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Water Intake and Fluid Regulation in the Horse

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Abstract

The aim of this thesis was to study factors affecting water intake and fluid regulation in the horse, both at rest and in connection with exercise. Exercising horses sometimes show little inclination to drink despite extensive fluid losses and free access to drinking water. The results from this thesis confirm that a lack of an osmotic thirst stimulus (i.e. increase in plasma sodium concentration) reduces the post-exercise voluntary water intake in the horse. The water supply method had a great influence on the daily water intake. The daily intake was about 40% higher when horses were drinking from buckets than from an automatic water bowl (float valve, 3 L/min). In a two-choice preference test all horses showed a strong preference for buckets compared to an automatic bowl (pressure valve, 8 L/min). Different strategies for voluntary rehydration during endurance exercise were tested in a field trial. It was found that horses offered a saline solution to drink (9g NaCl/L) had the highest fluid intake and regained 84% of their body weight losses (<3h post-exercise) compared to 46% in the horses drinking only water. To give concentrated salt paste (NaCl) did not increase water intake and since there were signs of an altered fluid distribution between body compartments, this strategy cannot be recommended. This thesis shows that it takes several days to fully regain fluid balance after an exercise-induced fluid loss (10-15 kg). When horses were supplemented with 10 L of saline solution, fluid balance was regained already on the exercise day. Plasma aldosterone increased during the recovery phase in all horses with fluid losses above 10 kg which were not supplemented with NaCl in connection with exercise. The exercise-induced changes in plasma concentrations of atrial natriuretic peptide and arginine-vasopressin were influenced by hydration status which suggest that both hormones are involved in blood flow control during exercise.

Key words: aldosterone, arginine-vasopressin, atrial natriuretic peptide, dehydration, electrolytes, exercise, faeces, fluid balance, fluid regulation, horse, hyperhydration, plasma osmolality, plasma sodium, sweat, thirst, urine, water intake, water source

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“ A man may well bring a horse to the water,
But he cannot make him drinke without he will.”

Contents

Abbreviations

ANP - atrial natriuretic peptide
AVP - arginine-vasopressin
BW - body weight
Cl - chloride
CONST- constant velocity exercise test (40 min, no incline, 65-70% HR_{max})
DM - dry matter
DEH - dehydration trial, no access to drinking water for 24 h
ECF - extracellular fluid
HH - hyperhydration trial, 12 L of water was given via a naso-gastric tube
ICF - intracellular fluid
INCR - incremental exercise test (6-9 m/s, 6.25% incline)
K - potassium
N - normohydration trial
Na - sodium
PAC - plasma aldosterone concentration
PCV - packed cell volume
pK - plasma potassium concentration
pNa - plasma sodium concentration
pOSM - plasma osmolality
PV_{calc} - calculated plasma volume (formula see *Materials and methods*)
TPP - total plasma protein concentration

Appendix

Papers I-VI

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Nyman S., Jansson A., Lindholm A., and K. Dahlborn (2000) Fluid shifts and water intake in horses - effects of hydration status during two exercise tests. Submitted.

II. Nyman S., Kokkonen U.M. and K. Dahlborn (1998) Changes in the plasma concentrations of atrial natriuretic peptide in the exercising horse in relation to hydration status and exercise intensity. Am. J. Vet. Res., 59, 489-494.

III. Nyman S., Hydbring E., and K. Dahlborn (1996) Is vasopressin a "stress hormone" in the horse? Pferdeheilkunde, 12, 419-422.

IV. Nyman S., Jansson A., Dahlborn K., and A. Lindholm (1996) Strategies for voluntary rehydration in horses during endurance exercise. Equine vet. J., Suppl. 22, 99-106.

V. Dahlborn K., Nyman S., and A. Jansson Exercise-induced changes in water, sodium, and potassium balances in the horse - effects of ambient temperature and saline loading. In manuscript.

VI. Nyman S. and K. Dahlborn (2000) Effect of water supply method and flow rate on drinking behavior and fluid balance in horses
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Introduction

Background

The primary use of horses has changed with time. The number of horses in Sweden is not known, but figures varying between 200 000 and 300 000 are mentioned. Most of these horses are used for competition or pleasure purposes.

Feral horses spend most of their time grazing and their alimentary tract is adapted to a regular flow of digesta (Clarke et al. 1990; Feist & McCullough 1976). These horses range over wide areas every day, but only occasionally, they perform high intensity physical activities, for example to avoid threats from predators (Feist & McCullough 1976). In the way most stabled horses are kept today, they often have difficulties in performing their natural behaviour. For example, they are fed a concentrated diet a few times a day, they experience large and regular sweat losses from exercising all year around and are often changing environment when transported to racing grounds or other competitions. It is quite common that horses during these stressful situations refuse to drink or at least reduce their voluntary water intake. Since drinking for a feral horse is a time of increased risk, as predators might wait for them at the watering sites, one reason for this behaviour might be evolutionary. The fact that horse sweat is iso- or hypertonic to plasma (McCutcheon et al. 1995) and sweat losses thereby do not always stimulate thirst by an increased plasma sodium concentration (pNa), can be another reason for the inefficient rehydration sometimes seen in connection with exercise (Carlson 1979). Therefore both psychological and physiological factors can be involved in this behaviour. Loss of appetite is one of the first signs to notice if a horse does not drink enough water, but the underlying cause for this is not always understood by the trainer/rider.

The physical demand on different categories of horses is more diverse today than when horses were used for farming or transportation. At the same time, the physiology of the horse has not changed as much as the demands we put on them. Therefore, to avoid clinical problems, as well as welfare-problems, the handling of the horse in connection with training and racing is very important and should be adjusted to the individual horse and the specific purpose it is used for. As an example, endurance rides (25-160 km/day) are getting more and more popular. This is a form of event, which challenges fluid balance of the horse in an obvious way and has also led to a greater interest in this area of research. For the racehorse of all disciplines, there is a very small difference between being a winner or not being placed at all. Therefore, for the racing industry there is an economical interest in improving management procedures. Finally, last but not the least, for the horse and its owner an increased knowledge about factors that can improve the welfare of the horse is always of great interest.

How do we offer drinking water to horses?

Many handbooks on horse-management give detailed advice on how to water horses (Hinton 1978). Although, there are very few scientific data on the effect of water supply method on water intake in horses. The water content of fresh grass is about 80%, thus horses on pasture get proportionally more water from the feed, than when they are stabled and fed hay or other dry feed stuffs (10-15% water) (Cymbaluk 1989; Scheibe et al. 1998). For the stabled horse drinking water is therefore the most important fluid source, but the way these horses are offered water varies (Hinton 1978).

Water can be offered continuously from a bucket, water trough or from an automatic watering system. Sometimes, horses are only offered water intermittently, for example when they are fed (Freeman et al. 1999). Water can be withheld for many reasons, and as an example, one trainer motivated the restricted water supply in the stable with the fact that it was easier to muck out if less urine was produced (Nyman, unpublished observation). The state of hydration of a horse prior to exercise can sometimes be altered deliberately by the trainer or owner, for example by restricting feed or water before exercise. This is done in the belief that it will enhance performance capacity by reducing the gastrointestinal fill and thereby the weight to be carried. The management of the horse prior to exercise (transportation, change of environment, changer of water supply mode) can sometimes unwittingly affect hydration status by a reduced voluntary water intake. On the other hand, it is well documented from papers on human athletes that dehydration can impair performance capacity (Saltin 1964; Walsh et al. 1994). In the horse, dehydration (3% BW) was found to reduce cardiac output and increase core temperature during prolonged, low-intensity exercise (Geor & McCutcheon 1998). Similar investigations have not been made in the horse during more intense exercise.

It is often recommended in different handbooks to restrict water intake post-exercise since they are concerned that clinical problems, such as colic and laminitis, could otherwise develop (Hinton 1978). However, there are no scientific data to support these suggested consequences of horses drinking large volumes of water after exercise.

Regulation of fluid balance

Fluid balance

The total water content of the horse is about 60-70% of body weight (BW) (Carlson 1987; Julian et al. 1956). The major part of this fluid (~2/3) is located within the cells and is called the intra-cellular fluid (ICF) volume. The rest is the extra-cellular fluid (ECF) volume, which can be divided into the intra-vascular fluid (~1/4 of ECF), the interstitial fluid, and the lymph. In the horse, the amount of fluid in the gastrointestinal tract can be substantial. In ponies a fluid content of 143 ml/kg BW was found (Meyer et al. 1992) which corresponds to around 70 L in a 500 kg horse. The daily volume of water entering the large intestine in the horse is approximately equal to the total extra-cellular fluid volume of the animal, of which 95% is reabsorbed (Argenzio et al. 1974).

The maintenance of body water content is paramount to physiological wellbeing, since water is involved in almost all chemical processes in the body and therefore necessary for life. Dehydration of more than 15-25% of BW is life threatening, but long before that occurs, different clinical signs will develop. Water is continuously lost from the body by faecal and urinary routes as well as by cutaneous and respiratory evaporation (insensible losses). In the lactating mare, milk production can cause daily fluid losses corresponding to 2-3.5% of BW (Bouwman & van der Schee 1978). These ongoing losses need to be compensated for by intake from feed (including metabolic water) and drinking water. The definition of water balance is when water losses and intake matches and the body weight is maintained. Body fluid homeostasis is normally regulated within narrow limits by secretion of the anti-diuretic hormone, arginine-vasopressin (AVP), regulating water absorption in the kidneys and by the thirst mechanism initiating drinking.

Arginine-vasopressin (AVP) and thirst

AVP is a peptide hormone first isolated and identified in pituitary neural lobe extracts from cattle by du Vigneaud *et al.* (1953) and a few years later also in the horse (Acher et al. 1958). AVP is synthesised in the cell bodies of the supraoptic and paraventricular neurons and transported to nerve fibre endings in the posterior lobe of the pituitary. It is stored in secretory granula and is secreted in response to both osmotic (i.e. increased plasma sodium concentration, pNa) and hypovolemic stimulation. The effect of the hormone is anti-diuretic and at very high plasma levels it also acts as a vasoconstrictor and thereby increases blood pressure (Oliver & Schafer 1895; Schwartz & Reid 1981). The antidiuretic effect is achieved by an increased permeability for water of the distal parts of the renal tubules and collecting ducts and thereby more concentrated urine is formed. AVP has also been found to be involved in regulation of adrenocorticotropic hormone (ACTH) in horses and thereby in their response to stress (Alexander et al. 1996)

When water is freely available, most mammals satisfy the need for water by anticipated, habitual, or prandial drinking (Fitzimons 1979). Thirst is, like AVP release, stimulated both by an increase in plasma osmolality and hypovolemia and the neuronal system that controls thirst is located in the hypothalamic region of the brain (Andersson 1971, 1978). In water deprivation, cells in this area, which are sensitive to changes in osmo-concentration, shrink and thereby a sense of thirst is evoked. Hypovolemia can also stimulate thirst, both directly via baroreceptors situated in the large vessels and atria, and in response to angiotensin II (via renin release from the kidneys), but the hypovolemic stimulation is less sensitive than the osmotic. Cessation of drinking before any significant absorption of water has taken place, has been attributed to regulatory mechanisms in the pharynx or the upper gastrointestinal tract in other animal species than the horse (Maddison et al. 1977; Towbin 1949). Although, final satiety of thirst must await the restoration of body fluid to normal (Fitzimons 1979).

Hormonal regulation of fluid balance and the role of sodium

Sodium is the major cation in the extracellular fluid (ECF). The sodium content of the ECF is therefore of crucial importance for maintaining its volume. A steroid hormone, aldosterone, acts to conserve body sodium content. Its release from the adrenal cortex is induced by an increase in plasma potassium concentration (pK), increased plasma renin followed by the subsequent hormonal cascade in the renin-angiotensin-aldosterone-system (RAAS), as well as a decrease in pNa. Aldosterone regulates reabsorption of sodium in the kidney and absorption of sodium from the alimentary tract. In contrast to humans, aldosterone does not seem to have an effect on the sweat composition (i.e. reducing sodium concentration) when given intravenously to horses (Jansson 1999).

Atrial natriuretic peptide (ANP) was first recognised by de Bold in the early 1980s (de Bold et al. 1981). This hormone is stored in vesicles within the walls of the atria and is released in response to an increased atrial stretch or pressure. The effect generated by ANP is to lower blood pressure by natriuresis (at very high plasma levels), cause a rapid vasodilation and an increased transfer of fluid out of the intravascular space (de Bold 1985).

Water is freely diffusible over most biological membranes. The total body content of water and electrolytes (mainly sodium) affects the relation between the body fluid compartments. After water deprivation both ECF and ICF volume will be reduced. If total body water content increases, for example during hyperhydration, ECF will be diluted, AVP and thirst will be inhibited and the urine production increased. Hypernatremia, inhibits aldosterone secretion, stimulates AVP secretion and increases water intake, after which the extra sodium is excreted by the kidneys. Depending on the severity of hypernatremia and availability of drinking water, fluid will be drawn from the ICF and cause cellular dehydration. On the other hand, a lowering of the extra-cellular sodium content will draw water into the ICF from the ECF. This will cause a reduction in plasma volume without changes in pNa. Thus, sodium depletion in the horse cannot be estimated from measuring only pNa. However, a recent study on athletic horses showed that the relation between faecal concentrations of sodium and potassium, as well as measuring plasma aldosterone concentration (PAC) during the night and early morning could be useful tools (Jansson 1999).

