order of 60 g/female per d.

Renal solute excretion

The urinary concentration of Na was not affected by water supply, and 24 h excretion was higher for E than for N animals. This indicates that regulation of Na excretion is less accurate in lactating than in dry animals, and therefore the lactating female may be expected to be more susceptible to changes in the Na concentration of the food. The pattern for excretion of K suggested that physiological stage and environmental effects have less influence on the excretion of this electrolyte.

Urinary cortisol and catecholamines as stress indicators

Cortisol and catecholamine excretion rates in urine over 24 h were used as possible indicators of stress, since blood sampling can be stressful to the animals, and levels found in plasma, therefore, may be of limited value. From the results achieved, it appears that neither water supply nor T_a influenced cortisol excretion. It may, however, be doubted whether cortisol is specific enough to be a good measure of stress. Hence, Olsson *et al*. (1995) found that positive stress could result in plasma concentrations similar to those given by negative stress. Catecholamines were not affected by period but were affected to some extent by water supply; however, the results only represent every second animal, and only urine collected during the last day of each period, which of course limits their reliability. Therefore, the present results cannot be taken as any clear evidence regarding the degree of stress that the high T_a had inflicted on the animals.

Conclusions

A prolonged period of high T_a may be hazardous for lactating mink because of a decrease in ME intake which, if persisting over an extended time period, may lead to excessive weight loss and possibly result in nursing sickness. Therefore, if a heatwave occurs during the lactation period, all possible precautions to cool the environment and to secure the supply of high quality food and drinking water must be taken in order to sustain dam and kit welfare and performance.

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References

Amtsblatt der Europäischen Gemeinschaften L 279 (1971) *17 Official Journal of the European Communities* **L279** (1971), p. 995.

Brouwer E (1965) Report of sub-committee on constants and factors. *Proceedings of the 3rd Symposium on Energy Metabolism. European Association for Animal Production Publication* no. 11, pp. 441–443 [KL Blaxter, editor]. London: Academic Press.

Chwalibog A (1991) Energetics of animal production. Research in Copenhagen, review and suggestions. *Acta Agriculturæ Scandinavica* **41,** 147–160.

Chwalibog A, Glem-Hansen N & Thorbek G (1980) Energy metabolism in adult mink in relation to protein-energy levels and environmental temperature. *Proceedings of the 8th Symposium on Energy Metabolism. European Association for Animal Production Publication* no. 26, pp. 283–286 [LE Mount, editor]. London: Butterworths.

Clausen TN, Olesen CR, Hansen O & Wamberg S (1992) Nursing sickness in lactating mink (*Mustela vison*) I. Epidemiological and pathological observations. *Canadian Journal of Veterinary Research* **56,** 89–94.

Farrell DJ & Wood AJ (1968) The nutrition of the female mink. III. The water requirement for maintenance. *Canadian Journal of Zoology* **46,** 53–56.

Fisher RA (1935) *The Design of Experiments.* Edinburgh: Oliver and Boyd.

Hansen BK (1997) The lactating mink (*Mustela vison*) - Genetic and metabolic aspects. PhD Thesis, Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen.

Hansen BK & Berg P (1998) Mink dam weight changes during the lactation period I. Genetic and environmental effects. *Acta Agriculturæ Scandinavica, Section A, Animal Science* **48,** 49–57.

Hansen M (1974) Ny og bedre metode til påvisning af plasmacytose (New and improved method to diagnose Aleuthian disease). *Dansk Pelsdyravl* **37,** 209–211.

Hansen NE, Finne L, Skrede A & Tauson A-H (1991) Energiforsyningen hos mink og rev (The energy supply of mink and foxes). *NJF utredning/rapport* no. 63, DSR Forlag, Landbohøjskolen, Copenhagen.

Juokslahti T (editor) (1987) *Vitamins in the Nutrition of Fur Bearing Animals*, pp. 1–70. Basle: Roche A/S.

