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Original Research

Effect of Food Availability on the Physiological Responses to Water Deprivation in Ponies

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ABSTRACT

Six ponies were deprived of drinking water and food and compared over 24 hours with nondeprived ponies, ponies deprived of water but with food available, and ponies deprived of food but with water available. When food was eaten during water deprivation, plasma osmolality rose 4% from 284 mOsm/kg to 295 mOsm/kg. During water and food deprivation, plasma osmolality failed to rise, even over 24 hours, and usually fell. Packed cell volume was higher when food but not water was available. Food and/or water deprivation had no significant effect on plasma protein concentration. When food was available, the ponies drank three times more water (13.1 ± 2.1 kg) than when water but not food was available (3.5 ± 1.4 kg). Blood volume changes were calculated from packed cell volume and plasma protein data, and it was found that blood volume did not change significantly with deprivation. Urine volume did not vary with deprivation, but free water clearance changed significantly, falling when food but not water was available. Under these conditions, blood volume is maintained, but the mechanisms are not clear. When deprived of both drinking water and food, ponies failed to develop the hyperosmolality expected under these conditions. Water deprivation while food is available is a more powerful challenge to water and electrolyte homeostasis than deprivation of both food and water.

1. Introduction

The mechanisms of initiation and termination of water drinking, that is, of thirst, have most often been studied in animals deprived of drinking water. It is sometimes assumed that the effects of water deprivation are always the same: an inevitable decrease of total body water and a consequent rise in osmolality of extracellular fluid as well as a decrease in blood volume [1]. Little notice has been made of the feeding conditions imposed during such water deprivation. An earlier study indicated that neither plasma osmolality nor blood volume of pigs falls when they are deprived of food, but only if water is also withheld [2]. Interest in this aspect of thirst arose from studies of the causes of prandial drinking. Typically, many animals, in particular humans, ponies, and rats, consume approximately three-fourths of their daily water in close temporal association with eating [3-5]. The welfare of horses either during transport or when food or water might be withheld is also a concern, and we wish to determine the least physiologically stressful means to restrict water. The objective of these experiments was to describe the pattern of changes in fluid balances with water deprivation in the presence or absence of food.

There are real-life situations in which these conditions occur. The animals can have food but no water. This occurs under drought conditions, when grass is available but water holes have dried up [6], and in the winter, when horses eat a lot to keep warm but their water source is frozen. The transported horse usually has hay available but is often heat stressed as well as water deprived. The confined neglected horse may have neither food nor water. The condition of water without food happens when a horse

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is fasted perioperatively. This can also happen in a desert environment when all the grass close to a water hole has been consumed.

Our hypothesis was that the presence of food affects the dehydration status of the animal but that the response of the horse may differ from that of other species, including other equine species such as the donkey or zebra. To test this hypothesis, we measured plasma protein, packed cell volume (PCV), blood and urine osmolality, and urinary as well as plasma sodium and potassium in ponies under four conditions: water but no food; food but no water; neither food nor water; food and water.

2. Methods

This experiment was approved by Cornell University's Institutional Animal Care and Use Committee.

2.1. Animals and Housing

Six male castrated Shetland-type ponies (age range: 8-13 years; mean weight = 287 ± 17.2 kg) were used. They were kept for the 24 hours of each experiment in raised 1.82 × 0.8 m metabolism stalls that allowed collection of urine in a pan placed beneath the center of the stall. The urine was strained through a screen and cheesecloth to eliminate any hay or other debris. Sterile catheters were placed in the jugular vein before each experiment was begun to facilitate and render painless frequent blood sampling. Hay was available ad libitum, except during the no food (NF) conditions. Water was presented in a bucket and the amount drunk measured; there was minimal spillage. The ponies were released in a pasture for 3-5 days between experiments. All the experiments were carried out in the summer.

