Population Parameters of Equine Urine pH and Methods of Acidification

T. W. Wood, T. J. Weckman, P. A. Henry, S.-L. Chang, W. Hyde,* J. W. Blake and T. Tobin

Kentucky Equine Drug Testing and Research Programs
Department of Veterinary Science
Maxwell H. Gluck Equine Research Center
University of Kentucky
Lexington, Ky 40546-0099

and

Veterinary Diagnostic Laboratory
College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

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Abstract

Currently available evidence suggests that the urine pH of horses maintained on pasture is consistently alkaline and that of a substantial proportion of post-race urine samples of racing horses is acidic. Since urine pH can greatly affect the urine concentration of some drugs, uncertainties are created when data generated in grazing horses are compared or extrapolated to racing horses. Our investigation of the urine of grazing horses shows that the mean pH level is about 7.9 and that if their diet is supplemented with grain their mean pH level drops to about 7.4 There appears to be no significant effect of time of day or year on urine pH levels in horses. However, horses taken from pasture and supplemented with grain in a stalled environment will show a slight decrease in urine pH. Urine samples subjected to different initial storage treatments are quite stable with regard to pH for 48 hrs. but then showed a marked increase. The potential of ammonium chloride, ascorbic acid, lactic acid and methionine to rapidly acidify the urine of research horses was investigated using both oral and intravenous routes of administration. While all methods tested showed varying degrees of efficacy, oral administration of ascorbic acid proved to be the safest and most effective method to model the rapid acidification of urine seen in post-race samples.

Introduction

The pH of urine has long been known to exert an effect on the distribution of weak acids and bases between blood and urine (Goodman and Gilman,1980). These principles are well established in medicine and are used in therapeutic situations to alter the distribution of drugs in the body and either reduce their toxicity or aid in their elimination (Doull, Klaassen and Amdur, 1980).

Post-race urine samples from racing horses show a bimodal distribution of pH levels, ranging from a low of about 4.5 to a high of 9.0 (Houston et al, 1985).

This is a very wide range of values and on first evaluation would appear likely to affect the distribution of drugs between blood and urine. In fact, it has been shown that the concentrations of phenylbutazone and its metabolites in post-race urine samples are highly dependant on urine pH (Houston et al, 1983).

Preliminary investigations conducted by Dr. Walter Hyde at the Iowa State University Veterinary Diagnostic Laboratory indicate that a similar situation may exist in racing dogs. However, the range of pH values does not appear to be as severe, and the values do not fit a biomodal distribution as clearly as the equine post-race urine samples. Nevertheless, exercise induced acidification of the urine in racing dogs, and the accompanying effect on drug distribution, may pose a problem similar to the situation in horse racing.

A major problem in studying the effect of urine pH on the distribution of drugs and drug metabolites in horse urine is that no useful model for approximating the effect of exercise on urine pH exists. Beyond this, no study on the normal ranges of urine pH in non-exercised horses has been reported. In this study we investigated several parameters pertaining to the pH of urine in non-exercised horses and evaluated numerous laboratory models to approximate the acidic urine seen in a substantial proportion of post-race samples.

Materials and Methods

Experimental

Population distributions were obtained from research herds consisting of Thoroughbred, Standardbred and Quarterhorse mares. In acidification experiments, Thoroughbred or Standardbred mares weighing approximately 500 kg were used. All urine samples were collected by direct bladder catheterization. Determination of pH levels was accomplished immediatly following collection by use of a portable pH meter (Corning M107). The meter was calibrated daily using standard pH buffers and the results were checked periodically by alternative techniques to insure accuracy. All acidifying agents used were obtained from Sigma Chemical Co., St. Louis, Mo. All grain mixtures were obtained from Farmers Feed Mill Inc., Lexington, Ky. When applicable, statistical analysis was accomplished by analysis of variance or by use of the student's t test.

Population Parameters

Urine samples were collected over a 30 day period from 35 mares maintained on pasture alone and from 30 mares maintained on pasture along with daily grain supplementation. The effect of hay alone verses hay plus grain supplementation was tested in six mares kept in box stalls for five days. A group of ten mares that were maintained on pasture alone were monitored the first week of each month to determine any possible effect of season on urine pH.