Klein M & Hoffman L (1989) Bioenergetics of protein retention. In *Protein Metabolism in Farm Animals, Evaluation, Digestion, Absorption, and Metabolism*, pp. 404–440 [HD Bock, BO Eggum, AG Low, O Simon and T Zebrowska, editors]. Berlin: Oxford Science Publications and Deutscher Landwirtschaftsverlag.

Madej A, Forsberg M & Edqvist L-E (1992) Urinary excretion of cortisol and oestrone sulfate in pregnant mink fed PCB and fractions of PCB. *Ambio* **21,** 582–585.

Maksimov AP (1973) A rational regimen of watering for mink. *Nutrition Abstracts and Reviews* **44,** 1974 Abstr.

Neil M (1988) Effects of dietary energetic composition and water content on water turnover in mink. *Swedish Journal of Agricultural Research* **18,** 135–140.

Neil M (1992) Supplementary dietary water to mink in lactation and early kit growth. *Swedish Journal of Agricultural Research* **22,** 125–129.

Nes N, Einarsson EJ & Lohi I (1987) *Beautiful Fur Animals - and their Colour Genetics.* Hillerød, Denmark: Scientifur.

Olesen CR, Clausen TN & Wamberg S (1992) Compositional changes in mink (*Mustela vison*) milk during lactation. *Norwegian Journal of Agricultural Sciences* Suppl. 9, 308–314.

Olsson K & Dahlborn K (1989) Fluid balance during heat stress in lactating goats. *Quarterly Journal of Experimental Physiology* **74,** 645–659.

Olsson K, Josäter-Hermelin M, Hossaini-Hilali J, Hydbring E & Dahlborn K (1995) Heat stress causes excessive drinking in fed and food deprived pregnant goats. *Comparative Biochemistry and Physiology* **110**A, 309–317.

Sørensen HJ (1995) Water turnover in pregnant and lactating female mink. MSc Thesis, Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen.

Statistical Analysis Systems (1985) *SAS User's Guide: Statistics*. Cary, NC: SAS Institute Inc.

Tauson A-H (1988) Varied energy concentration in mink diets. II. Effects on kit growth performance, female weight changes and water turnover in the lactation period. *Acta Agriculturæ Scandinavica* **38,** 231–242.

Tauson A-H (1994*a*) Postnatal development in mink kits. *Acta Agriculturæ Scandinavica, Section A, Animal Science* **44,** 177–184.

Tauson A-H (1994*b*) High dietary level of polyunsaturated fatty acids and varied vitamin E supplementation in the reproduction period of mink. *Journal of Animal Physiology and Animal Nutrition* **72,** 1–13.

Tauson A-H (1997) Prolactin profiles in pregnant, lactating and non-mated female mink (*Mustela vison*). *Journal of Reproduction and Fertility*, Suppl. 51, 195–201.

Tauson A-H, Sørensen HJ, Wamberg S & Chwalibog A (1998) Energy metabolism, nutrient oxidation and water turnover in the lactating mink (*Mustela vison*). *Journal of Nutrition* **128** (In the Press).

Wamberg S (1994) Rates of heat and water loss in female mink (*Mustela vison*) measured by direct calorimetry. *Comparative Biochemistry and Physiology* **107**A, 451–458.

Wamberg S, Clausen TN, Olesen CR & Hansen O (1992) Nursing sickness in lactating mink (*Mustela vison*) II. Pathophysiology and changes in body fluid composition. *Canadian Journal of Veterinary Research* **56,** 95–101.

Wamberg S & Tauson A-H (1998) Daily milk intake, body growth and body water turnover in suckling mink (*Mustela vison*) kits. *Comparative Biochemistry and Physiology A* **119**, 931–939.

Wustenberg W & Wustenberg M (1988) Reducing heat stress in mink production units: basic principles of environmental control. In *Biology, Pathology and Genetics of Fur Bearing Animals. Proceedings of the 4th International Scientific Congress in Fur Animal Production*, pp. 130–135 [BD Murphy and DB Hunter, editors]. Toronto, ON: International Fur Animal Science Association.