2.2. Blood and Urine Analyses

Blood samples were taken into preheparinized sterile tubes and immediately refrigerated. PCV determinations were made in duplicate using a microhematocrit technique (Micro-capillary reader, Damon/IEG Division, Needham Heights, MA). Plasma protein concentration was estimated to within ±0.1 g/dL with a handheld refractometer (Veterinary Refractometer, AO Veterinary Instruments Helsinkistrasse 9, Ch-4023, Basil Friedlander SZ). Plasma and urine osmolality were determined in duplicate on a 2-mL plasma sample using a freezing point 30 osmometer (Osmette A, Precision Systems, Inc., 16 Tech Circle, Natick, MA). This instrument has a precision of ±0.5 mOsm/kg. All these measurements were made on the same day as the experiment. Sodium and potassium levels in plasma and urine were measured using a flame photometer (model 443, Instrumentation Laboratory).

2.3. Experimental Procedures

Blood parameters were measured under four conditions: (i) control: water and food available ad libitum (WWWF); (ii) no water but with food (NWWF); (iii) no water no food (NWNF); and (iv) water available but no food (WWNF). The order of treatments was randomized. Experiments began at 6 A.M. Blood samples were taken hourly until 22 hours, after which they were taken only when the animal urinated. This timing of blood samples was for determination of urinary clearance values. However, to compare and summarize the results of separate experiments, the results of the blood samples taken at regular intervals, that is, at the beginning of the deprivation period and at 22 hours (1320 minutes) were used for statistical analysis. Data were not available for all the ponies for hours 23 and 24.

Each time the pony urinated, another blood sample was taken, and total urine volume was measured. Deprivation experiments were of 24 hours duration or the time of the next urination after the 6 A.M. sampling at the end of the 24 hours deprivation period. Urine volume excreted during the 24 hours deprivation was also measured and samples taken for sodium, potassium, and osmolality determination.

2.3.1. Estimations of Blood Volume Changes

Although neither blood nor plasma volumes were measured directly in these experiments, changes in blood volume could be estimated by calculation. This was done for comparison of the change between initial volume at the beginning of the deprivation period and volume at the end of the period. These calculations were done as previously described [2], based on changes in plasma protein and PCV values. An assumption that could not be verified was that there was no loss of plasma protein.

In brief, the ratio between initial blood volume (V_1) and the blood volume (V_2) at the end of a deprivation period can be estimated. The rationale follows: V_2 will equal the sum of its plasma volume (PlV_2) and total red blood cell volume ($RBCV_2$). PlV_2 can be estimated in terms of PlV_1 (initial plasma volume) on the assumption that the total amount of plasma protein in the vascular system does not change between the beginning and end of the deprivation period. In that case, PlV_2 equals PlV_1 times the ratio $PlPr_1/PlPr_2$, where PlPr indicates the respective plasma protein concentrations. PlV_1 will equal $[1 - PCV_1]$ times V_1. PCV_1 is initial PCV. $RBCV_2$ will equal V_2 times PCV_2.

Therefore:

$$V_2 = [1 - PCV_1][P1P_{r1}/P1P_{r2}]V1 + (PCV_2)V_2$$

And, by rearrangement: $V_2/V_1 = [1 - PCV_1]\ [P1P_{r1}/P1P_{r2}]/[1 - PCV_2]$. Here a ratio greater than 1 indicates a greater end blood volume compared with initial volume. The results can be expressed as a percentage, for example, a ratio of 1.05 will indicate a 5% greater volume at the end of the deprivation period than the initial volume.

In addition to the assumption of a constant total amount of plasma protein within the blood vascular system, there are other assumptions implicit in these calculations. The method of calculation takes into account the possible addition or subtraction of red cells, as they may be stored in the spleen and released on eating or drinking. However, it is assumed that only insignificant amounts of plasma would be added with those additional red cells. All methods of estimating blood or plasma volumes contain such assumptions, accounting in part for their imprecision.

2.4. Clearance

Clearance was calculated for osmolytes, by dividing the quantity (flow rate) of urine (mL/min) times the concentration

in the urine by the plasma concentration. Free water clearance was calculated by subtracting the ratio of urine osmolality (U_{osm}) to plasma osmolality P_{osm} times urine flow (V) from urine flow (V). Clearance of water = V − (U_{osm}/P_{osm}) V. Solute clearance was calculated by dividing the rate of excretion of the solute by the plasma concentration.