Fluid balance-effects of exercise

When a horse performs short, intensive exercise the evaporative losses during exercise are seldom very great due to the short duration. However, if all fluid losses are taken into account, for example during transportation, anticipatory sweating and post-exercise losses, the total loss can be substantial. A high ambient temperature will further increase these fluid losses (Jansson et al. 1995). In contrast to human sweat, horse sweat is hyper- or isotonic compared to plasma and the sodium- (Na), potassium- (K), and chloride (Cl) concentrations are about 140, 30, and 150 mmol/L, respectively (McCutcheon et al. 1995).

During endurance exercise 10-15 L of fluid can be lost every hour (~2-3% BW in a 500 kg horse) (Carlson 1987). If these losses are not replaced the horse will soon develop clinical signs of dehydration (loss of gastro-intestinal sounds, a soon develop clinical signs of dehydration (loss of gastro-intestinal sounds, a high, persistent heart rate (HR), prolonged capillary refill time) and electrolyte imbalances (Carlson 1975). Despite severe dehydration and free access to drinking water these horses sometimes show little inclination to drink and Carlson (1979) suggested that this voluntary dehydration could be caused by an ineffective thirst stimulus due to a lack of an increase in plasma osmolality.

During intensive exercise heat production can increase 40 to 60-fold over the basal level (Carlson 1983), since about 80% of the energy required is converted into heat (Brody 1945). Although this does not pose a problem during exercise of short duration, it can represent a major threat during longer events and endurance exercise. If dehydrated horses were allowed to continue to exercise, severe clinical problems and heat stress (depending on ambient temperature and humidity) would soon develop, since hyperthermia and dehydration have an additive effect (Geor & McCutcheon 1998). This is due to the fact that when blood volume is reduced, there may be difficulty in meeting the requirement for a high blood flow to both muscles and body surface and thereby impair heat dissipation.

Hormonal regulation during exercise and recovery

Exercise poses a great challenge on both the cardiovascular system and fluid balance regulation. Hormones involved in the regulation of blood pressure, plasma volume, and fluid balance, such as AVP, angiotensin II, and ANP are all released in the horse during exercise (McKeever & Hinchcliff 1995). The first two hormones named can have a hypertensive effect since they cause vasoconstriction (AVP only at very high plasma levels), and ANP has a hypotensive effect. However, it is not fully understood how these hormones interact during exercise and whether they are affected mostly by state of hydration or exercise intensity.

The body's main sodium-saving hormone, aldosterone is also released during exercise (Guthrie et al. 1982). It has been debated whether a decreased pK, angiotensin II (RAAS), a decrease in pNa or a combination of these factors are responsible for its release in the horse during physical activity.

External factors affecting voluntary water intake in horses

Ambient temperature

The end products of aerobic energy metabolism are CO_2, H_2O, and heat. Homeothermy requires that heat produced or gained from the environment equals heat loss to the environment. Heat loss from the body surface is achieved by radiation, convection, conduction, and evaporation. At high ambient temperatures, the only mechanism by which heat can be lost from the body is through evaporation. Evaporation increases the daily fluid loss from the body either as water through insensible losses or as both water and electrolytes in sweat. The daily insensible water losses in a horse in a thermoneutral environment fed the maintenance requirement has been estimated to 16 ml/kg BW (Tasker 1967).

In free-ranging horses, a high ambient temperature increased the frequency but not the duration of drinking bouts (Crowell-Davis et al. 1985). The correlation between high ambient temperature and water intake was less clear in a study investigating water consumption of Prezwalski horses (*Equus ferus przewalski*) in a semireserve (Scheibe et al. 1998). The probable explanation for this was that even if the highest water intake was registered during warm periods, the forage had a higher dry matter content in the winter, which confused the interpretation of the data.

Diet and feeding regimen

Water intake has been correlated to dry matter (DM) intake in studies performed on stabled horses. In 1968, Fonnesbeck found a water to feed ratio (L/kg DM) of 3.6:1 for horses fed an all roughage diet and 2.9:1 for horses on a hay-grain diet. In a retrospective study of horses fed various diets a similar relation was found between water and DM, 3.2:1 and 2.0:1, with a hay or all pelleted diet, respectively (Cymbaluk 1989). In free ranging Przewalski horses, there was a tendency but not a significant correlation between water intake and both DM intake and ambient temperature (see above).

Sedentary ponies were found to drink most (89%) of their daily water periprandially (defined as 10 min before to 30 min after feeding) (Sufit et al. 1985). When sedentary horses were fed twice a day, 60-80% of the daily water was consumed within 3 h after feeding (Heilemann 1985). In the same study it was found that when the horses were fed moistured mixed feed, their daily fluid intake decreased for more than the volume added to the feed (water to feed ratio decreased from 3.3 to 1.8 L/kg DM). In athletic horses kept under training conditions feeding regimen (2 vs. 6 times/day) did not influence daily water intake, although water intake was more evenly distributed when the horses were fed 6 times/day (Jansson & Dahlborn 1999). A positive correlation between sodium and water intake was also found in these horses (Jansson & Dahlborn 1999). On the contrary, no correlation was found between sodium and water intake in sedentary ponies (Schryver et al. 1987).

Water availability

Drinking frequency of feral horses' ranges from several times a day to once every second day (Feist & McCullough 1976). In situations where there is a long distance between grazing areas and watering sites, large volumes of water can be drank on one occasion. A positive correlation between water availability and water intake has been found in grazing dairy cattle (Castle & Watson 1973). It is not known whether this is the case for the stabled horse. In an investigation of sedentary brood mares fed grass hay and oats (ambient temperature 5-10°C), no significant difference in water intake between intermittent and continuous water delivery systems were seen (Freeman et al. 1999). However, since all horses in this study had a low urine volume (11-14% of water intake) irrespective of water supply mode, it can not be excluded that even the continuous supply (water depth 2.5-5 cm, float control) might have restricted water intake. According to Swedish legislation, water to animals should be offered at least twice daily and free access is recommended (LSFS 1989:20, §28).

Water quality

Water taste has been found to affect water intake in horses transported to a new environment (Mars et al. 1992). The water smell and taste can differ due to naturally occurring compounds, like iron and sulphur, or to its content of actinomycetes (Nyman 1994). Copper originating from the water pipes can give the water a bitter taste, especially in areas with soft and acid water. Chlorine is often used for purification of water, especially in urban areas. Water with a high chlorine content gets a very distinct smell and is sometimes avoided by horses (Nyman, 1994).

Another quality parameter, water temperature, affected water intake in horses during cold weather conditions, where the warmer water was preferred (Kristula & McDonnell 1994). In contrast, no preference for water temperature was seen during hot summer weather (+15-29°C) (McDonnell & Kristula 1996).

In 1992 a survey was carried out in ten Swedish training stables where water quality was tested every month and both microbiological and chemical analyses were carried out (Nyman 1994). The most common cause of water quality problems was that the water bowls and troughs were not properly cleaned. It was also found that some stables had varying water quality due to leaking pipes in their water supplying system. In one stable waste water had polluted the ground water and in this case the well could not be used for a long time period. The tests used for evaluating water quality, are a risk assessment and when quality problems are suspected repeated testing are needed. There are, today, no threshold values for water quality parameters for horses, but according to Swedish legislation drinking water should not be suspected to be unwholesome or in any way harmful to an animal (SFS 1985:295, §3).

Which is the optimal fluid replacement strategy in connection with exercise?

Voluntary intake of water and electrolytes

Whether it is possible to rely on voluntary intake of water and electrolyte solutions or if electrolytes have to be administered to the endurance horse as a paste has long been debated (Carlson 1975; Dusterdieck et al. 1999; Lindinger & Ecker 1995; Schott II et al. 1997). When fluid is lost by passive evaporation, only water is lost. This will result in a hypovolemia with an increased plasma osmolality (i.e. plasma sodium). Sedentary water deprived ponies (19 h) showed an increase in plasma osmolality (pOSM) of 3% and in total plasma protein concentration (TPP) of 6% (Sufit et al. 1985). When drinking water was offered again they compensated for the water deficit by drinking an amount equal to, or greater, than they would have consumed over the same time period if water had been freely available. If active heat dissipation by sweating is needed sodium, among other electrolytes, is lost and in the horse sweat losses can result in dehydration and hypovolemia without a change in plasma electrolyte concentration. In the study by Sufit *et al.* (1985), hypovolemia without an increase in pOSM achieved by furosemide treatment (2 mg/kg), were less effective in stimulating thirst and only about 60% of the fluid losses were replenished by voluntary drinking. This illustrates that there is a risk that thirst is less effectively stimulated when only a hypovolemic stimulus is present. Thus, exercise-induced fluid losses caused by a high sweat rate differ from losses caused by water deprivation. If these losses are replaced by drinking only water, an uptake will occur and thus the plasma will be diluted. This will reduce arginine-vasopressin levels, and the urine production will increase. Simultaneous replacement of electrolytes losses is therefore important for a successful post-exercise rehydration (Lindinger & Ecker 1995). However, by which electrolytes and how this supplementation is best carried out is still controversial.

Administration via a naso-gastric tube?

Several investigations on the effect of administering fluid to horses via a nasogastric tube prior to, during or after exercise have been made (Jansson et al. 1995b), (Sosa-León et al. 1995a), (Sosa-León et al. 1995b), (Sosa-León et al. 1996), (Geor & McCutcheon 1998), (Marlin et al. 1998a), (Marlin et al. 1998b). In one experimental setting, the horses were not exercised, but a pre-trial fluid deficit was induced by giving 1 mg/kg BW of furosemide i.m. (Sosa-León et al. 1995b). These authors found that fluid tonicity had a major effect on the uptake and elimination of fluids, but fluid temperature (5, 21, or 37 °C) did not affect absorption or elimination. Inclusion of glucose, which has been shown to be beneficial for fluid uptake in humans (Wapnir & Lifshitz 1985), did not enhance absorption in these horses (Sosa-León et al. 1995b). The timing and amount of fluid, electrolyte content, exercise intensity and duration, as well as ambient conditions vary in the experiments cited above and the different results are discussed under results and comments.

Aims of the thesis

The general aim of the thesis was to increase the knowledge about factors affecting voluntary water intake and fluid regulation in the horse, in the hope of improving management procedures and the welfare of the horse.

- How does a change in hydration status prior to exercise (of different intensities and durations) affect plasma sodium concentration, plasma osmolality and total plasma protein concentration and will these changes affect the post-exercise water intake in horses?
- Does the water supply method affect the daily water intake, drinking behaviour, and fluid balance?
- Will supplementation with salt paste (NaCl) or saline affect the voluntary fluid intake and restoration of fluid losses in horses performing endurance exercise?
- How are exercise-induced fluid losses restored in horses and is supplementation with saline (0.9%) beneficial for the recovery rate?
- Are plasma levels of atrial natriuretic peptide (ANP), arginine-vasopressin (AVP) or aldosterone affected by hydration status and/or type of exercise?

Materials and methods

Most methods are described or referred to in each paper but some details of general interest are discussed below.

Animals and experimental design

In all papers, except for paper IV, Standardbred horses were used. In paper IV ten out of thirteen horses were Arabians, two were Arabian crossbreeds, and one a Standardbred. A relatively low number of horses (n=4) were used in paper I, II, III, and V, but to compensate for that the horses were used as their own controls. We would also like to quote a citation from Alexander and co-workers (1996) "The horses vary in age, size, previous management" ..."when such a disparate group shows a significant response it is likely to represent a basic operating principle" (Alexander et al. 1996). In papers I, II, IV, and V some individual values are presented and discussed.

Exercise

In all papers, except for paper VI, all horses were undertaking regular exercise. In paper VI, the horses were instead let out in sand paddocks for three hours per day. The advantage of studying sedentary horses in this case was that variable exercise-induced fluid losses would not affect the interpretations of the results.

In papers I, II, III, and V all horses performed exercise of different intensity and duration on a treadmill (Sato Treadmill, Uppsala, Sweden). The following exercise tests were used; in papers I and II a constant velocity test (CONST, 40 min, no incline, heart rate (HR) ~165 beats/min), in papers I and III an incremental exercise test (10 min, 6-9 m/s, 6.25% incline), in paper II an intense constant velocity test (12 min, HR ~200, 6.25% incline), and in paper V a simulated warm-up phase (23.5 min, 2.5% incline, mainly submaximal) and a race-phase (26 min, 2.5% incline, HR ~205 beats/min) for a Standardbred trotter. In paper IV the horses performed a simulated endurance ride (62 km, speed ~3.9 m/s) on roads and tracks. To standardise the exercise performed in paper IV as far as possible between treatment groups; the horses were divided into four start groups where each treatment was represented.

How to measure fluid balance

In papers V and VI fluid balance was measured as intake (drinking water + water content in the feed) versus output (faecal water + 0.9 times urinary output). The insensible losses (evaporation from skin and lungs) were not measured, but are represented by the net figure (net = intake - output). The water arising from metabolic oxidation was estimated in papers V and VI, by calculation of water derived from oxidation of fat (1.1 ml/g), carbohydrate (0.6 ml/g), and protein (0.4 ml/g). During exercise, the evaporative losses were measured as the body weight (BW) loss, which was corrected for faecal losses in paper V. To use uncorrected BW loss can somewhat overestimate evaporative losses. Since the evaporative losses includes both sweat and respiratory water losses, 2/3 of the BW loss in paper IV and 0.7 of the corrected BW loss in paper V were accounted for as sweat losses (Hodgson et al. 1993).

Collection of urine and faeces

In papers V and VI a total collection of urine and faeces was made. A collection harness was used which allowed the horses to stay in their ordinary boxes, earlier described in Jansson (1999). The horses were accustomed to the harnesses before the experiments began and readily accepted them. This collection method was preferred to metabolism stalls, as the horses could move around freely. However, it demands frequent emptying to ensure that urine is not spilled in the case the horses would lie down.