Possible diurnal effects on urine pH were investigated in six mares kept in box stalls and monitored around the clock. Fifteen urine samples were checked for pH values and then subjected to three different storage treatments for 24 hrs. Treatments consisted of freezing, refrigeration at 4°C or storing at room temperature. At the end of the 24 hr. treatment period, all samples were maintained at room temperature in capped specimen containers and pH levels monitored until the levels rose substantially above baseline.

Acidifying Agents

Intravenous (IV) infusion of lactic acid (30% aqueous solution) and L-ascorbic acid was tested for effects on blood and urine pH in six horses. The effects of feed supplementation with methionine and L-ascorbic acid were evaluated in six mares. Corn oil or liquid molasses was used as a vehicle for addition of the agents to a grain mixture. Control horses received the grain mixture plus the vehicle. Using cross-over designs, crystalline ammonium chloride, D-L methionine, L-ascorbic acid and water as a control were administered to a total of eight horses.

Results and Discussion

Analysis of urine samples from 65 research horses maintained at pasture shows a distribution of urine pH that is linked to the presence or absence of dietary grain supplementation. A population of 35 mares maintained at pasture year round without any form of grain ration had a mean urine pH value of 7.94 (Fig 1A). When we examined a second group of grazing horses on the University of Kentucky farm which were being fed 2.5 lbs. of grain mixture daily we found their mean urine pH to be 7.42 (Fig 1B) .

The data suggests that this significant difference in the pH values between the two groups is related to their diet. It is interesting to note that the grain fed group shows a more "classically defined" normal distribution than the group maintained on pasture alone. With or without grain supplementation, our data suggest that grazing horses can be expected to show slightly alkaline urine on a consistant basis. As can be seen in Fig 3 and Fig 4, urine pH levels in grazing horses appear to be relatively stable during any given 24 hr. period and also over a period of months. However, a close examination of Fig 4 shows that the pH levels tended to be lower during the hot summer months but statistical analysis suggests that there is no trend or significant differences.

When grazing horses are placed in box stalls and fed hay plus a grain supplementation their urine pH showed a transient drop when compared to horses fed hay alone (Fig 2). In many equine pharmacokinetic studies, grazing horses are placed in box stalls for the 4 or 5 days required for the study (Tobin, 1981) . If the kinetics of the drug in question can be influenced by urine pH, care should be taken in the feeding of these horses. Individual research laboratories should develop a uniformity to their feeding schedules to insure reproducibility of kinetic data.

Since urine samples collected for chemical analysis are usually not processed immediately, we tested for pH stability of these samples when subjected to routine storage treatments (Fig 5). It would appear that urine samples will remain stable with regard to pH for 48 hrs. In addition, we have periodically thawed stored samples and checked for pH fluctuations and have found minimal variation in samples frozen for as long as ten months.

As stated earlier, the pH of many post-race urine samples are acidic with some samples as low as 4.5. This acidification would appear to be a result of heavy exercise (Snow, 1983). The differences in urine pH levels between grazing horses and horses following heavy exercise have traditonally been accorded little weight in the area of interpretation of urinary concentrations of drugs. However, recent evidence has shown that the pH of the urine can have a marked effect on the distribution of phenylbutazone in the horse (Houston et al, 1985).

These observations than give rise to the question of how one might rapidly change the urine pH in grazing non-exercising horses to mimic the pH profiles seen in post-race samples. The classic method of altering the pH of the urine of a horse is to administer ammonium chloride (Evans and Lambert, 1974). As can be seen in Fig 6, after a single oral administration of ammonium chloride the pH of the urine drops over a 24 hr period to a low of about 6.4. The magnitude of this effect falls short of the desired 4.5 pH level and we therefore decided to examine other methods.