2.5. Statistical Analyses

The General Linear Model procedures of the SAS system [7] were used to assess the significance of the results summarized in the figures. The factors used in the analysis were horse and treatment. SAS contrast statements were used to test for significance between control values and each of the deprivation conditions. Paired *t*-tests were also applied to specific differences of interest.

3. Results

The ponies drank a mean of 13.1 ± 2.1 kg of water in the 24 hours when both food and water were available and 3.5 ± 1.4 kg when water but not food was available ($t = 10.08$, $P < .001$).

3.1. Changes in Blood

Figure 1 is a plot of plasma osmolality, plasma protein, and PCV during a typical experiment.

3.1.1. Osmolality

Changes in plasma osmolality over the 24 hours deprivation period are shown in Figure 2. The value at each hour is the change from the initial value at 0 hour. The osmolality at time 0 was 285.1 ± 1.56 mOsm/kg. There was a significant effect of condition on plasma osmolality ($F_{8,15} = 4.32$, $P = .007$) at 22 hours deprivation. The osmolality was significantly higher in the NWWF (296.4 ± 4.79 mOsm/kg) than in the WWNF (284.4 ± 1.81 mOsm/kg) ($P = .004$) and higher than in the WWWF (285.6 ± 1.87 mOsm/kg)

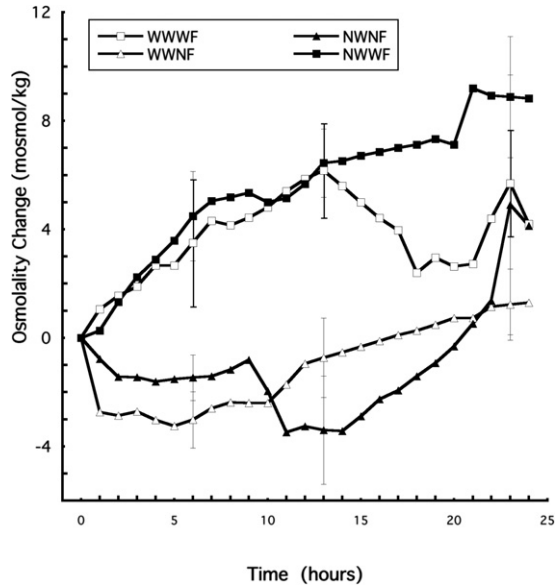

Fig. 2. Plot of plasma osmolality changes from initial value (time 0) during deprivation periods for the four conditions over 24 hours. Vertical lines are the standard error of the means at 12 and 22 hours.