Effect of feeding

Since feeding influences fluid regulation by the production of salivary and gastrointestinal juices, and can during some circumstances activate RAAS (Clarke et al. 1988), feeding was controlled during the experiments. In papers I, II, and III no feed was offered during the exercise trials. In paper IV, no feed was allowed until 1 h post-ride when all horses were given 2 kg of grass hay. In paper V, no feed was given until 2 h post-exercise when 1/3 of the concentrates and 2.8-3.5 kg of hay was given, depending on the BW of the horse. In paper VI blood was sampled every hour (except for 01.00, 03.00, and 05.00 h) during each treatment and effects of feeding on TPP, pNa, and pOSM were registered.

Plasma volume changes

In contrast to humans, packed cell volume (PCV), haemoglobin or red cell count can not be used in horses to calculate changes in plasma volume during exercise, as horses mobilise their splenic reservoir of red blood cells at the onset of exercise (Persson 1967). Instead, dye dilution techniques are commonly used when determining plasma volume in exercising horses. Both Evan's blue (T-1824) and indocyanine green (ICG), which both are bound to plasma protein (principally albumin) have been used for this purpose (Parry et al. 1989; Persson 1967). However, neither of these dye dilution techniques are suited for evaluating plasma volume changes during a dynamic process like exercise. Evan's blue has a too long half-life and the half-life of ICG is short but reported to be highly variable, 3.1 to 6.9 min, (Parry et al. 1989) and 5.4 to 13.8 min (Engelking et al. 1985).

During exercise a temporary move of protein-poor fluid out of the intravascular space, caused by the increase in hydrostatic pressure is seen (McKeever et al. 1993b). In this thesis we have therefore used total plasma protein concentration (TPP) as an indicator of relative plasma volume changes. This is only correct if one assumes that no major changes in TPP content are seen during exercise or recovery. However, an addition of proteins to plasma during exercise by an increased lymphatic protein return has been suggested during certain circumstances in exercising human subjects (Senay 1970). Also the contrary, a small net loss of plasma proteins has sometimes been found in human subjects performing intense exercise, but in other similar experimental settings no such loss was seen (Harrison 1985). However, for one of the aims of this thesis, to compare relative changes in plasma water after different exercise intensities and states of hydration in the same individuals, TPP is a useful indicator.

In paper I the following formula modified from van Beaumont *et al.* (1972) was used to calculate percent changes in plasma volume (PV_{calc}):

$$PV_{calc} = -[(TPP_{time} - TPP_{pre\text{-}treatment}) / TPP_{time}] \times PV_1$$

where the pre-treatment plasma volume (PV_1) is set to 100%.

The correlation between PV_{calc} and TPP is y = -1.09x+0.36, where x = PV_{calc} and y= TPP, $R^2 = 0.99$. To compare relative changes in TPP from papers II, III, IV, V and VI with PV_{calc} in paper I, this formula is used.
The fluid shifts from the intra-vascular space were estimated by comparing relative changes in TPP, pOSM, and pNa (Fig 3-5).

Water supply methods and drinking behaviour

It was found in paper I that the horses drank 45% more (ml/kg BW) from buckets than from an automatic water bowl (FV3, float valve, 3 L/min), therefore only buckets were used in papers II to V. In paper VI buckets, FV3 and an automatic bowl fitted with a pressure valve were used. All water supplying devices used in the thesis are shown in Fig 1 a-c.

To measure water consumption from the buckets, graded ones were used. The accuracy was ±0.5 L. The evaporative losses from the buckets were negligible. The buckets were secured to the wall by a steel device and could not be tipped (Fig 2c). Consequently, since none of the horses in the experiment practised hay-soaking, spillage was not a problem.

In paper VI videotape-recordings were made for later analysis of the horses drinking behaviour. For safety reasons (horses are explorative animals) the cameras were placed on the upper part of the box wall and from that angle it was possible to get a clear view of the horse when drinking but not always to count their swallows (Fig 1d).

Choice experiments, as the preference test in paper VI, can together with comparative studies help to answer questions raised such as whether different water supply methods will affect water intake in the horse (Houpt 1991). One of the weaknesses of choice experiments was avoided in paper VI by alternating the left-right placement of the water devices. Another possible weakness was avoided by accustoming all horses to the devices used, since animals might be reluctant to choose an item or food, of which it has no previous experience (Houpt 1991).

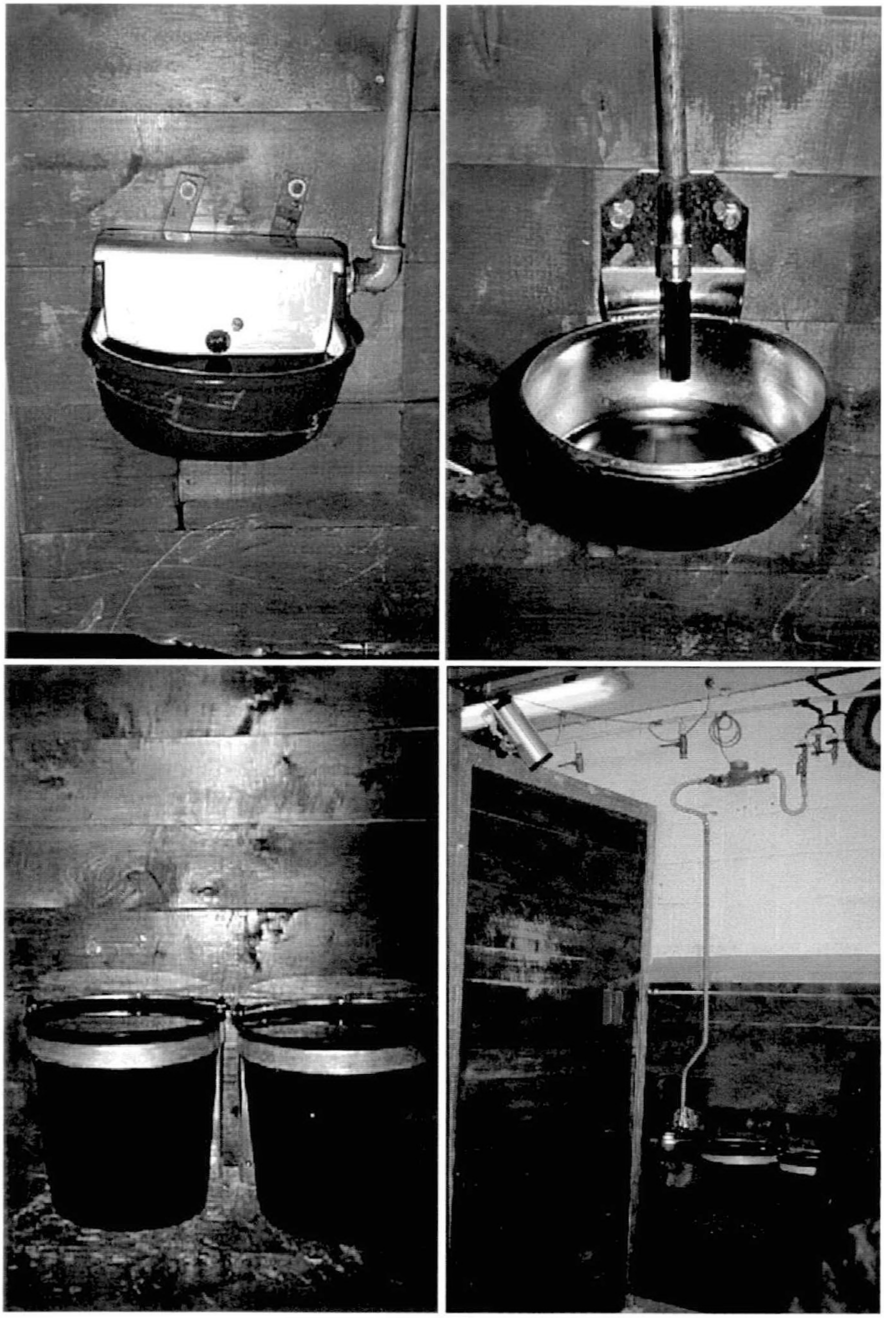


Fig 1. Automatic water bowl fitted with a float valve, minute flow 3 L (FV3, top left), automatic water bowl fitted with a pressure valve, minute flow 8 L (PV8, top right), c) buckets, 2x20 L (B, bottom left), and the placement of the videocamera in paper VI (bottom right).

Use of a naso-gastric tube

Water or saline was administered to the horses via a nasogastric tube in papers I, II, III, and V. This method was chosen to ensure that the same amount of fluid was given to each horse every time. With one exception the horses accepted the amount of fluid given, 10 or 12 L, without any signs of discomfort. The only adverse reaction seen in paper I (1 occasion out of 24) might have been caused by an unpleasant distension of the gut, since the horses were not fasted or thirsted beforehand. It could also have been a stress reaction induced by tubing. To evaluate if tubing can induce a stress response, paper III was performed and data from the same experiment concerning the effect of tubing and type of restraint are also found in Hydbring *et al* (1996).

Results and comments

Factors affecting daily water intake (Papers I, V, and VI)

Effects of water supply method and dry matter intake

The daily water intake ranged in paper I from 25-55 ml/kg BW, in paper V from 43-69 ml/kg BW, and in paper VI from 30-66 ml/kg BW. The individual daily water intake/kg BW in papers I, V, and VI is presented in Table 1.

Table 1. Mean (±SE) individual daily water intake (ml/kg BW) in a total of 14 horses. In paper I (horse A-D, 16 days in each treatment), in paper V (horse E-H, 5 days in each treatment, saline solution included), and in paper VI (horse I-N, 6 days in each treatment).

	Paper I			Paper V (B)				Paper VI		
	B	FV3		20°C	35°C	35°C+F		B	PV8	FV3
A	43±1	27±1*	E	43±4	52±3	51±7	I	52±2	57±1	38±2*
B	55±2	46±3*	F	64±2	64±3	63±3	J	46±2	51±2	39±1*
C	42±1	25±1*	G	59±3	66±3	69±3	K	50±3	49±3	36±3*
D	53±2	37±1*	H	51±1	50±1	50±1	L	42±3	43±1	35±2*
	-	-		-	-	-	M	55±2	41±3*	30±1*
	-	-		-	-	-	N	66±3	52±3*	46±2*
M	**48±1**	**33±1***		**54±2**	**58±2**	**58±3**		**52±2**	**49±1**	**37±1***

*PV8= an automatic water bowl fitted with a pressure valve and a minute flow of 8 L. FV3= an automatic water bowl fitted with a float valve and a minute flow of 3 L. B=buckets (2x20 L, refilled twice daily). 20 °C, and 35 °C, refers to ambient temperature during exercise and +F to pre-exercise saline loading (for details see paper V). * Significantly different from B within paper (P<0.05).*

The horses in paper I were exercised twice a week (exercise-induced fluid loss/week ~10 kg during the pre-trial period), horses in paper V were exercised once a week (exercise-induced fluid loss/week ~10-15 kg), and horses in paper VI were not exercised.

When mean daily water intake from B and FV3 were compared the water intake per kg BW was 45% and 41% higher from B, in papers I and VI, respectively. In paper VI it was found that the mean daily water intake from an automatic bowl fitted with a pressure valve (8 L/min) was not significantly different from the intake from buckets.

In paper I the dry matter (DM) of the daily feed intake was 8.4 kg (35% concentrates), in paper V it was 8.7-10.6 kg (32% concentrates), and in paper VI it was 7.6-8.1 kg (6-11% concentrates). The water intake (L) to feed DM (kg) ratio varied between 1.4 and 2.9 to 1 in paper I, between 2.2 and 3.5 to 1 in paper V, and between 2.0 and 3.7 to 1 in paper VI. The individual ratio is summarised in Table 2.

Table 2. Individual water to feed ratio (L/kg DM) in a total of 14 horses. In paper I (horse A-D, 16 days in each treatment), in paper V (horse E-H, 5 days in each treatment, saline solution included), and in paper VI (horse I-N, 6 days in each treatment).

	Paper I			Paper V (B)				Paper VI		
	B	FV3		20°C	35°C	35°C+F		B	PV8	FV3
A	2.4	1.5	E	2.2	2.6	2.6	I	3.5	3.7	2.5
B	3.3	2.9	F	3.2	3.2	3.2	J	2.9	3.2	2.5
C	2.5	1.4	G	3.0	3.4	3.5	K	3.1	3.0	2.1
D	3.2	2.3	H	2.6	2.6	2.5	L	3.0	3.1	2.5
	-	-		-	-	-	M	3.7	2.8	2.0
	-	-		-	-	-	N	3.7	2.9	2.5
M	**2.8**	**2.0**		**2.8**	**2.9**	**2.9**		**3.3**	**3.1**	**2.3**

For explanations of symbols see Table 1.

Comments: It is clear from our results that there was a considerable variation in daily water intake between individuals, even if the ambient conditions were the same within each paper. This finding is supported by earlier studies of stabled (Tasker 1967) and free-ranging horses (Scheibe et al. 1998).

The water supply method had a greater influence on daily water intake than dry matter intake. The intake from FV3 was lower than from B in all 10 horses tested (Paper I and VI). The mean daily intake from PV8 did not differ significantly from B, although in two out of six individuals, the intake from B was higher than from both FV3 and PV8. The composition of the diet (hay vs. concentrate) might explain some of the difference in water intake between studies, as the intake per kg dry matter was highest in paper VI where a higher proportion of roughage was given. This is in accordance with previous investigations of the correlation between water intake and diet composition (Cymbaluk 1989; Fonnesbeck 1968). The effect of a weekly exercise loss of fluid of between 10 and 15 L corresponds to a calculated daily extra fluid loss of between 2.8 and 4.3 ml/kg BW in a 500-kg horse. This means that the difference in exercise-induced fluid losses cannot explain the difference in water intake between trials. Two horses out of four in paper V increased their mean daily intake over a five day period, when exercised in 35°C compared to in 20°C.