Based on the fact that many grains are high in methionine content (Church and Pond, 1976) and that methionine has been used as a urinary acidifier (Leman and Relman, 1959), we investigated the effect of large amounts of this amino acid in the diet of the horse. We conducted several feeding experiments with methionine added in excess to the diet of stabled horses. Fig 8 shows that methionine added to the feed can cause acidification but there exists considerable variation among individuals with regard to palatability, absolute change in urine pH and onset of acidification. In subsequent feeding experiments with ascorbic acid (Fig 8) added to the feed, we observed similar wide variations in efficacy.

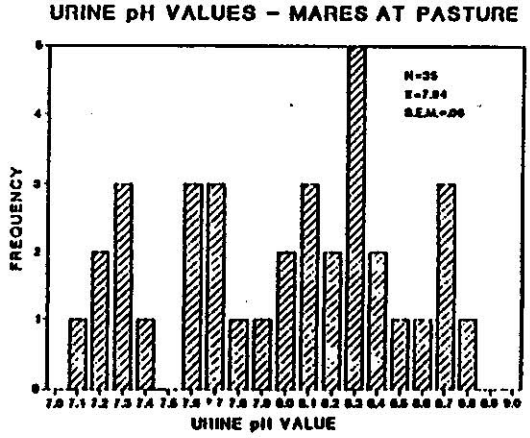
Since the drop in pH seen in racing horses is thought to be a result of excess lactic acid in the blood, we decided to explore the effects of IV infusions of lactic acid, as well as ascorbic acid. In our studies with lactic acid we found it to be of little practical value to simulate the racing situation as it proved to be dangerous and lacking efficacy. Simarily, we found IV infusions of asorbic acid to have little or no effect on urine pH (Fig. 7.). In addition, when infused, both acids can cause transient loss of membrane integrity of blood vessels and can thus lead to severe edema of the surrounding tissue. Based on our studies, we can not recommend infusions of acidifying agents as a safe or practical method as a research tool.

Following our limited success with ammonium chloride, we conducted a series of cross-over within subject experiments of gastric intubation with ammonium chloride, methionine, ascorbic acid and water (Fig 9) . A comparison of these agents shows that all can lower urine pH following a single administration. However, ascorbic acid is clearly superior in producing acidic urine in the shortest time frame. Fig 10 illustrates that

this effect of ascorbic acid is dose dependent, with 1kg per horse producing reliable results without evidence of harm to the animal. All animals were closely watched for gastric discomfort or other side effects and we observed no complications with this method. Since ascorbic acid is eliminated unchanged in the urine once its maximal renal tubular reabsorptive capacity is exceeded (Toggenburger et al, 1981), its potential for toxicity and interference with drugs metabolized in the liver is very low. We therefore believe that this method clearly has potential both as a therapeutic strategy and as a research tool for rapidly acidifying the urine in the horse.

Fig. 1 *Frequency distribution of urine pH values in 65 grazing horses at the University of Kentucky. The upper panel (A) shows a distribution of urine pH values from 35 mares maintained on pasture only. The lower panel (B) shows a distribution from 30 mares which were being supplemented with 2.5 lbs of grain per day.*

A.



B.

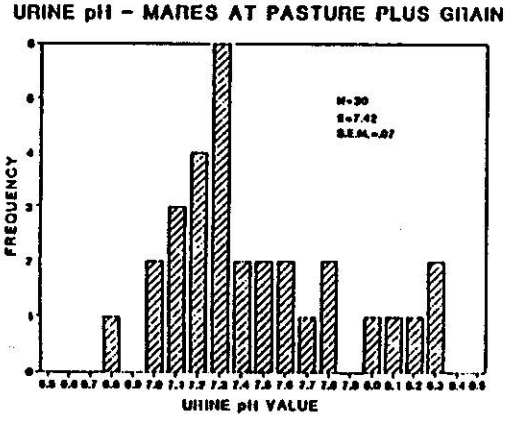


Fig. 2 *Effect of grain supplementation on equine urine pH. The solid triangles (▲-▲) show urine pH values in horses fed hay only. The solid squares (■-■) show urine pH values of horses fed hay plus 5 lbs. of grain per day.*