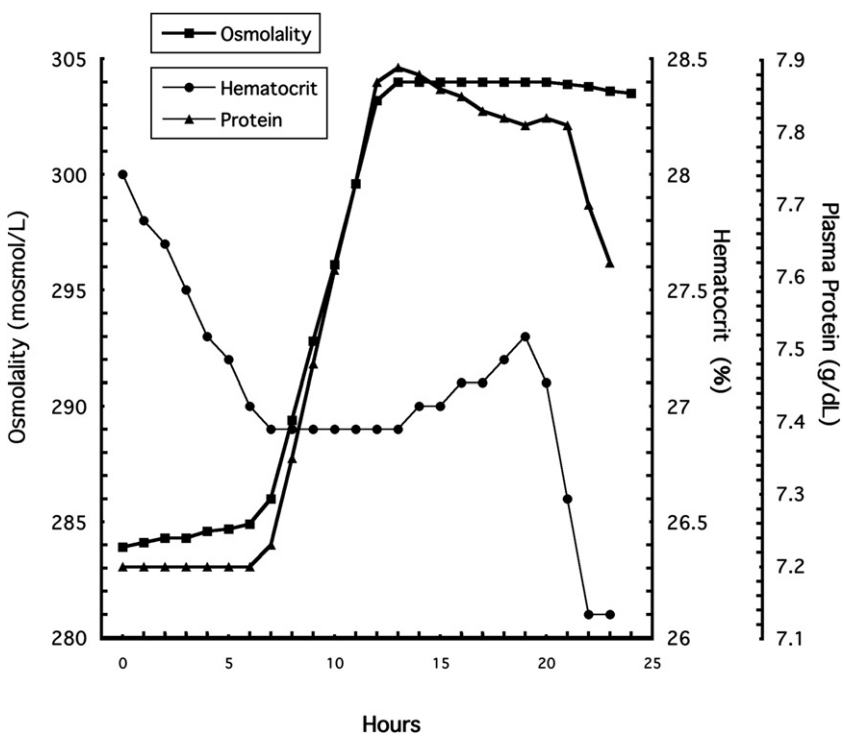

Fig. 1. Plot of blood plasma osmolality for one pony during a water deprivation period of 24 hours with food freely available. Water was removed at time 0 and blood samples were drawn hourly and after 20 hours whenever the pony micturated spontaneously.

condition ($P = .007$). By the 19th hour, the rise of osmolality in Figure 2 in the NWWF condition reached 7.3 mOsm/kg, whereas osmolality in the NWNF condition fell to 0.9 mOsm/kg.

3.1.2. Plasma Protein

Plasma protein concentration at time 0 was 7.06 ± 0.15 g/dL. Changes of plasma protein concentration with time and condition are shown in Figure 3. There was a significant overall effect on plasma protein at 22 hours ($F_{8,15} = 11.68$, $P < .0001$), but none of the conditions were significantly different. Plasma protein (Fig. 3) continued its steady rise in all conditions but was most pronounced in the NWWF, attaining +0.8 g/dL above the initial value by the 14th hour.

3.1.3. Packed Cell Volume

The changes in PCV are illustrated in Figure 4. The PCV at time 0 was 31.4% ± 2.3%. The PCV fell when food was not available. The PCV remained low in the NWNF condition. At 22 hours, there was a significant over all effect on PCV ($F_{8,15} = 5.47$, $P = .002$). NWNF (28.3% ± 1.3%) was significantly different from NWWF (31.2% ± 1.6%) ($P = .012$) and WWWF (30% ± 2.1%) ($P = .008$).

PCV continued its downward trend (Fig. 4) under the NF condition to the fifth hour and then rose, whereas it rose over the whole course of the experiment in the NWWF condition. Finally, although control levels of all parameters varied closely about a mean level during the first 12 hours, as the dark phase of the second 12 hours began, all showed shifts, presumably owing to lowered food and water intake during the night.

3.1.4. Potassium and Sodium Changes

The potassium levels in the plasma differed significantly with condition, falling 0.5 mEq/L from baseline values with food deprivation ($F_{8,15} = 3.32$, $P = .02$) (Table 1). Potassium was lower in the NWNF than in the WWWF ($P = .01$) and the NWWF ($P = .0009$) condition. Sodium levels rose with water deprivation, increasing 4.8 mEq/L in the NWWF condition. At 22 hours, there was a significant overall effect on sodium ($F_{8,15} = 3.99$, $P = .01$). Sodium levels were higher in the NWWF than in the WWWF condition ($P < .001$), and levels in NWNF were significantly lower than those in NWWF ($P = .005$).

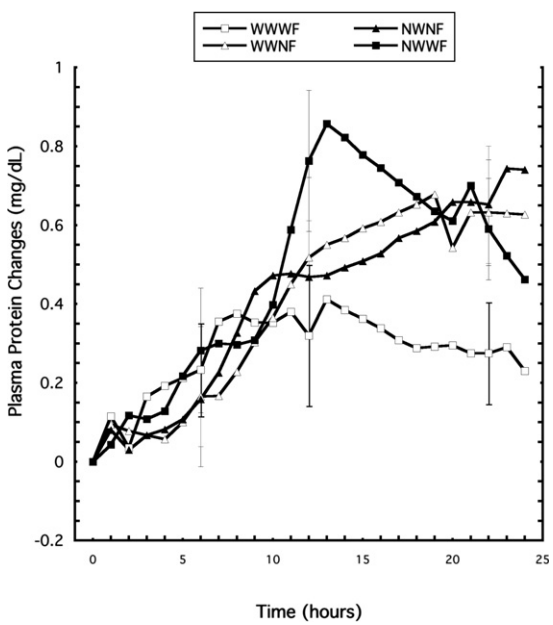

Fig. 3. Plot of plasma protein concentration changes from initial values (time 0) during deprivation periods for the four conditions for 22 hours. Vertical lines are the standard error of the means at 12 and 22 hours.

