



## Original Research

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

Maira L. Norris DVM, Katherine A. Houpt VMD, PhD, T. Richard Houpt VMD, PhD

Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

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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 ( $13.1 \pm 2.1$  kg) than when water but not food was available ( $3.5 \pm 1.4$  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.

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## 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

Corresponding author at: Katherine A. Houpt, VMD, PhD, Animal Behavior Consultants of Northern Michigan, 3065 East Dixon Lake Road, Gaylord, MI 49735.

E-mail address: [kah3@cornell.edu](mailto:kah3@cornell.edu) (K.A. Houpt).

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 =  $287 \pm 17.2$  kg) were used. They were kept for the 24 hours of each experiment in raised  $1.82 \times 0.8$  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  $\pm 0.1$  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  $\pm 0.5$  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 ( $PIV_2$ ) and total red blood cell volume ( $RBCV_2$ ).  $PIV_2$  can be estimated in terms of  $PIV_1$  (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,  $PIV_2$  equals  $PIV_1$  times the ratio  $PIPr_1/PIPr_2$ , where  $PIPr$  indicates the respective plasma protein concentrations.  $PIV_1$  will equal  $[1 - PCV_1]$  times  $V_1$ .  $PCV_1$  is initial PCV.  $RBCV_2$  will equal  $V_2$  times  $PCV_2$ .

Therefore:

$$V_2 = [1 - PCV_1][PIPr_1/PIPr_2]V_1 + (PCV_2)V_2$$

And, by rearrangement:  $V_2/V_1 = [1 - PCV_1][PIPr_1/PIPr_2]/[1 - PCV_2]$ . 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

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