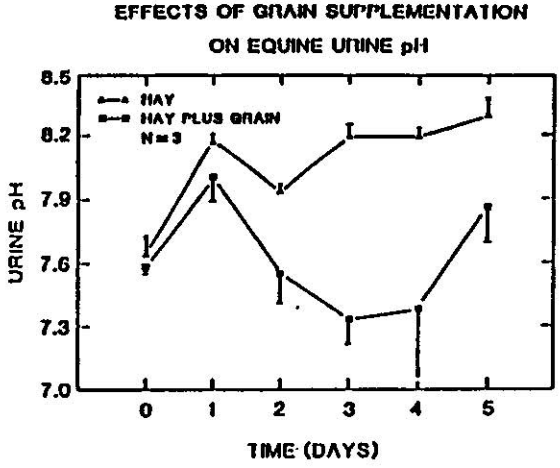


Fig. 3 *24 hr profile of urine pH levels of six mares kept in box stalls. The points represent the mean pH values.*

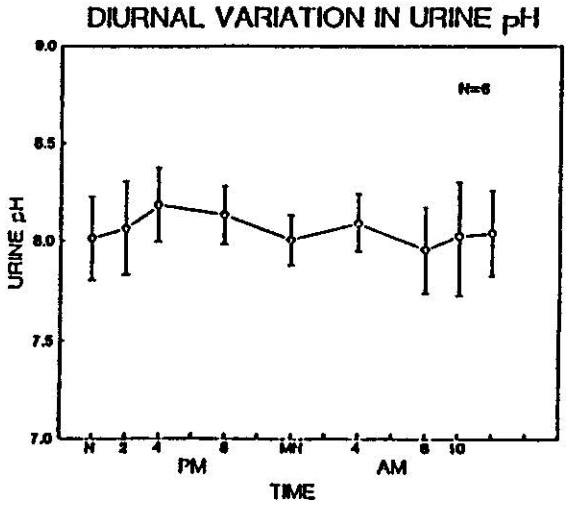


Fig. 4 *Monthly profile of urine pH levels collected from 10 mares the first week of each month. The points represent the mean pH values of the group.*

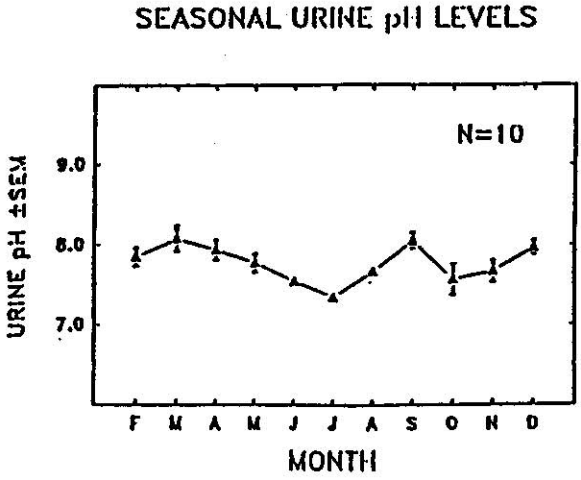


Fig. 5 *The effects of storage on equine urine pH stability. Urine samples from 5 horses were subdivided into 3 treatment groups and stored for 24 hours and then were monitored while being kept at room temperature.*

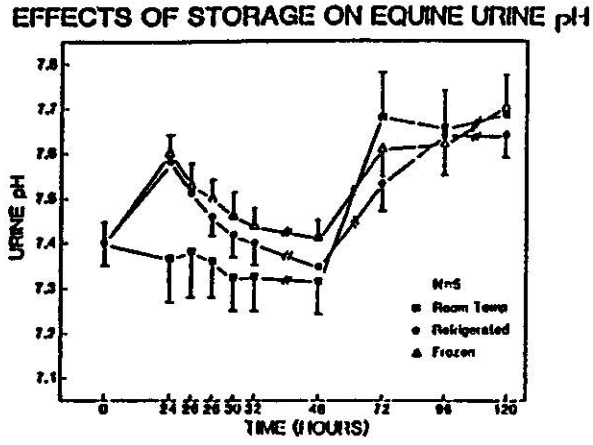


Fig. 6 *The effects of gastric intubation of ammonium chloride on urine pH. Two Thoroughbred mares were dosed in a cross-over design with water as a control (HOH,■-■), .16g NH4Cl/kg body weight (▲-▲) and .33g NH4Cl/kg body weight (●-●).*