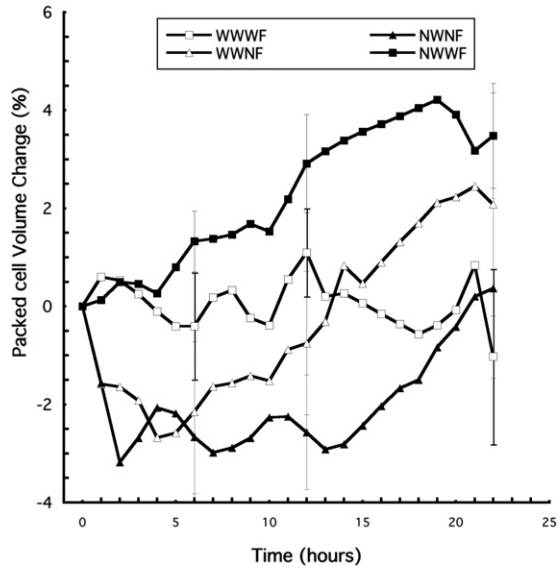

Fig. 4. Plot of packed cell volumes changes from initial values (time 0) during the deprivation periods for the four conditions for 24 hours. Vertical lines are the standard error of the means at 12 and 22 hours.

3.2. Urine Changes

Urine volumes voided during the 24 hours deprivation periods were nearly identical in all conditions, in that 7-8 L were voided (Table 2). Total urinary osmolyte excretion differed significantly ($F_{8,15} = 5.25$, $P < .003$). Urinary osmolyte excretion in the condition of WWWF differed from that of both NWNF and WWNF ($P = .01$ and $P = .03$, respectively); NWNF differed from NWWF ($P = .002$) and NWWF differed from WWNF ($P = .006$). Free water clearance also differed significantly among conditions ($F_{8,15} = 3.23$, $P = .024$). Free water clearance was significantly higher in the WWWF than in the NWWF condition ($P < .034$), and both the NWNF and the WWNF conditions

Table 1
Electrolyte values of ponies deprived of food and/or water for 22 hours

Condition	K mEq/L	Na mEq/L
WWWF	3.89 ± 0.11	136.7 ± 0.63
NWWF	3.59 ± 0.18[a]	141.5 ± 0.42[a,b]
WWNF	3.64 ± 0.10	136.9 ± 0.8
NWNF	3.33 ± 0.10[a]	138.1 ± 1.05

WWWF, with water with food; NWWF, no water with food; WWNF, with water no food; NWNF, no water no food; ± SEM.
[a]Differs significantly from NWNF.
[b]Differs significantly from control WWWF.

Table 2
Urinary excretion values for ponies deprived of food and/or water for 22 hours

Condition	Urine Output L	Free Water Clearance mL/min	Osmolytes mOsm/L	Total K mEq	Total Na mEq
WWWF	7.26 ± 0.78	−9.6 ± 4.8[a]	7250 ± 639	778 ± 36	5.4 ± 0.8
NWWF	7.10 ± 0.49	−18.0 ± 7.3	8401 ± 719	3785 ± 633	235.0 ± 110.
WWNF	7.13 ± 1.17	−15.7 ± 7.0[a]	5137 ± 626[b]	778 ± 196	19.7 ± 11.6
NWNF	7.41 ± 0.55	−8.6 ± 2.1[a]	4560 ± 694[b,c]	2249 ± 721	179.4 ± 82

WWWF, with water with food; NWWF, no water with food; WWNF, with water no food; NWNF, no water no food; ± SEM.
[a]Differs significantly from NWWF.
[b]Differs significantly from control WWWF.
[c]Differs significantly from WWNF.

were significantly different than NWWF ($P = .0029$ and $P = .003$, respectively). There was no difference in urinary sodium excretion with condition, but urinary potassium excretion differed significantly with condition ($F_{8,12} = 5.02$, $P = .007$), although none of the contrasts were significant.