Drinking behaviour (Paper VI)

Diurnal drinking pattern

The drinking patterns and water intake of three out of six individuals from paper VI are shown in Fig 2a-c. Most of the daily water was consumed immediately after feeding and hardly any between 23.00 and 08.00 h with the feeding regimen used. The duration of each drinking bout was shorter in horse I than in horse J and this was consistent for each water supply method. Horse N tended to increase both the duration and the frequency of the drinking bouts with FV3, compared to the other two water supplying methods.

One hour post-feeding in paper VI there was a mean increase across all treatments in TPP of 7-11%, in pNa of 2-3% and in pOSM of 3-5%. This is the probable physiological explanation for the fact that most of the daily water was drunk post-prandial in this trial. The feeding of hay has previously been found to increase TPP by 12%, while no such changes were seen when concentrates were fed (Kerr & Snow 1982).

Comments: The research horses at our Department are all Standardbred trotters raised in training stables. The most common water supply method in this type of stable in Sweden is different types of automatic water bowls. At the time this study was performed all stables at the Department were fitted with a FV3 bowl. Except for one horse (M), which arrived six weeks before the study all horses had therefore been watered from the FV3 bowl for a time period of between six months and two years. Since the horses were accustomed to the FV3 bowl (and presumably other types of bowls depending on their background, see above) we do not believe that the strong bucket preference was a learned behaviour but rather caused by the accessibility of water. It is noticeable that horse J, did not drink after the 08.00 h feeding, despite the same amount of feed being given at each meal. The reason for this is not clear but might be caused by the fact that this individual was more eager to be let out in the paddock than the other horses.

Fluid shifts in connection with feeding like those found in paper VI, have earlier been reported in ponies and horses fed pelleted feed at maintenance requirement level (Clarke et al. 1988, 1990). These authors also found that the transient hypovolemia seen when the horses were fed a single large feeding activated RAAS (Clarke et al. 1988). In contrast, no major post-prandial fluid shifts nor any increases in PAC were seen in athletic horses fed either 2 or 6 times per day (Jansson & Dahlborn 1999). The explanation for the discrepancy between these findings are probably that the latter horses, which were fed grass hay and oats at double their maintenance requirement, fed less voraciously than the former. With a longer eating time, equilibrium between secreted and absorbed fluid was probably reached.

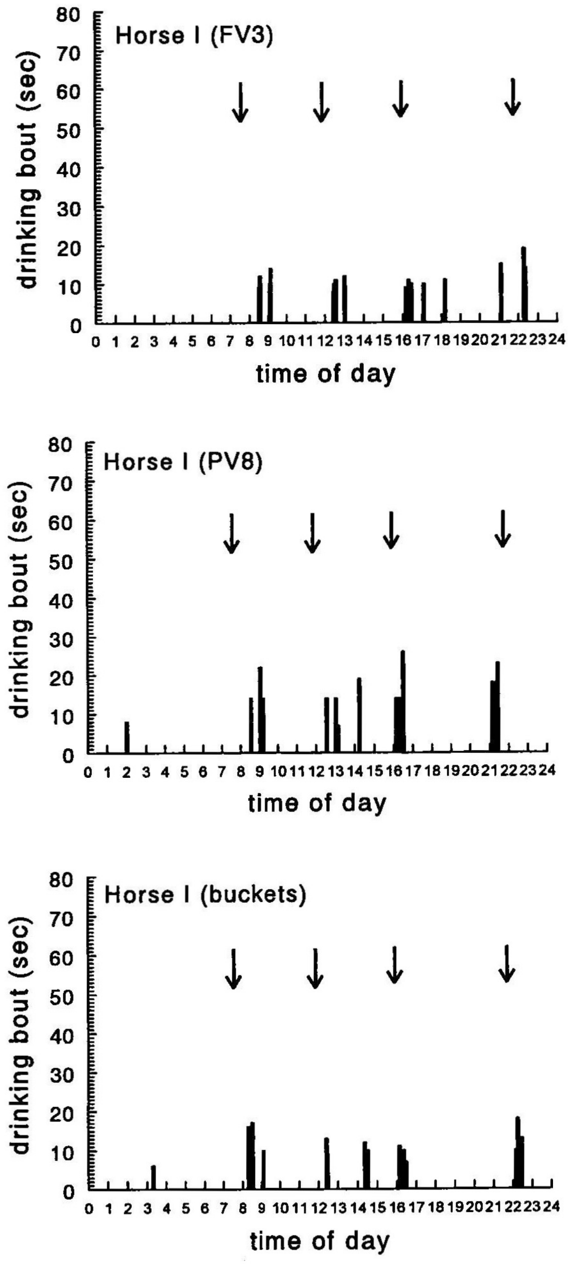
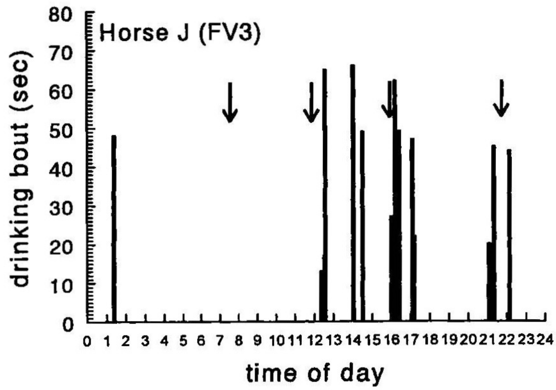
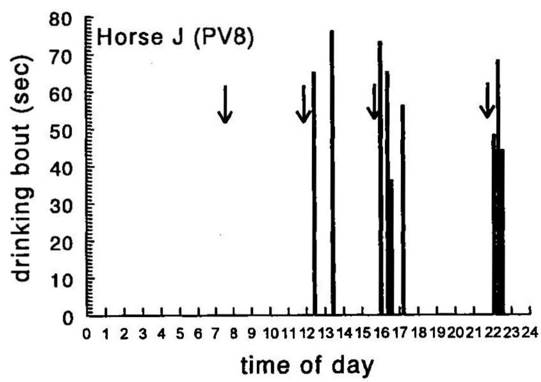
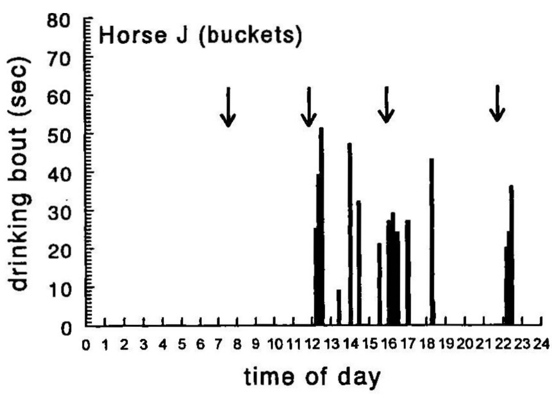
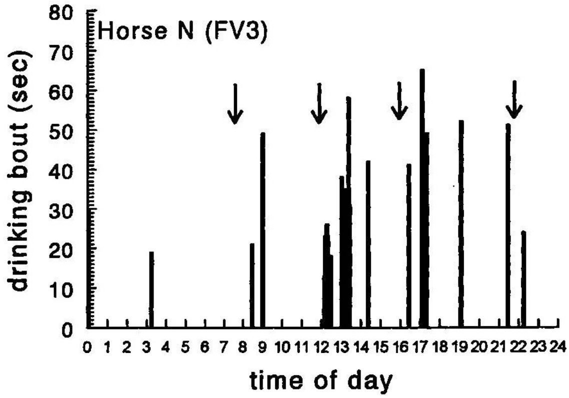


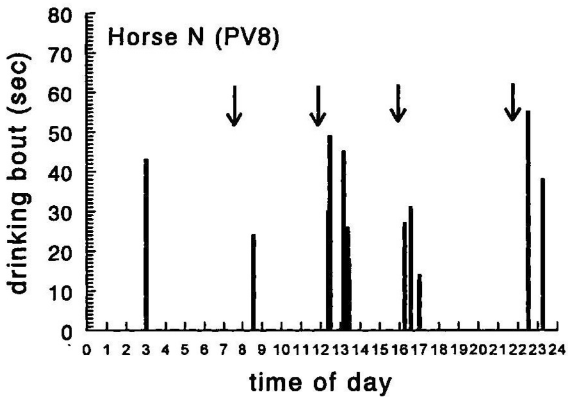
Fig 2. Diurnal drinking pattern in three horses (I, J, and N) in paper VI. FV3, PV8, and B refers to water supply methods (see page 17).

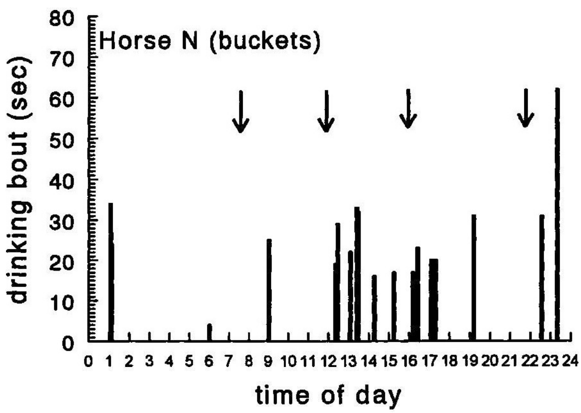

Horse J (FV3)
drinking bout (sec)
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day


Horse J (PV8)
drinking bout (sec)
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day


Horse J (buckets)
drinking bout (sec)
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day


Horse N (FV3)
drinking bout (sec)
80 70 60 50 40 30 20 10 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day


Horse N (PV8)
drinking bout (sec)
80 70 60 50 40 30 20 10 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day


Horse N (buckets)
drinking bout (sec)
80 70 60 50 40 30 20 10 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
time of day

Fluid balance and effects of exercise (Papers I, II, III, IV, V and VI)

Fluid balance in sedentary and exercising horses (Papers V and VI)

The routes for daily fluid losses can be presented as percentage of daily water intake. Results from papers V and VI are shown in Table 3 together with some data found in the literature. The metabolic water in papers V and VI was calculated to be 2.7 and 2 L/day, respectively, and this is not included in the figures found in Table 3.

Table 3. Mean daily water intake (L), water excreted in faeces and urine in % of water intake and volume (L), estimated insensible losses in % of water intake and volume (L), and faecal:urinary water loss ratio. In paper V 20°C and 35°C refers to ambient temperature during exercise, and F to pre-exercise fluid loading (saline). In paper VI PV8 is an automatic water bowl fitted with a pressure valve and a minute flow of 8 L, FV3 is an automatic water bowl fitted with a float valve and a minute flow of 3 L, and B buckets.

Paper	Water intake (L)	Urinary water % (L)	Faecal water % (L)	Insensible[a] (%) (L)	Faecal:urinary water loss ratio
V 20°C	26.4	18 (4.7)	49 (13.0)	33 (8.7)	2.7
35°C	28.4	15 (4.4)	44 (12.4)	41 (11.6)	2.9
35°C+F[b]	28.2[b]	17 (4.8)	42 (11.9)	41 (11.5)	2.5
VI* B	28.6	31 (8.9)	46 (13.2)	23 (6.5)	1.5
PV8	26.2	33 (8.6)	50 (13.1)	17 (4.5)	1.5
FV3	20.7	38 (7.9)	61 (12.7)	1 (0.1)	1.6
Tasker**, 1967	24.7	20 (4.9)	57 (14.0)	23 (5.8)	2.8
Cymbaluk[#], 1988					
-grass hay	-	27	55	18	2.0
-legume hay	-	44	32	24	0.7
-grain	-	56	29	15	0.5

*[a]insensible losses = 100 - (% water in faeces + % water in urine) in paper V sweat losses/5 days are included [b]saline included *water intake is the mean intake on day 6 and 7 in each treatment **sedentary horses, 10-20°C, mean BW 440 kg (Tasker 1967) # horses or ponies, 6 h daily in a paddock, 15-20°C (Cymbaluk 1989)*

Comments: The routes for daily fluid losses are affected by feed composition (Cymbaluk 1989). When the diet is based on grass hay the major route for fluid losses are the faeces. This is true both in paper V and VI, but in paper V the insensible losses (here including sweat losses divided by 5 days) are higher since these horses exercised once a week. Neither the daily water intake nor the faecal water differed between paper V and paper VI (B and PV8), and thus the urinary water excretion was proportionally decreased in paper V.

Even if the daily water intake was significantly lowered from FV3 in paper VI, the faecal and urinary losses (L) were only slightly reduced (ns). The net volume left for insensible losses were almost zero and there was a tendency of a reduced BW from day 1 to 7 in this treatment. However, since TPP, pNa or pOSM did not differ between water supply methods no appreciable change in plasma volume could be detected. Therefore the most probable explanation is that these horses, like water deprived ponies in a study by Meyer (1996), absorbed fluid from the gastro-intestinal tract in order to maintain plasma volume. This is further supported by the fact that faecal water (L) was slightly reduced in the FV3 treatment. For this relatively short period and for sedentary horses this strategy could probably be used without ill effects. However, for longer periods or when fluid balance is challenged by exercise a reduction in urinary volume or an increase in water intake, or both, is needed to maintain fluid balance.