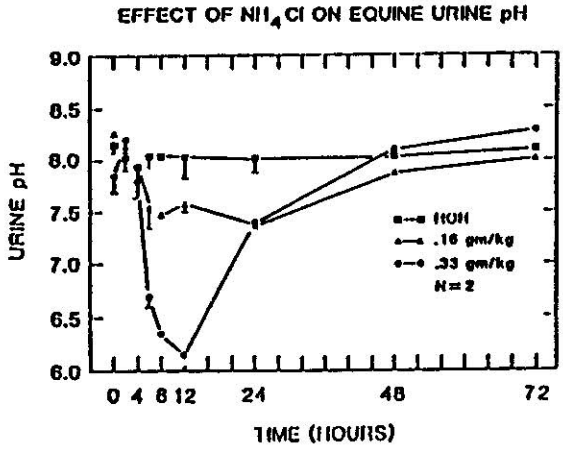


Fig. 7 *Comparison of the effects of IV (■-■,200 gm) and oral (●-●, 1 kg adminstration of ascorbic acid on equine urine pH.*

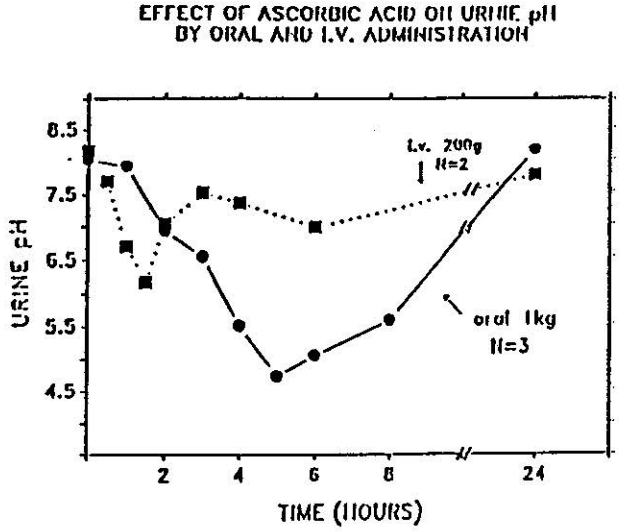


Fig. 8 *Effect of methionine and ascorbic acid on equine pH when fed in the diet. Each treatment group contained 4 mares. On days 0 and 1 the mares were fed a grain mixture plus liquid molasses as a vehicle for the methionine (500g) and the ascorbic acid (500g). Control mares received grain plus the vehicle. On days 2,3,4, and 5 the mares received the grain mixture only. The points represent the mean urine pH.*