3.3. Blood Volume

Changes in blood volume between the start of the deprivation period, that is, at time 0, and the end of the 22 hours deprivation conditions were calculated. A small insignificant decrease (3.3%) occurred during the control experiment where the pony had food and water freely available. When both water and food were withheld, blood volume fell by more than 7.2% from its initial value at 0 hour. When water but not food was withheld, blood volume fell by 7.4%. When both food and water were withheld, the decrease was 6.3%. The decreases in blood volume in the three deprivation conditions were not significantly different from control.

4. Discussion

The most dramatic effect of the different deprivation conditions was the marked rise of plasma osmolality when food was consumed during the water deprivation period (Figs. 1 and 2). This indicates that the primary stimulus to drinking during water deprivation with food available was the rise in osmolality. The changes in these experiments approximate the rise of plasma osmolality needed to stimulate drinking, as has been reported for the pony [8].

Pigs and horses are species in which a volatile spleen releases its store of red cells in response to mild stimuli, such as eating [9]. In an earlier study, it was found that simply eating a spontaneous meal resulted in addition of a significant volume of red cells in pigs, presumably from the spleen, resulting in a constant blood volume during a meal despite shifts of fluids from the plasma into the digestive tract [10]. A fall of PCV when food was withheld occurred in the ponies of this study (Fig. 4), indicating a fall of total red cell volume.

The study of food and water deprivation in pigs showed a decrease of both plasma volume and total red cell volume, resulting in a large fall of total blood volume during the NWNF condition [2]. The lack of significant effect of water deprivation on plasma protein and volume indicated that horses are better able to maintain homeostasis during water deprivation than pigs. This comparison is particularly interesting because the experiments were performed in the same laboratory using the same analytical equipment. In horses, the larger hindgut containing 10-20 L of fluid may buffer water deprivation [11]. Water restriction for 20 hours reduced the amount of water in the gastrointestinal tract of horse by 13% [12]. The extracellular fluid volume is 240 mL/kg in ponies [13]. Other equids have not been studied under all four conditions, but in ponies deprived of food and water for 72 hours at high environmental temperatures (maximum 33°C), plasma protein as well as osmolality rose and plasma volume fell [14]. Donkeys have been deprived of water but not hay in several studies [15-17], but have not been deprived of both food and water. Physiological measurements have not been made on any other equid, although the long journeys and times between drinking opportunities indicate that studies on onagers and zebras would be of interest [18].

PCV measurements in water-deprived ponies (Fig. 4) showed a distinct dichotomy: with food available, PCV levels were maintained; without food, PCV fell. Splenic stores of red cells are released on eating, and this may be the explanation of the maintained PCV levels when food was available. The obverse process may be occurring during the NWNF period: that is, red cells are removed from the circulating blood volume and put into splenic storage. Plasma osmolality fell steadily throughout the 12 hours (Fig. 2). This picture of osmolality and plasma proteins falling and predominantly being below initial levels suggests dilution of plasma proteins and electrolytes. If there is an expansion of plasma volume, then the fall of PCV could in part be simply a dilution process, but sequestration of red cells in storage sites such as the spleen could also be involved. The final rise of plasma proteins suggests a contraction of plasma volume. The accompanying final rise of PCV could be the result of this decrease of plasma volume or of splenic release of red cells in the NF conditions. The condition of WWNF rounds out the experimental design of this study. Here both plasma proteins and PCV values decreased during the first 7 or 8 hours of food deprivation, and then both tended to rise during the final hours.