Fluid losses and fluid shifts during exercise (Papers I, IV, and V)

During the short (10 min) incremental test in paper I ~6 kg of fluid was lost, and during the longer (40 min) constant velocity test ~15 kg was lost, but with no differences between the states of hydration (hyperhydration or dehydration). In paper V the evaporative losses were almost 40% higher when the same exercise test was performed in 35°C than when in 20°C. The calculated evaporative losses were reduced when saline was supplemented before exercise in 35°C, compared to when the same test was performed without supplementation.

The effect of exercise on relative plasma volume changes is reflected in the TPP curves (Fig 3-5). During the INCR in paper I, the net increase in TPP did not differ between hydration status but since the dehydrated horses started out at a higher level, the total increase by the end of exercise was over 25% compared to about 15% in the other two treatments. During the CONST, TPP increased less (10%) in HH than in N (17%) and DEH (14%) ($P<0.05$). In paper IV, TPP had increased by 10% after 42 km in the saline treatment, while only a slight increase in TPP was seen in the other two treatments. In paper V, the maximal exercise-induced increase in TPP (seen after the "race") was 10% after saline administration compared to 22% without. The relative changes in pNa and pOSM were smaller than in TPP except for in paper IV (see *Comments* below).

Comments: Fluid loss during exercise was affected by exercise intensity, duration, and ambient temperature. The effect of hydration status varied. According to BW losses in paper I, there were no difference in evaporative losses between state of hydration. This is in accordance with previous findings where no differences in exercise-induced BW losses were seen between horses either dehydrated for 30 h (-6% BW), given furosemide (1.1 mg/kg, -4.4% BW), and a control group performing submaximal exercise (Naylor et al. 1993). In contrast to this and the results from paper I, saline supplementation prior to exercise reduced the evaporative losses. Since blood temperature did not differ between 35°C and 35°C+F (Jansson et al. 1995b), it appears that the increased body fluid content in the latter trial attenuated the need for active heat dissipation, possibly by a greater heat storage capacity.

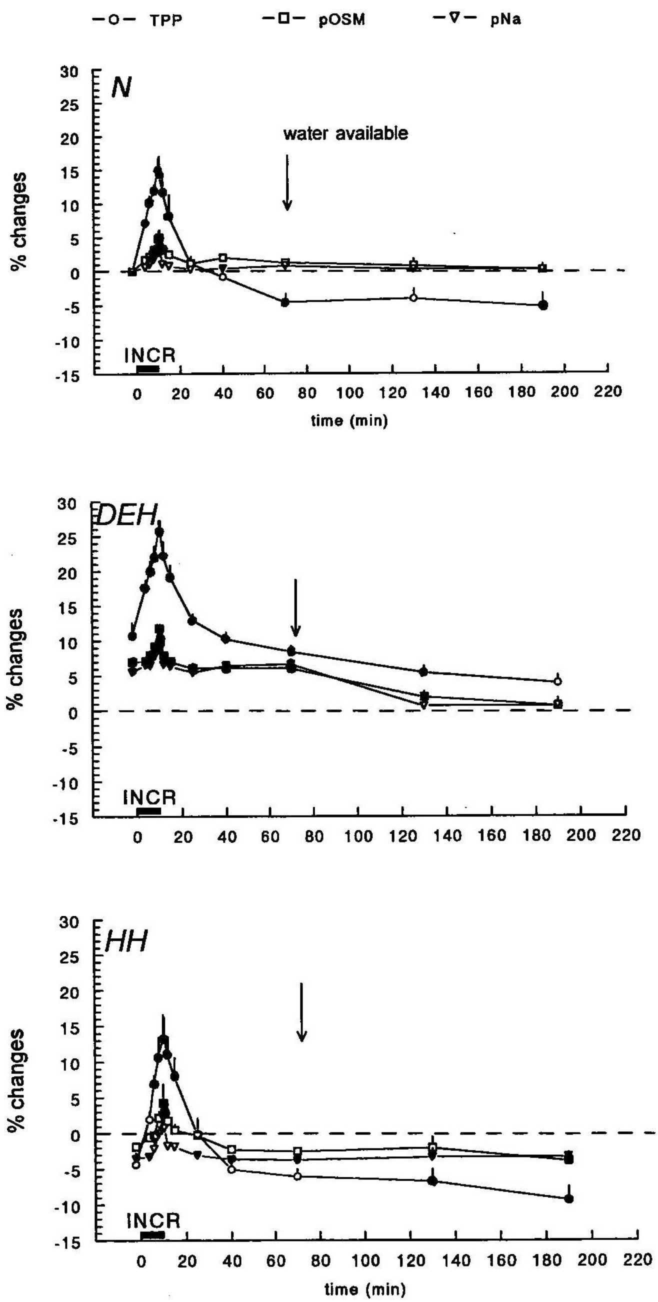


Fig 3a) Relative changes (±SE) in total plasma protein concentration (TPP), plasma osmolality (pOSM), and plasma sodium concentration (pNa) in paper I when horses performed an incremental exercise test (INCR) during normohydration (*N*), dehydration (*DEH*), and hyperhydration (*HH*). Filled symbols are significantly different from pre-treatment. (P<0.05).

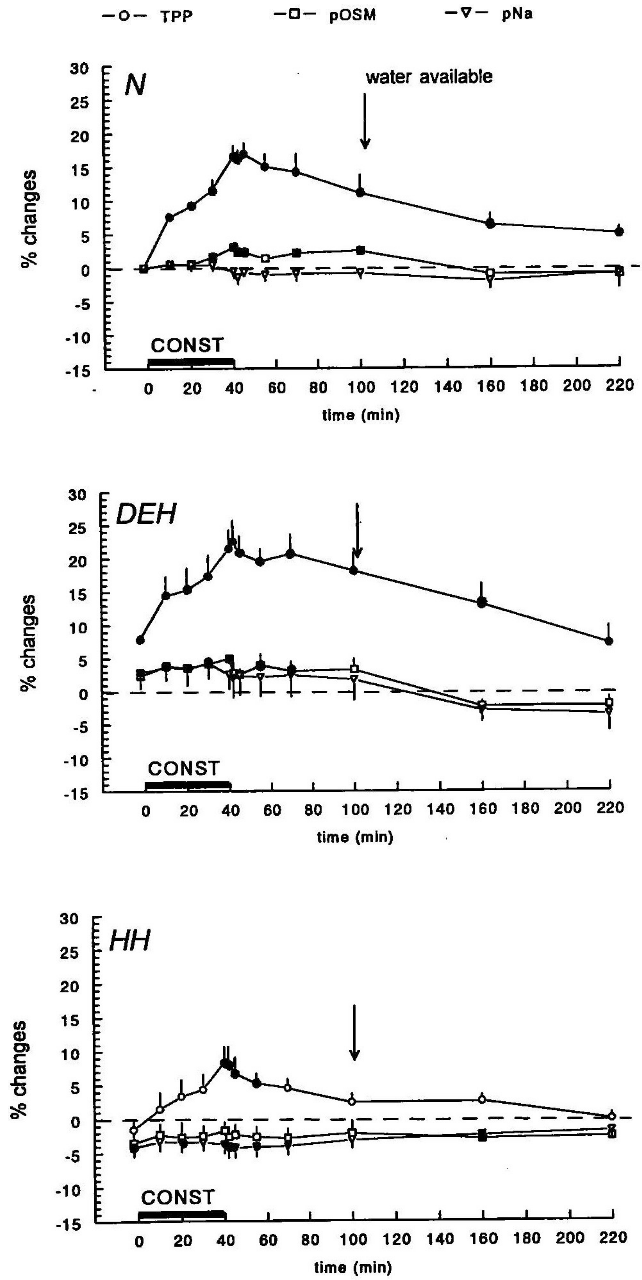


Fig 3b) Relative changes (±SE) in total plasma protein concentration (TPP), plasma osmolality (pOSM), and plasma sodium concentration (pNa) in paper I when horses performed a 40-min constant velocity exercise test (CONST) during normohydration (*N*), dehydration (*DEH*), and hyperhydration (*HH*). Filled symbols are significantly different from pre-treatment. ($P<0.05$).

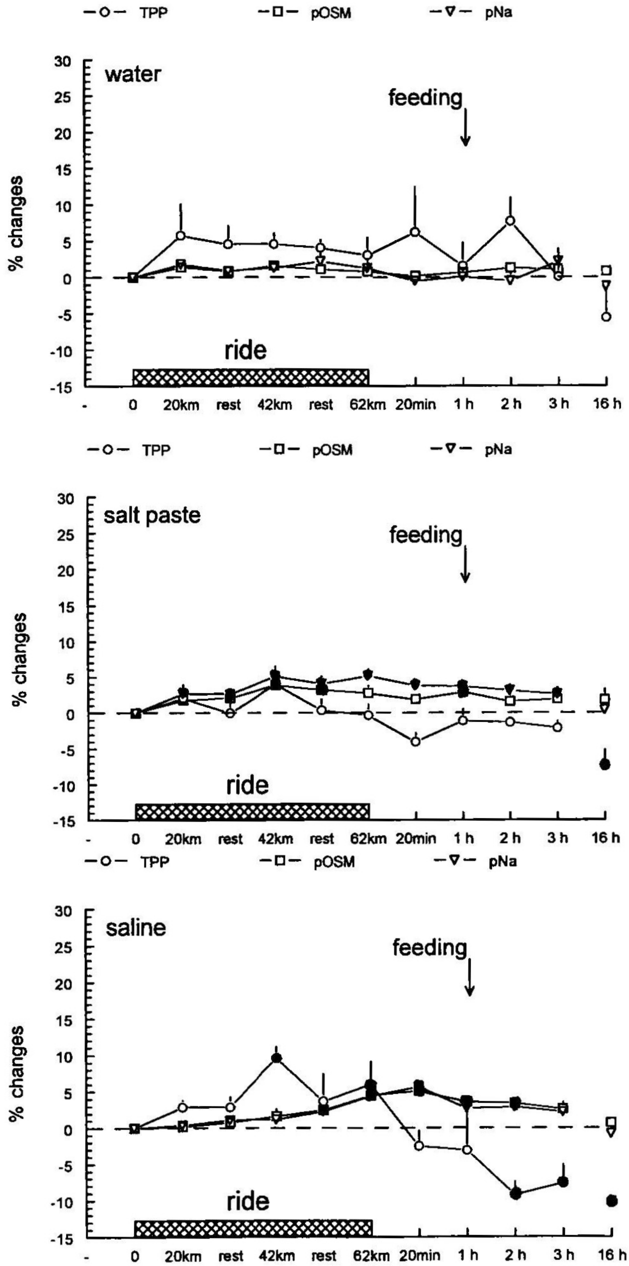


Fig 4. Relative changes (±SE) in total plasma protein concentration (TPP), plasma osmolality (pOSM), and plasma sodium concentration (pNa) in paper IV when horses performed endurance exercise with only water, saltpaste and water, or saline to drink and Filled symbols are significantly different from pre-treatment. (P<0.05)

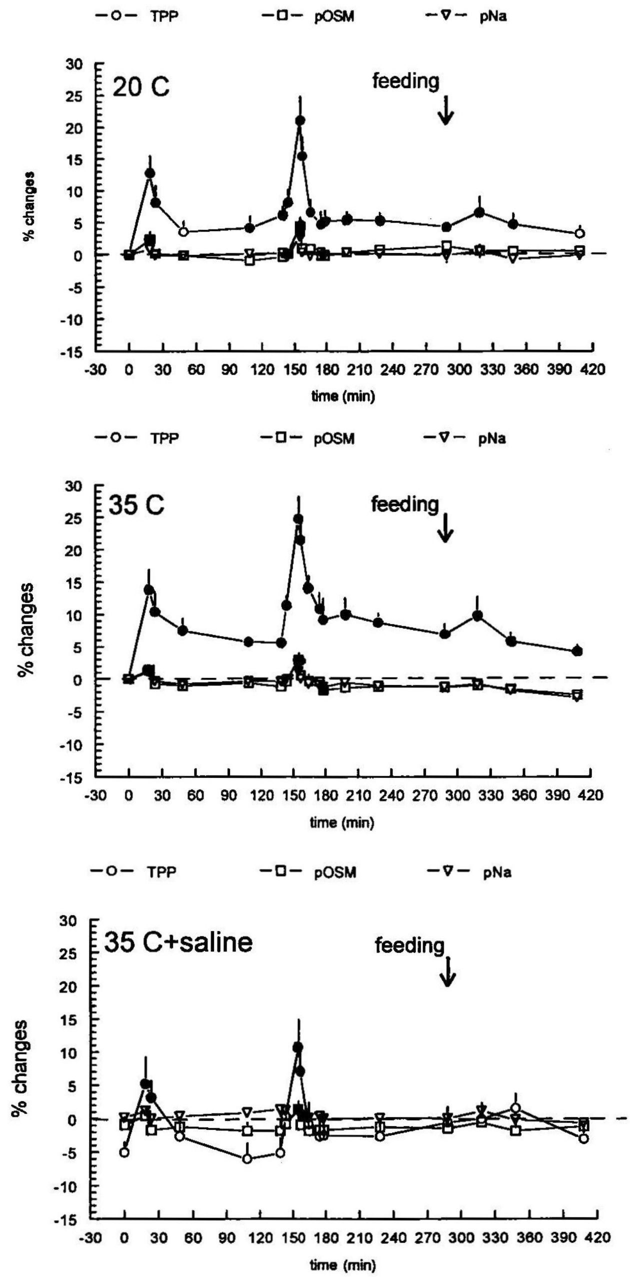


Fig 5. Relative changes (±SE) in total plasma protein concentration (TPP), plasma osmolality (pOSM), and plasma sodium concentration (pNa) in paper V when horses performed two phases of exercise in paper V in 20°C, 35°C, and 35°C with pre-exercise supplementation of saline (35°C+F). Filled symbols are significantly different from pre-treatment. ($P<0.05$)

The exercise-induced changes in TPP can reflect exercise intensity as well as a true body water loss or gain affecting plasma volume. Exercise intensity is correlated to increases in blood pressure, which is the probable explanation for that the magnitude of the relative increases found in TPP clearly follows the intensity (Fig 3 and 5) (McKeever et al. 1993b). During the CONST the continuous increase in TPP was lower than in the other two treatments suggesting that fluid was ab sorbed during exercise.

