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Handling Method Influences Equine Urinary Calcium and Nitrogen

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Abstract: This study was conducted to examine the effects of temperature, time, location and acidification on urinary Ca and N. Urine was collected from 4 horses into a clean bucket and poured through cheesecloth. Urine was stirred and 5 mL was pipetted into vials and tightly capped. Twenty-one different treatments were evaluated with combinations of the following variables: urine held in total collection apparatus (yes or no), acid (none, added immediately, or added after urine was frozen and thawed), time held and temperature until freezing (frozen immediately, 6 h at 25°C, 6 h at 10°C, 12 h at 25°C, or 12 h at 10°C). Sample preparation was completed within 45 min. Acid was added at a rate of 20 μL of 30% Hcl mL^{-1} urine. All samples were frozen at -4°C. Urinary Ca was higher when acid was added compared to no acid regardless of holding time, temperature, or location ($p<0.01$). The addition of acid prior to freezing tended to result in samples having higher Ca than samples with acid added after thawing ($p = 0.07$). There was no effect of holding time or temperature on Ca ($p = 0.86$). Urine N was unaffected by the addition of acid to the sample ($p=0.22$). Urinary N tended to be lower when urine was placed in the total collection apparatus for either 6 or 12 h than when urine was not placed in the apparatus ($p = 0.06$) and urinary N was higher when acid was added after thawing compared to before freezing ($p<0.01$). There was no difference in N based upon holding time ($p=0.77$). In this experiment, all samples were tightly capped and N was not able to volatilize. In the field, samples may sit open to air for a time after collection. Therefore, a portion of the study was repeated to test samples left uncapped. Sample processing was conducted as indicated before except that each holding time and temperature combination was repeated with an uncapped vial. Urine from 4 horses was collected and evaluated with 27 treatments. Five combinations of holding time and temperature were examined (frozen immediately, held for 6 h at 25°C, held for 6 h at 6°C, held for 12 h at 25°C, held for 12 h at 6°C). The same acidification methods as indicated before were used. Urine held uncapped for either 6 or 12 h had a higher N ($p<0.01$) than samples held for either 6 or 12 h while capped. These data suggest that urinary Ca is more sensitive to the addition of acid than urinary N while N is more sensitive to evaporation; however urine handling methods influence results of both.

Key words: Horse, calcium, nitrogen, urine

INTRODUCTION

Mineral and nitrogen balance are studied under varying nutritional conditions[1-3]. In order to correctly evaluate the results of a study, it is critical that mineral and nitrogen concentrations measured during a balance study are accurate. Ambient temperature and type of holding container often vary between studies. Many types of collection devices exist for collecting urine from horses over a period of time. Some total collection devices hold the urine in a semi-closed environment[3], while others collect into a relatively open environment[4,5]. Since urine is not typically frozen as soon as it is voided from the horse, the way it is handled from the time the horse urinates to the time the urine is frozen could affect the amount of Ca or N measured in the urine at a later time. The objective of this study was to determine if urine handling (including time, temperature, acidification, and type of environment) prior to analysis alters Ca and N concentration in equine urine.

MATERIALS AND METHODS

Experiment 1: Three mature geldings were used for urine collection. These horses are used in a horsemanship class and have a habit of urinating during class. The horses were followed around the arena until they exhibited signs that they were preparing to urinate. Urine was collected into a clean 18.9 L bucket and then transferred immediately to 500 mL plastic bottles and tightly capped. The urine was then poured through three layers of cheesecloth to remove any hair or debris. The urine was stirred to suspend any precipitate and 5 mL of urine was pipetted into 7 mL vials which were then tightly capped. An additional 500 mL of urine was poured into a Total Collection Device (TCD) for later sampling. The

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TCD used was a horse Anappy@ (Equisan, Australia). Urine from each of the 4 horses was blocked by horse and evaluated with twenty-one treatments. Seven combinations of holding time and temperature were examined (frozen immediately, held in capped vials for 6 h at 25°C, held in capped vials for 6 h at 6°C, held in capped vials for 12 h at 25°C, held in capped vials for 12 h at 6°C, held in TCD for 6 h at 25°C, held in TCD for 12 h at 25°C). Three acidification methods were evaluated for each combination (no acid, acid added just before freezing, or acid added after urine was thawed). Sample preparation was completed within 45 min of collection. Acid was added at a rate of 20 μL of 12 M Hcl mL^{-1}urine. All samples were frozen at -4°C until analyzed. Calcium was measured on a Unicam 989 atomic absorption spectrophotometer (Thermoelemental, Waltham, MA) and N was determined by a Leco FP-2000 (St. Joseph, MI) using AOAC method 990.03.

Statistical analysis: Differences between treatments were determined by orthogonal contrasts using the mixed model procedure in SAS 8.0. Differences were considered significant at $p<0.05$ and trends were evaluated at $p<0.10$. Results are expressed as means SEM.

Experiment 2: Two mature geldings and two mature mares were used for urine collection. These horses were driven for 2 h and then allowed to drink water. They were placed into stalls with fresh shavings. All four horses urinated within 20 min. As soon as urination began, a clean 18.9 L bucket was placed into the stream to collect the urine. Sample processing was conducted as done in Experiment 1 except that each holding time and temperature combination was also repeated with an uncapped vial. Urine from each of the 4 horses was blocked by horse and evaluated with twenty-seven treatments. Five combinations of holding time and temperature were examined (frozen immediately, held for 6 h at 251 C, held for 6 h at 61 C, held for 12 h at 251 C, held for 12 h at 61 C). Each holding time and temperature combination had one capped vial and one uncapped vial. There were 3 acidification methods evaluated for each combination (no acid, acid added just before freezing, or acid added after urine was thawed).