Urine volume excreted during the 22 hours deprivation periods was used as an index of water loss from the body on the assumption that rate of water loss in expired gases, by diffusion through the skin, and in feces was relatively small and constant. Although urine volumes voided during the 22 hours water-deprivation periods were nearly identical, the clearance of water and osmolytes differed significantly with condition (Table 1). Free water clearance fell in the NWWF condition in comparison with the others. Osmolyte excretion was lower in the NF condition irrespective of whether water was available. The reduced clearance of water is probably an effect of vasopressin that rose from

1.4 pg/mL to 4.3 pg/mL in ponies after 24 hours water deprivation with hay available [19]. Fecal output and water content would have been a valuable measurement of the contribution of gastrointestinal fluid to maintenance of blood volume. Donkeys subjected to chronic dehydration, causing a loss of 6% of their body weight, exhibited changes in their large intestinal structure that increase absorptive and secretory processes [20].

The amount of water drunk was more than three times greater when food was available as compared with amount drunk when no food was available. There are several reasons for the increased water intake when food is available. The extracellular space is reduced by secretion of isotonic digestive juices, and the osmotic content of the food causes cellular dehydration and ultimately loss of body fluid from the osmotic diuresis that ensues [21,22]. As shown in Figures 1 and 2, a marked rise of plasma osmolality develops when food is available during water deprivation, but this is not accompanied by a significant hypovolemia. In contrast, when food is not available, plasma osmolality tends to fall. This emphasizes again the relatively greater effectiveness as a thirst stimulus of cellular dehydration due to the rise of osmolality. Houpt and Yang [2] also found that plasma osmolality rose when water but not food was withheld from pigs.

There are a variety of studies of the effects of dehydration on horses, but none have compared horses with and without food available. Tasker et al. [23] deprived horses of both food and water for a week. The 400-kg horses lost 40 kg of body weight and their plasma protein rose, but there was no effect on PCV. Sneddon et al. [24] deprived horses of water but not pelleted feed for 72 hours and found an increase in osmolality from 285 mOsm/L to 310 mOsm/L, an increase in PCV from 36% to 44%, and an increase of plasma protein from 6.5 g/dL to 7.8 g/dL. Vasopressin increased and aldosterone decreased with 72 hours dehydration in the horses [20]. Aldosterone probably decreased in this study under the NWWF condition because both plasma and urinary sodium rose. It was found, in an earlier study, that vasopressin increased after 24 hours dehydration with hay available [19]. The fall in plasma potassium in the NF conditions is probably an indication of lack of dietary potassium.

After 30 hours of transportation by truck and without food or water during hot weather, horses were severely dehydrated. They exhibited significant increases in both osmolality (up to 330 mOsm/L) and plasma protein (up to 10 g/dL) [25], indicating both hyperosmotic and hypovolemic changes. They lost 50 kg body weight due to water restriction and water loss from sweating. Other less severe forms of water deprivation occur when horses are water but not food restricted during commercial estrogen production [26]. There was no change in plasma protein or PCV but osmolality rose from 282 mOsm/kg to 293 mOsm/kg when water was restricted to 3 L/100 kg/day. Horses are also dehydrated when treated with furosemide before a race to prevent exercise-induced pulmonary hemorrhage. In this case, plasma protein rises from 7.2 g/dL to 7.8 g/dL and PCV from 33% to 36%, but osmolality is unaffected because both sodium and water are lost in the urine in response to the diuretic [27].

Food intake falls when most animals, including horses, are water deprived. For example, in an earlier study from this laboratory, ponies had a 6% change in plasma osmolality and an 11% increase in plasma protein after 36 hours of water deprivation. The ponies were offered hay every 4 hours. They consumed 11 kg when water was available and 7 kg when deprived. The donkeys, a desert-adapted species, ate 8 kg when water was available and 7 kg when water deprived [15].

In conclusion, these simple experiments highlight the need to consider the feeding conditions in any study of the effects of water deprivation. There is a positive correlation between feeding and drinking. The ponies drank less when no food was available and ate less when no water was available. Despite these behavioral changes, there were physiological consequences: a marked hyperosmolality when food but no water was available. Ponies appear to be able to moderate the effects of dehydration and preserve blood volume possibly by increased absorption from the gastrointestinal tract and reducing free water clearance in the kidney.

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