In papers I and V, the relative changes in pOSM and pNa were always smaller than the relative changes in TPP (Fig 9a and c). This has been described earlier (Carlson 1987) and has been attributed to a transient shift of plasma water due to the increased osmotic activity within the muscle tissue observed during high-intensity exercise (Green et al. 1984). Although, in paper IV (Fig 4) this was not true in the salt paste treatment, where pNa and pOSM were significantly increased compared to resting levels towards the end of the ride, while TPP was not changed. This is indicative of an altered distribution between body fluid compartments, where water is driven from the cells to the ECF by osmotic forces and causes cellular dehydration.

During exercise and for at least 4 h post-exercise in paper V, the TPP level was lower after administration of saline, compared to exercise in the same temperature (35°C) without saline supplementation. The pNa was not higher in the saline treatment, and the only increase in pNa was seen during the "race" in phase 2. The packed cell volume (PCV) was lower between the two exercise phases in the saline supplemented horses. These data suggest that saline induced an expansion of plasma volume which lasted throughout the experiment. A similar response was seen in the group of horses drinking saline in paper IV, although not significant until post-exercise since the horses drank most towards the end of the ride. In contrast to the salt paste treatment in paper IV, no signs of cellular dehydration were seen when water and sodium was given simultaneously as saline.

Effects of using a naso-gastric tube (Paper III)

In paper III, where saline was given to resting horses via a naso-gastric tube, there was a tendency for TPP to decline 30 min after administration began, and at 60, 90 and 120 min after administration started the TPP values were significantly lower than pre-treatment (Fig 6a). Just to enter the treatment room, caused an increase in PCV and TPP in all treatments, reflecting an activation of the sympathetic nervous system (Hydbring et al. 1996).

In the same study insertion of a naso-gastric tube in combination with upper-lip twitching increased plasma cortisol, β-endorphins, and AVP, which indicated a stress response in the studied horses (Hydbring et al. 1996). Surprisingly, no increase in β-endorphins was seen when only lip-twitching or tubing were used (Hydbring et al. 1996). A similar response to that of β-endorphins was seen in AVP (the lip twitching data are based on unpublished data from only two horses, Fig 6b).

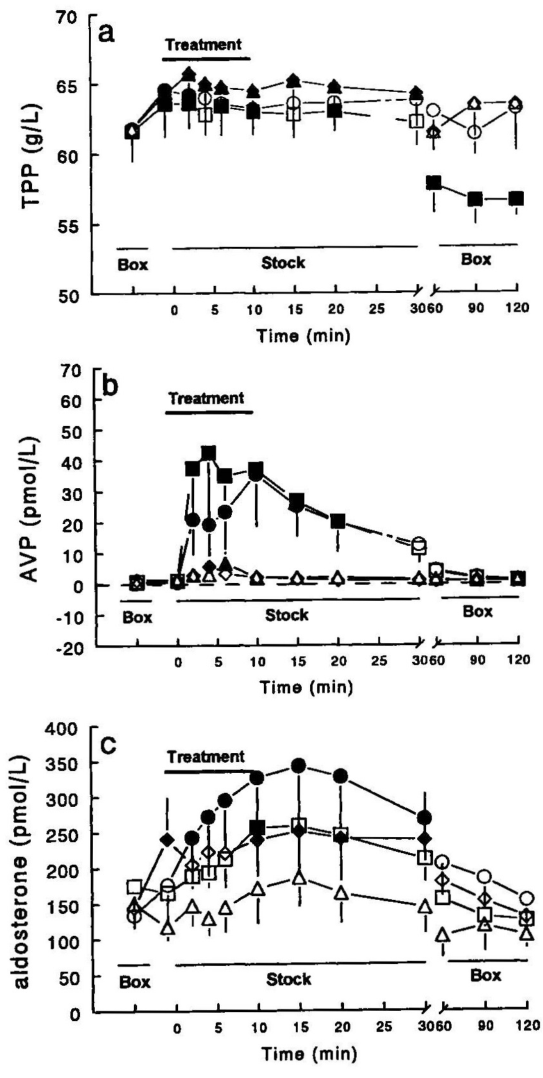


Fig 6. Mean (±) plasma concentrations of a) total plasma proteins, b) arginine-vasopressin, and c) aldosterone during the following treatments (same experiment as paper III). Upper-lip twitching (-◊-), naso-gastric tubing and ear-holding (-Δ-), naso-gastric-tubing and upper-lip-twitching (-O-), and naso-gastric-tubing, upper-lip-twitching and administration of 10 L of saline (-□-). Filled symbols are significantly different from pre-treatment. ($P<0.05$)

PAC was also measured in these horses, and an increase was seen in response to both upper lip twitching and the combination of upper lip twitching and tubing (both with or without fluid), but no increase was seen when the tube was inserted without lip-twitching (Fig 6c) (unpublished data).

Comments: A stress response was seen when nasogastric tubing was combined with upper lip twitching. Lip twitching in the horse is believed to activate the parasympathetic nervous system (Lagerweij et al. 1982). The suppressed response in AVP and β-endorphins when upper lip twitching was not combined with a stressor or when tubing was not combined with upper lip twitching is not clear. Although, it is fair to assume that most of these responses were abolished by the time the horses entered the treadmill in papers I, II and III.

A slight increase in PAC was seen whenever the lip twitch was used but no increase was seen when the tube was inserted with another type of restraint (ear holding). A similar pattern to aldosterone was also found in cortisol (Hydbring et al. 1996), and therefore an effect of adrenocorticotropic hormone (ACTH) could be speculated on. On the other hand, no increase in PAC was seen in thoroughbred horses given ACTH intravenously (Guthrie et al. 1982).

Hormonal response to exercise (Papers I, II, III, IV, and V)

Aldosterone (Papers I, III, IV, and V)

An increase in PAC was seen during exercise in all trials where PAC was measured (papers I, IV, and V), except for when the horses had been supplemented with sodium prior to exercise (salt paste in paper IV and saline in paper V). In paper I no differences in PAC were found between states of hydration during exercise. In the two dehydration trials, the fast post-exercise rehydration with water induced a second increase in PAC, significant at 2 h. In paper IV an exercise-induced increase in PAC was seen in horses drinking water or saline.

Comments: PAC has previously been reported to be correlated to exercise intensity as it increased linearly during an incremental exercise test (McKeever et al. 1992). However, the results from papers I and V suggest that the duration can be as important. In paper I the PAC level was similar after 10 min of exercise whether incremental or submaximal exercise had been performed, and in paper V the increase was similar in both exercise phases (23.5 and 26 min) in spite of different intensities. Although, since the above-mentioned studies are descriptive rather than mechanistic, it cannot be excluded that several mechanisms, depending on type of exercise, are responsible for the exercise-induced increase seen in PAC, i.e. being both pK and a lower body sodium content caused by sweating.

It is clear from our results that the exercise-induced increase PAC is dependent on the sodium status of the horse, since when sodium was supplemented either as a paste or as a saline solution in paper IV or V, respectively, the release of al dos terone was abolished or suppressed. In paper IV, drinking saline did not influence PAC until after the ride, while when saline was given 1 h prior to exercise in paper V, a suppression of PAC was seen already during exercise. This discrepancy is most likely caused by the saline drinking horses in paper IV starting to drink late in the ride while, in paper V, most of it was already absorbed when exercise commenced.

The post-exercise increase in PAC seen in both dehydration trials in paper I is probably a result of a dilution of plasma caused by water absorption from the gut. This causes a reduced delivery of sodium ions to the macula densa that will activate RAAS. This is also the most probable explanation for the increase in PAC seen the day after exercise, both in water drinking horses in paper IV, and in paper V after exercise in 20°C and 35°C without saline supplementation.

ANP and AVP (Papers II, III, and V)

The plasma concentrations of ANP increased during both exercise trials in paper II. The area under the plasma ANP concentration curve was significantly greater during exercise when the horses were hyperhydrated with 12 L of water than when dehydrated for 24 h before exercise (-3% BW). The maximal plasma ANP value induced during the very intense 12-min constant velocity test (HR ~200 beats/min) was similar to that of the less intense 40-min constant velocity test (65-70% HR_{max}), but the post-exercise return to baseline was slower in the former.

Despite an increase in pOSM of 20 mosm/kg in the dehydrated horses in paper III, there was no significant increase in AVP prior to exercise. However, exercise caused an increase in AVP in both paper III and paper V. The increase in AVP was significantly higher during exercise phase 2 (35°C) when no extra fluid had been given, compared to when saline was supplemented.

Comments: A similar result as in paper II, was found in horses performing an incremental exercise test with or without a furosemide-induced reduction in plasma volume (Hinchcliff & McKeever 1998). The body weight deficit and the increase in TPP in their study was of the same magnitude as in the dehydrated horses in paper II, and a similar reduction in plasma ANP concentration was seen. These authors advocated that the effect on ANP was most likely attributed to the reduced plasma volume (and right atrial pressure) and not on a pharmacological response of furosemide. This was confirmed in a third treatment where right atrial pressure and ANP did not differ from control when plasma volume was restored to pre-treatment values by an infusion (Hinchcliff & McKeever 1998). ANP and its vasodilatory actions may be involved in the regulation of the intra-vascular volume during physical activity and as suggested by EXP II in Paper II, probably also after strenuous exercise in the horse.

The smaller increase in AVP found in the saline supplemented horses in paper V compared to when the horses were not given saline, suggests that AVP can be another factor involved in the distribution of the effective blood volume during exercise (Stebbings et al. 1994). This is further supported by data from an earlier investigation where a reduction in plasma volume induced by furosemide treatment, significantly enhanced the AVP response in exercising horses (McKeever et al. 1993a). As the plasma levels of these counteracting hormones were affected by hydration status during exercise, it suggests that they both have an effect on hemodynamics during exercise.

In contrast to our expectations AVP increased to a much higher level (almost 30-fold) in the hyperhydrated horses than in the dehydrated or normohydrated horses (both showing a 10-fold increase) during incremental exercise in paper III. The elevated plasma AVP towards the end of exercise in this treatment might have been an effect of having fluid still placed in the upper gastro-intestinal tract during intensive exercise.

Rehydration in connection with exercise (Papers I, IV, V and VI)

Individual post-exercise rehydration (Papers I, IV and V)

In paper I, a similar fluid loss was seen in both *N*-CONST and *DEH*-INCR, but in the former it was caused mostly by a sweat loss and in the latter mostly by a body water loss. The voluntary water intake within three hours post-exercise corresponded to 76, 73, 59, and 86% (*N*-CONST) and 116, 92, 127, and 123% (*DEH*-INCR) of the BW loss in horse A, B, C, and D, respectively. This shows that the nature of dehydration affects post-exercise rehydration.

In *DEH*-CONST, 76, 121, 89, and 147% of the BW losses had been replenished by water intake at 3 h post-exercise, in horse A, B, C, and D, respectively. Already during the first 15 min after water was available again, 45, 86, 43, and 93% of the fluid deficit was replenished by drinking in horse A, B, C, and D. This shows that there is a considerable individual variation also in the rate of rehydration. Even here seemed the nature of dehydration, reflected by either a simultaneous change in TPP and pNa or just in TPP, affect post-exercise rehydration (Fig 7a).

In paper IV, the voluntary water intake in litres was not higher in the group given a concentrated salt paste, compared to the group offered only water despite an increase in pNa. However, the ratio between total fluid intake (<3 h post-exercise) and the total BW loss corresponded to 46±4%, 68±8%, and 84±3% in treatment water, salt paste and saline (0.9%), respectively, and was significantly lower in water than in both salt paste and saline treatments.

In paper V, the horses drank significantly more (<4 h post-exercise) after exercising in 35°C than in 20°C. This difference was eliminated when saline was administered before exercise in 35°C. The individual difference in water intake : evaporative fluid loss ratio in paper V is shown in Table 3. In 35°C+F the total fluid intake (including saline) corresponded to 180, 167, 144, and 176% of the evaporative losses.

Table 4. Individual water intake (<4 h):evaporative fluid loss ratio (L/kg) in paper V where 20°C and 35°C refers to ambient temperature during exercise, and F to fluid loading (saline).