Statistical analysis: Differences between treatments were determined by orthogonal contrasts using the mixed model procedure in SAS 8.0. Differences were considered significant at $p<0.05$ and trends were evaluated at $p<0.10$. Results are expressed as means SEM.

RESULTS AND DISCUSSION

Experiment 1: Urinary Ca was higher when acid was added compared to when no acid was added (1.52 mg mL^{-1}

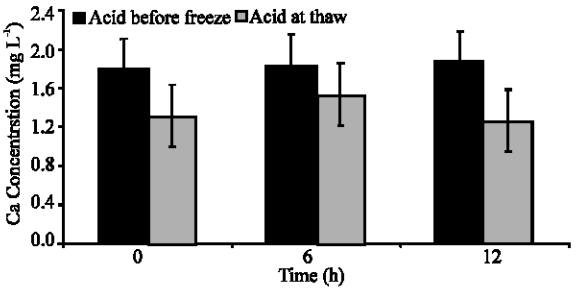

Fig. 1: Urinary calcium concentrations when acid was added before freezing or after thawing. There was a trend for an overall treatment difference (p=0.07), but no difference between time

0.18 vs. 0.59 0.18, respectively) regardless of holding time, temperature, or location ($p<0.01$). The addition of acid prior to freezing tended to result in samples having higher Ca than samples in which acid was added after urine was thawed ($p = 0.07$; Fig. 1), though no other variables had an effect. Urine N was unaffected by the addition of acid to the sample (1.04% 0.26 vs. 1.05% 0.26; $p = 0.22$; Fig. 2). There was a trend for urine placed in the TCD for either 6 or 12 h to have lower N than urine in a tightly capped vial (1.02% 0.01 vs 1.05% 0.01; $p = 0.06$). Urinary N was higher when acid was added after thawing compared to before freezing (1.06 % 0.01 vs. 1.01% 0.01; $p<0.01$). There was no difference in N with either a 6 or 12 h holding time (1.03% 0.01 vs. 1.04% 1.01; $p = 0.65$).

Acidification of samples lowers the urinary pH thus preventing the Ca from precipitating out of the solution. In this study, acid was added at a rate of 20 μL of 12 M Hcl mL^{-1} urine, however other acids may have a similar effect. Wall *et al.*,[2] acidified urine samples to a pH of 2.0 with HCl while Palmgren Karlsson *et al.*,[3] used 10% sulfuric acid to lower urine pH below 3.0. Calcium seems to be sensitive to when the samples are acidified since

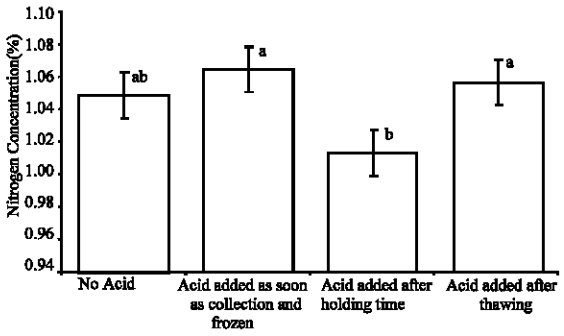

Fig. 2: Nitrogen concentrations when acid was added at different times during Experiment 1. There was a trend for a difference between no acid and acid before (p=0.06). Bars with different letters differ ($p<0.05$)

samples acidified after thawing tended to have a lower Ca concentration when compared to samples acidified before freezing. On the other hand, urinary N was not affected by the addition of acid, but it was affected by when the acid was added to the sample. When acid was added after a 6 or 12 h holding time, samples had lower urinary N than when acid was added after thawing the sample. One explanation for this could be that the pH of the sample was basic and at a solution pH of 9.2 ammonia occurs in the forms NH_{4+} and NH3(aq) equally[6]. If the N was in a gaseous form when the cap was removed to add the acid, some N would have been lost - thereby decreasing the N left in the sample. When acid was added after thawing, the sample was still cold and the N likely did not have a chance to volatilize prior to the addition of acid. Vials were tightly capped and N was not able to volatilize, which may explain why other differences were not seen. Neither holding time nor temperature had an effect on either Ca or N content of the urine. These holding times and temperatures were thought important because they mimic how the urine is handled during a collection. The urine may be placed into a cooler or a refrigerator before it is pooled or taken to the lab for freezing. In this first experiment, all samples were tightly capped. In the field, samples may sit open to air for a time prior to collection. Therefore, a portion of the study was repeated to test what happens when the samples are left uncapped.

Experiment 2: Samples held uncapped for either 6 or 12 h had higher N ($p<0.01$) than samples held for either 6 or 12 h while capped (0.736% 0.001 vs. 0.725% 0.001). There was no difference between uncapped samples that had acid added and those that had no acid added (0.736% 0.002 vs 0.734% 0.002; $p = 0.23$).

Uncapped samples had a higher N content than capped samples presumably due to evaporation of water from the urine. Samples left on the counter uncapped for 12 h had a 2.7% reduction in volume due to evaporation; however uncapped samples in the refrigerator only exhibited a 1.7% loss. When an open collection environment is used to collect urine, researchers may wish to evaluate the amount of evaporation taking place in order to assure that N values accurately reflect the urine concentration.

CONCLUSIONS

In order to accurately measure calcium, it is important to add acid to the urine prior to freezing. Nitrogen, however, seems to be much more sensitive to evaporation than to acidification. The urine handling methods discussed here should be considered when researchers are measuring the nitrogen and calcium content of equine urine. Holding time and temperature did not affect the samples, however temperatures equivalent to a hot summer=s day were not tested. It is important to correctly handle urine in order to be able to accurately report data for balance trials.

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