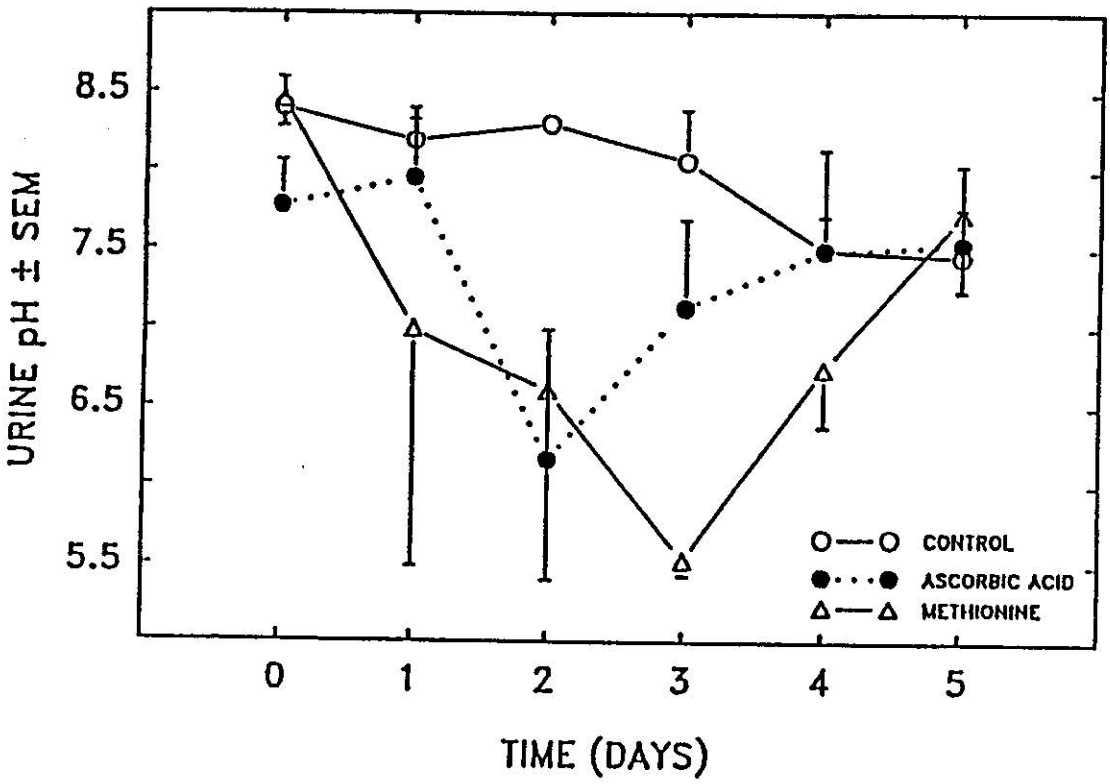


Fig. 9 *Effects of gastric intubation of 160 g ammonium chloride (Δ-Δ), 500 g methionine (▽-▽), 1 kg ascorbic acid (□-□) and 2 liters of water (○-○) on urine pH. A total of eight horses were used in a series of cross-over designs.*

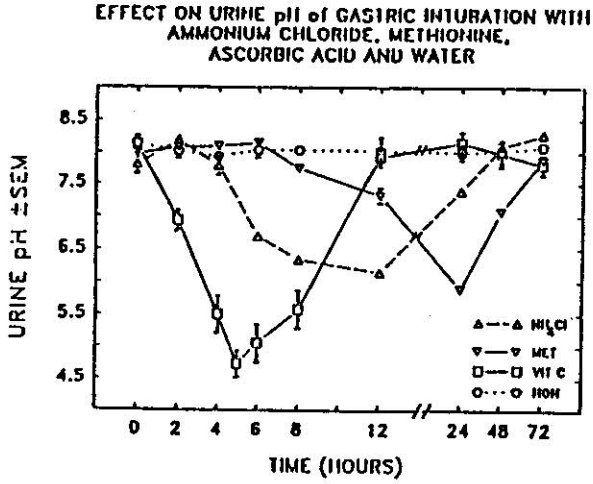
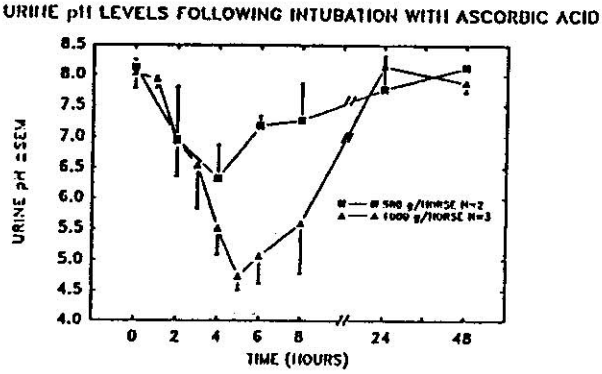


Fig. 10 *Dose-response effects of ascorbic acid on urine pH following gastric intubation.*



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Discussion

PIERRE BEAUMIER We did a lot a studies with alfalfa cubes which are very high in salicylates content which presumably would be metabolized down to salcylic acid which would lower the pH. Have you done anything on alfalfa cubes or anything with high alfalfa content?

TW No, we haven't. As a matter of fact, in the experiments we used where we fed hay, we were using a grass hay, we particularly stayed away from alfalfa hay because of the large amounts of protein and possibly different acids in the hay.