Treatment	Horse E	F	G	H	Mean
20 °C	134%	67%	34%	71%	**76%**
35 °C	99%	92%	73%	98%	**90%**
35 °C+F[a]	98%	79%	59%	81%	**79%**
Mean[a]	**110%[a]**	**79%**	**55%**	**83%**	**82%**

[a]*Saline is not included*

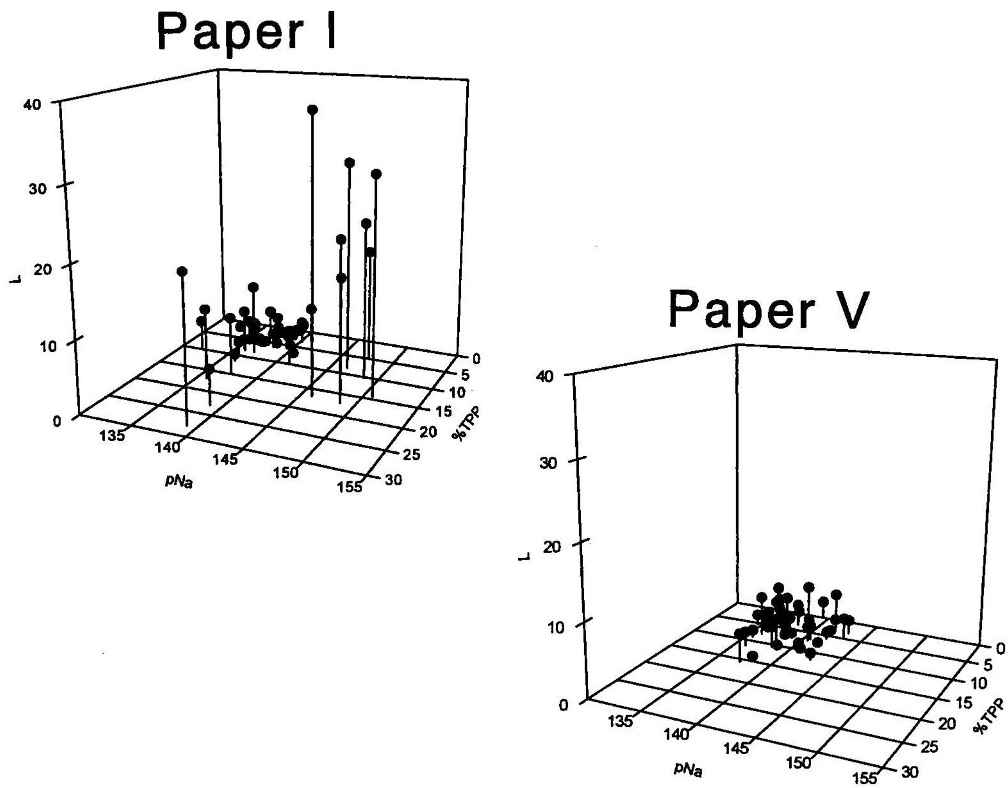


Fig 7. Individual postexercise water intake plotted against the relative change in total plasma protein concentration (TPP%) and plasma sodium concentration (pNa) in paper I and V.

Comments: There was a great individual difference in post-exercise voluntary water intake. The nature of dehydration affected both the rate and efficiency of rehydration. The combination of an osmotic and hypovolemic thirst stimuli (seen in both dehydration trials in paper I) seem to stimulate rehydration more effectively than the slight changes in both TPP and pNa seen in paper V (Fig 7b).
The mean post-exercise water intake in paper I matched relatively well the fluid losses, but this was not true on an individual level.

Our results suggest that giving NaCl as a paste was not beneficial for maintaining fluid balance in horses performing a simulated endurance ride, as they did not increase their water intake. However, in comparision with horses drinking only water, salt paste treated horses had a higher water intake:body weight loss ratio. In contrast to our results Düsterdick *et al.* (1999) concluded that giving a concentrated electrolyte paste increased water intake in horses performing a simulated 60 km endurance ride on a treadmill. The high water intake in their study might be due to that these horses performed less intensive exercise in familiar surroundings and that they were offered longer rest breaks and were fed hay.

Not only the water intake but also the efficiency differs between the rehydration strategies tested. Despite the fact that the mean water intake corresponded to the mean body weight loss, TPP was still increased at 3 h post-exercise in both dehydration trials and in N-CONST (paper I). In paper IV a slower restoration of BW losses was found in the group of horses drinking only water. The results from both papers IV and V show that post-exercise rehydration with water is less effective than when sodium is given simultaneously as a saline solution.

Voluntary intake of saline (Paper IV)

All the owners of horses in paper IV had before the trial been told to offer their horses saline solution to drink in connection with exercise. About 50% of the horses had willingly accepted to drink saline and only these horses were candidates to be included in this treatment. The high fluid intake in this treatment is somewhat surprising, since earlier studies by Randall et al. (1978) showed that even solutions with NaCl concentrations lower than those used in our study (0.6 %) were strongly rejected by foals in a preference test. It has previously been suggested that it is possible to train horses to drink saline if this is offered to exercising animals as the only fluid source (Kleps et al. 1992).

Comments: In a pilot trial on research horses (n=6), the taste preference for saline (0.9%) was tested in a two-choice preference test. The horses were offered fluid from buckets with the following combinations; water vs. water, saline vs. water, and saline vs. saline in a randomised fashion and with at least 4 days in between experiments where saline was used (Nyman, unpublished). The study was performed on sedentary horses given their maintenance requirement of sodium. When water and saline was offered simultaneously two out of six horses drank the same amount of water and saline, while four horses preferred water to saline. Interestingly, the total daily fluid intake increased with more than 50%, compared to water, when the horses were offered only saline in both buckets. This was probably caused by a persistent increase in pNa (unfortunately not measured) stimulating thirst when only saline was available.

Fluid supplementation in connection with exercise (Papers I, II, and V)

In this thesis the effect of pre-exercise administration of both water and saline was tested. The water load in papers I, II, and III was given close to exercise (30 min). There were signs of absorption by decreases in TPP, pNa, or pOSM at the onset of exercise and the post-exercise level in TPP were lower compared to in normohydrated horses. In paper V the saline solution was administered 1 h before the first exercise phase and a comparatively lower level of TPP than in the other two trials was seen from the onset of exercise until 4 h post-exercise.

Comments: It is clear both from our results and previous investigations that both the timing, the amount of fluid, its electrolyte content and type of exercise performed will affect the outcome of the treatment.

In all papers where water was administered blood plasma was diluted. Since this will increase urinary production, the water in papers I, II, and III was given 30 min prior to start of exercise. Our data shows that at least some of the water given in paper I had been absorbed when the horses entered the treadmill. According to a study performed on non-exercised horses the deuterium uptake had reached about 50% of its maximal value after 30 min when 4 L of labelled water was given via a naso-gastric tube (Marlin et al. 1998a). During the INCR the water load changed the physiologic response to exercise, since there was a greater plasma lactate accumulation and a tendency for an increased HR and PCV, possibly by the extra weight to be carried. During the CONST the greater plasma volume did not affect any of the measured performance indices except for a tendency to increase oxygen uptake.

When a sodium-rich hypo- to isotonic solution is used it is primarily distributed in the ECF volume. Accordingly, when 17.5 L of a slightly hypotonic solution was given to horses prior to low-intensity exercise (20°C, 50% relative humidity (RH)) a smaller net increase in TPP was seen compared to controls. However, under these temperate laboratory conditions, no differences in thermoregulatory or cardiovascular parameters were seen (Sosa-León et al. 1995a). When a simulated 2nd day of a three-day event was performed (25°C, 66% RH) with a preload of 26 L isotonic solution, plasma volume was expanded during exercise, HR was comparatively lower but the core temperature did not differ from that in controls (Sosa-León et al. 1996). In paper V, 10 L of saline was given 1 h before the start of exercise (35°C, 40% RH) and this resulted in a smaller change in plasma volume throughout both exercise phases. Both a comparatively lower plasma lactate concentration, and a lower HR during the recovery after the first exercise phase, than in control horses were seen. In the only study where the effects of giving water and an isotonic solution are compared, the authors conclude that there were no differences in reducing the physiological strain during exercise (34.5°C, 48% RH) between the two treatments (Geor & McCutcheon 1998). However, according to their data the reduction in plasma volume was greater with water at the end of exercise and pOSM was lower throughout the experiment. To summarise, it seems like pre-exercise fluid administration is most beneficial in warm ambient conditions.

General discussion

Factors stimulating post-exercise water intake in the horse

The results from this thesis confirm that an osmotic thirst stimulus (i.e. pNa) is important for post-exercise voluntary water intake in horses, as has earlier been suggested by Carlson (1979), among others. A combination of an osmotic and hypovolemic stimulus produced more rapid drinking and larger intakes of water in the post-exercise period than only a hypovolemic even if the latter, according to TPP, corresponded to a reduction in plasma volume of around 15%. With these results in mind, water should not be restricted post-exercise since the chance of an effective thirst stimulus is greatest immediately after exercise. No ill effects were seen in the dehydrated horses when drinking almost 30 L of water (10-12°C) within a single bout (paper I).

The reason for the unwillingness to drink in the horses given salt paste (paper IV) despite an increase in pNa is less clear. However, it might be explained by the absence of a simultaneous hypovolemic stimulus (no change in TPP) in these horses. Therefore, it is possible that a higher water intake had been seen in these horses if the administration of salt paste had awaited until a fluid deficit had de veloped. On the other hand, it could also have put the horse in an even worse situation by reducing the intracellular water furthermore if water intake was not increased.

Effect of water supply method and water availability

For the welfare of the horse and to minimise the risk for a reduced daily water intake, drinking water should always be available. From the results of this thesis it is evident that the way horses are offered water can affect their water intake and that an automatic bowl could not always be considered as free access to water. There are several possible explanations of why all ten horses in papers I and VI drank more from buckets than from the automatic bowl (FV3). The accessibility is higher in buckets since the water surface area and the water depth is greater. It is also possible for the horse to have a higher drinking rate from the bucket, which might better suite their natural drinking behaviour. Another possible factor can be that water taste change when the water is poured into a bucket (this is certainly true in areas with high levels of sulphur, iron, and chlorine).

In the preference test in paper VI only two horses drank from the PV8 when buckets were available. This preference for being watered from a bucket has also been found in practice, where some horses would not eat their night feed when offered water from an automatic bowl, but if provided with a bucket they ate (Nyman, unpublished observation). Since watering many horses from buckets is laborious and time-consuming, it would be of great interest to further elucidate which factors are the most important ones in determining water intake from an automatic system (for example water surface area, water depth, type of valve).

Since most of the daily water was consumed in connection with feeding, it is an absolute must that water is provided at meal times. It is a common practice in Sweden today, that horses are fed several times a day with grass hay in their paddocks without access to water (Nyman, unpublished observation). This way of separating feeding and availability of water have lead to repeated cases of impaction colic (Nyman, unpublished observation).

Hydration status during exercise - does it matter?

Hydration during exercise matters, but how, depends on the type of exercise to be performed and the ambient conditions. In paper I it was shown that to change pre-exercise hydration status by either hyperhydration or dehydration altered the horses' physiological response to exercise. Thus, for the welfare of the horse and to avoid the risk of an impaired performance capacity a normohydrated state should be aimed for before the commencement of exercise. In accordance with the results from paper I this is true even for horses performing exercise of a shorter duration, like race-horses.

For some categories, like endurance and three-day event horses, the pre-exercise hydration status is even more important since their exercise-induced fluid losses are higher. This means that they might need to go to the site of a competition several days in advance, to have time to compensate for the dehydration sometimes caused by trailoring stress (van den Berg et al. 1998) and adapt to the change of environment (Mars et al. 1992). It is also important to recognise early signs of a reduced water intake, such as loss of appetite, and if possible increase the accessibility and palatability of water, for example by offering it from a bucket.

The results from paper V shows that for horses performing exercise in warm ambient conditions, it is beneficial to minimise the exercise-induced dehydration, both in a cardiovascular (reduced heart rate) and a thermoregulatory perspective (smaller evaporative losses). This is further supported by results from Geor & McCutcheon (1998) where control horses performing prolonged low-intensity exercise had a reduced cardiac output and a higher blood temperature compared to fluid supplemented horses. One way to help the horse to reduce its evaporative losses when exercising in hot conditions, is to cool it by repeated application and removal of cold water (12°C) (Kohn et al. 1995) successfully used during the Olympic Games in Atlanta 96'.

Which is the best way of replenishing exercise-induced fluid losses in the horse?

It is evident from the results in papers IV and V that to replenish exercise-induced fluid losses with water only is less effective than to simultaneously restore both water and sodium losses with a saline solution. However, in practice, administration of fluids with a naso-gastric tube can be discussed from an ethical point of view and in many countries, including Sweden, this is not approved during competitions. What to do if the horse will not drink saline voluntarily? There are several possible ways to overcome this problem. It has been documented that the taste preference for sodium containing beverages in human athlete's change when sodium has been lost during heavy sweating (Brouns et al. 1995). This means that a sodium-rich beverage, which is rejected in a resting state, could be preferred in connection with exercise, although the reason for this is not fully understood. Therefore, the taste preference for saline should not be judged when the horse has not experienced any sweat losses. Another possibility for a horse that is reluctant to drink saline is to offer a hypotonic saline solution since these also contribute to the maintenance of plasma volume during exercise (Sosa-León et al. 1995a).

To use a concentrated salt paste and rely on voluntary water intake is a convenient method for the rider, but according to the results in paper IV, not beneficial for the horse. Although, to replace fluid losses in the post-exercise period, sodium can be given as NaCl added to the feed together with a simultaneous supply of fresh water.

Practical considerations in the experimental situation

The results from paper III clearly show that the handling of the horse in an experimental situation can effect many physiological parameters. This is a truth known by many but sometimes forgotten when an experiment is designed or described. For example, just to move the horse from its home box to the treatment room changed several parameters in paper III. It is also interesting that the type of restraint used in combination with naso-gastric tubing, upper-lip-twitching vs. ear-holding, induced very different responses for example in AVP.

Another problem could be that the hydration status of the experimental horse is ignored, and that some investigators still forget to inform the reader about the diet, feeding regimen, and water supply method used. The simplest way of assessing changes in body water content is by weighing the horse frequently. On a standardised diet based on the energy requirement for the size of horse and type of exercise performed, the daily variation in body weight is slight. The basal TPP value varies between individuals (58-70 g/L) and can like PCV values increase in response to stress (Hydbring et al. 1996). However, TPP reflects plasma water content (Carlson & Harrold 1977) and for a within animal comparison during a short time space it is a useful indicator of plasma volume changes

In connection with exercise many blood parameters can change within a very short time period due to fluid shifts or, in the horse, release of blood from the spleen. Therefore the timing of blood sampling is very important in these types of experiments. This is clearly illustrated in Paper I, where TPP fell up to 5% within 2 min post-exercise and were back to pre-exercise levels 15 min after stop.

Conclusions

- Hydration status affected the physiological response to exercise. The shifts of fluid from the intra-vascular space, indicated by changes in total plasma protein concentration, were dependent on both exercise intensity and hydration status. Plasma sodium concentration and plasma osmolality showed a slight and transient increase during intense exercise. The results confirm what has earlier been suggested, that a lack of an osmotic thirst stimulus (i.e. increase in plasma sodium concentration) reduces the post-exercise voluntary water intake in horses.

- The daily water intake was about 40% higher when horses were drinking from buckets than from an automatic water bowl fitted with a float valve (3L/min, FV3). In a two-choice preference test all horses showed a strong preference for buckets compared to an automatic bowl (pressure valve, 8 L/min). When the horses were drinking water from the FV3 bowl, the amount of water drunk only just covered for the water losses in faeces and urine.

- The horses offered saline solution (9g NaCl/L) to drink had the highest voluntary fluid intake. The ratio between total fluid intake (<3 h post-exercise) and the total BW loss was significantly lower in water (46±4%), than in both salt paste and saline treatments (68±8% and 84±3%, respectively). Concentrated salt paste (NaCl) increased plasma sodium concentration in horses during endurance exercise, but did not increase water intake. The persistently increased plasma sodium concentration and an unchanged plasma protein concentration, despite a low water intake, indicates an altered distribution between body fluid compartments and is therefore not recommended.

- In horses exercising once a week (fluid loss 11-15 kg), urinary output was significantly reduced on the exercise day and water balance was regained over several days. When the same horses were supplemented with 10 L of saline solution, fluid balance was regained already on the exercise day.

- The exercise-induced changes in plasma concentrations of atrial natriuretic peptide and arginine-vasopressin were influenced by hydration status which suggest that both hormones are involved in blood flow control during exercise. An increase in plasma aldosterone concentration was seen during exercise in all trials, except for when horses were given sodium (salt paste or saline solution) prior to exercise. Aldosterone was not affected by hydration status during exercise but was involved in the post-exercise regulation of fluid balance.

Populärvetenskaplig sammanfattning

Vatten är det fodermedel som hästar konsumerar mest av per dygn. Syftet med detta avhandlingsarbete var att öka kunskaperna om vilka faktorer som påverkar hästars vattenintag och vätskebalansreglering.

Bakgrund

Hästen är ett flockdjur som är anpassat till att äta 14-17 timmar per dygn (Feist & McCullough 1976). Den frilevande hästen äter ett foder (ffa betesgräs) som innehåller upp till 80% vatten. Hur ofta den uppsöker en vattenkälla beror såväl på avståndet till denna som omgivningstemperaturen. Ston som ger di söker oftare upp vatten och kan ibland initiera hela flockens förflyttning. Hästar kan ströva över stora områden för att söka betesplatser, tillgång på vatten och skydd för väder och vind. Eftersom hästen är ett bytesdjur har den utvecklat mycket goda fysiska förutsättningar för att, under någon eller några minuters tid, snabbt kunna fly från hotande rovdjur.

Tillvaron för den uppstallade hästen skiljer sig till stora delar från dess vilda anfaders. De flesta svenska hästar hålls uppstallade, åtminstone under vinterhalvåret, och utfodras med en begränsad mängd foder 2-4 gånger per dygn. Fodret, ofta i form av hö och havre, innehåller bara 15% vatten och för dessa hästar är dricksvattnet den absolut viktigaste vattenkällan. Vi vattnar våra uppstallade hästar på olika sätt. Många svenska stallar är idag utrustade med automatiska vattenkoppar, men vattning med hink eller ur gemensamma kar förekommer också. En av de frågor som besvaras i avhandlingen var om vattenintaget från olika vattenkällor skiljer sig och om det i sin tur påverkar vätskebalansen hos hästen.

Förutom de ovannämnda skillnaderna mellan uppstallade och frilevande hästar, tillkommer det faktum att många hästar utför mer eller mindre krävande fysiska prestationer eftersom de används i olika typer av tävlingsverksamhet. Hästens främsta sätt att göra sig av med den överskottsvärme som bildas vid muskelarbete är genom att svettas. För att tävla krävs regelbunden träning detta innebär att de dagliga förlusterna av kroppsvätska ökar betydligt. Hur hästen reglerar och återhämtar sig efter dessa förluster och om det är möjligt att påskynda detta förlopp är andra frågeställningar som berördes i denna avhandling. Dessutom studerades hur såväl ett överskott (vatten tillfört via en nässvalgssond) som ett underskott på vatten påverkade hästen under fysiskt arbete.

Vätskebalans och dess reglering

Eftersom vatten deltar i så gott som alla livsnödvändiga processer regleras mängden kroppsvatten inom snäva gränser hos såväl hästar som människor. Vätska förloras ständigt från kroppen via urin, träck och avdunstning från kroppsytan och andningsvägarna. Kroppen känner av redan mycket små förändringar i blodets salthalt, vilket vid en ökning leder till minskade vätskeförluster via urinen och till en stimulering av törst. Vid större vätskeförluster minskar även blodets volym vilket ytterligare bidrar till att minska urinvolymen och ökar törsten. Vid ett överskott av vätska i kroppen sker det omvända, blodets salthalt minskar och vätskeöverskottet utsöndras via urinen.

Vi människor utsöndrar en svett med en lägre salthalt än blodet vilket gör att blodets salthalt stiger när vi svettas och vår törst stimuleras. Hästens svett däremot, har samma eller högre salthalt än blodet vilket gör att blodets salthalt inte påverkas nämvärt av att svettas. Detta har ansetts som en trolig förklaring till att hästar inte alltid dricker under och efter arbete som medför stora svettförluster, till exempel vid de alltmer populära distansritterna (25-160 km per dag).

Hästen förlorar även elektrolyter (lösta salter) när den svettas, framförallt natrium, klorid och kalium. Hästens foder innehåller mycket kalium medan fodrets natriuminnehåll är lågt. Natrium och klorid (koksalt) måste därför ges i form av tillskott. Det har i ett tidigare avhandlingsarbete från Institutionen för Djurfysiologi, SLU, visat sig att det frivilliga intaget från en saltsten ofta inte ens täcker underhållsbehovet av salt utan måste tillsättas på annat vis, till exempel i fodret (Jansson 1999). Av resonemanget ovan framgår att blodets salthalt (framförallt natrium) är mycket viktig för att rätt kunna reglera kroppens vätskeinnehåll. Natrium är även viktigt för fördelningen av vattnet inom kroppen och brist på natrium leder till att blodets volym minskar.

Aktuella frågeställningar

- Vilka faktorer påverkar hästars vattenintag i vila och i samband med arbete?
- Har vattenkällan någon betydelse för hästars vattenintag, drickbeteende eller vätskebalans?
- Hur påverkar hästens vätskestatus dess fysiologiska svar på arbete?
- Kan man påverka hästars frivilliga vattenintag i samband med arbete?
- Hur påverkar fysiskt arbete hästens vätskestatus och hur återställes denna efter arbetet?

Vilka faktorer påverkar hästars vattenintag?

I djurskyddslagen det att djur skall vattnas minst två gånger per dag med tilläget "att de bör ha fri tillgång till vatten". I litteraturen anges ofta riktvärden för hästars vattenintag. Det bör poängteras att dessa skall ses som just riktvärden eftersom resultaten i denna avhandling visar att hästars vattenintag kan variera mycket mellan individer, trots att de har samma yttre förutsättningar (stalltemperatur, foderstat m.m.). Av de studerade faktorerna visade sig vattenkällan vara den enskilt viktigaste faktorn för hur mycket hästarna drack. När hästarna fick välja mellan att dricka ur en hink eller ur en automatisk vattenkopp (flöde 8 liter/ minut) föredrog alla att dricka ur hink. Bara två av sex hästar drack överhuvudtaget ur koppen. Hästarna drack också 40% mer per dag när de hinkvattnades än när de erbjöds vatten ur en självfyllande automatisk vattenkopp med ett minutflöde på 3 liter.

Det finns säkert flera förklaringar till att hinkvattning föredrogs, till exempel att vattenytan och vattendjupet är större och vattnets lukt och smak kan förändras eftersom vattnet "luftas" när man häller upp det i en hink. Den vilda hästen dricker mycket vatten under kort tid eftersom den vid vattenhålet är ett lätt byte för rovdjur. Att dricka ur en kopp med ett lågt minutflöde är inte förenligt med hästens naturliga drickbeteende. Dessutom påverkades hästarnas vätskebalans under den vecka som de drack ur flottörkoppen. En sådan vattenkälla måste anses som direkt olämplig för hästar.

Hur påverkar hästens vätskestatus dess fysiologiska svar på arbete?

I många handböcker om hästhållning förekommer goda råd om hur hästar bäst bör vattnas i förhållande till arbete. Tyvärr är råden ofta motstridiga och vetenskapliga data i detta område är få. För att studera detta i en experimentell miljö fick hästarna utföra två typer av arbetsprov på rullmatta, dels efter att 12 liter vatten givits med hjälp av en nässvalgssond och dels när de fått vara utan vatten i 24 timmar före arbetet. Försöket visade att både för mycket och för lite vatten förändrade hästens förutsättningar för att utföra fysiskt arbete.

I en annan studie fick hästarna genomföra ett arbetsprov som motsvarade en tävlingsdag för en travhäst med "värming", boxvila och "lopp". Även detta försök utfördes på rullmatta och hästarna gjorde samma test i två omgivningstemperaturer, 20°C och 35°C, samt ytterligare en gång i 35°C efter att ha fått fysiologisk koksaltlösning via en nässvalgssond före arbetet. Svettförlusterna ökade helt naturligt när omgivningstemperaturen ökade från 20°C till 35°C. När hästarna fick koksaltlösningen före arbetet i 35°C minskade svettförlusterna jämfört med när ingen vätska givits. Dessutom var såväl halten mjölksyra i blodet som pulsen lägre hos hästarna i det senare fallet. Sammanfattningsvis visar båda dessa försök att vätskestatus har betydelse vid fysiskt arbete.

Kan man påverka hästars frivilliga vattenintag i samband med arbete?

Distansridning är en tävlingsform där man rider sträckor mellan 25 och 160 km per dag. Målet är att tillryggalägga sträckan på snabbast möjliga tid. En veterinär avgör, bland annat av puls och vätskestatus, om hästen är i tävlingsmässigt skick. De hästar som dricker för lite under ritten klarar inte en sådan besiktning och blir direkt utesluten ur tävlingen. För att öka vattenintaget ges ibland elektrolyter direkt i munnen på hästen. Effekten av detta har inte tidigare studerats under verklighetsnära förhållanden.

I ett delarbete som utfördes i oktober månad 1995 studerades 13 distansrittshästar som utförde en kortare (62 km) distansritt i terräng. Hästarna var privatägda tävlingshästar med varierande rutin. Hästarna lottades till tre olika grupper. Varje häst fick antingen bara dricka vatten, gavs koksalt i munnen (3 doser om 30 gram) och fick dricka vatten eller erbjöds en saltlösning att dricka (9 g koksalt/liter vatten).

De hästar som fick saltlösningen hade det högsta vätskeintaget och återhämtade sin kroppsvikt snabbast. Hästarna som fick saltpasta i munnen drack inte mer än de hästar som bara fick vatten, trots en högre salthalt i blodet. Dessutom fanns det tecken på att vätskefördelningen mellan blod och celler hade förändrats på ett ofördelaktigt sätt hos de hästar som fått saltpasta. Denna behandling kan därför inte rekommenderas. De hästar som drack vatten återhämtade sina kroppsviktsförluster långsammast. Detta tyder på att vatten utan salt sänker blodets salthalt vilket gör att kroppen uppfattat det intagna vattnet som ett överskott gör sig av med det via urinen.

Hur påverkar fysiskt arbete hästens vätskestatus och hur återställes denna bäst efter arbete?

När hästar arbetar beror vätskeförlusterna till ca 30% på avdunstning ifrån andningsvägarna (enbart vatten) och till resterande ca 70% på svettförluster (både vatten och salter). I ett delarbete mättes intag och förluster av vatten, natrium och kalium genom att samla all urin och träck samt mäta intag av foder och vatten under 5 dagars perioder. Hästarna utförde ett arbetsprov per vecka som innebar en vätskeförlust på ca 12 liter. De fick dagligen 38 g koksalt i fodret vilket var beräknat att täcka deras dagliga underhållsbehov och veckans svettförlust. Under arbetsdygnet halverade hästarna nästan sin urinmängd och ökade vattenintaget något. Detta räckte dock inte helt för att återställa vattenbalansen utan den reglerades över flera dagar. Natriumhalten i urinen var mycket låg under arbetsdygnet och dygnet därefter och det saltsparande hormonet aldosteron var aktiverat. När samma hästar före arbetet gavs en mängd koksaltlösning som motsvarade vätskeförlusterna återställde de sin vätskebalans redan under arbetsdygnet. Detta visar att för hästar som skall utföra upprepade prestationer, som till exempel en tre dagars fälttävlan, är samtidig tillförsel av koksalt och vatten viktig.

Sammanfattningsvis: För hästens välbefinnande och förmåga att utföra arbete är det viktigt att i största möjliga mån bibehålla en normal vätskebalans. Två viktiga faktorer att tänka på är val av vattenkälla och att i samband med arbete samtidigt ersätta både vatten- och saltförluster.

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