JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Analysis in Horse Hair as a Means of Evaluating Selenium Toxicoses and Long-Term Exposures

T. Zane Davis,*[,†] Bryan L. Stegelmeier,[†] and Jeffery O. Hall[‡]

[†]Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1150 East 1400 North, Logan, Utah 84341, United States

[‡]Utah Veterinary Diagnostic Laboratory, 950 East 1400 North, Logan, Utah 84341, United States

ABSTRACT: Horses are very susceptible to chronic selenosis if grazed on seleniferous forages for a prolonged period. In this study, mane and tail samples from horses that exhibited classical hoof lesions of chronic selenosis were analyzed by inductively coupled plasma mass spectrometry for selenium (Se) content. The horses had grazed for 6 months, from approximately May 15 until November 15, each year for three grazing seasons in a pasture containing seleniferous forages and water sources with elevated Se concentrations. The segmented hair samples showed a cyclic pattern in Se concentrations in the mane and tail, which corresponded to entering and exiting the contaminated pasture. The Se concentration in the tail of one horse could be traced for three grazing seasons. These results demonstrate that in some cases hair samples can be used to determine Se exposure in horses for up to 3 years postexposure.

KEYWORDS: *selenium, horse, chronic selenosis, selenium hair analysis, toxicity*

INTRODUCTION

Selenium (Se) is an essential micronutrient that is required for the activity of many selenoenzymes and selenoproteins. Even though it is required by biological systems, Se can be toxic at relatively low concentrations. Hair samples have been used to determine the heavy metal concentrations in the hair of some species including horses.[1] Horses can suffer from chronic selenosis if too much Se is ingested in the diet over periods of weeks or months.[2−4] Most commonly these cases are associated with grazing on seleniferous plants that grow in high Se soils in the western United States, with one of the first documented cases being reported in livestock in 1860 by a U.S. Army surgeon that described a fatal disease in horses that grazed near Fort Randall, SD, USA.[5] Clinical signs of poisoning in affected horses may include dysplastic or corrugated hoof lesions, dull hair coat, mane and tail alopecia, and varying lameness, with severe cases resulting in untreatable hoof necrosis and sloughing resulting in euthanasia.[6] Potential exposure to sources of Se, observation of classic clinical signs, and chemical Se analysis of serum, whole blood, or hair are used to make a diagnosis of chronic selenosis. Although the exact mechanism of Se toxicity is unknown, one theory is that Se is incorporated in place of sulfur in biologically important molecules or that selenoamino acids are used in place of normal amino acids in proteins and thus disrupt normal cellular function and alter structural integrity of cellular components and products.[7] The substitution of selenomethionine for methionine in hair is believed to be the cause of Se accumulation in hair when exposed to excessive Se in the diet.

The purpose of this study was to use an actual field case of equine chronic selenosis to document the Se concentration in hair of horses that had been unknowingly exposed to elevated Se in the diet for approximately 6 months of each year for 3 years.

MATERIALS AND METHODS

Animals. Three quarterhorses (one 5-year-old gelding, one 9-year-old mare, and one 11-year-old mare) grazed in a Se-contaminated pasture, from approximately May 15 until November 15 for 3 consecutive years, near a large reclaimed phosphate mine. A small stream that originated at the nearby mine flowed through the pasture and was the primary source of water for the horses. The horses were used to help manage cattle on the adjacent summer range in southeastern Idaho. A fourth horse (a 15-year-old gelding) was also in the pasture but only for approximately 45 days during October and November of the third year and did not show any signs of clinical poisoning.

Hair Samples. Hair from the mane and tail of each of the four horses was collected on May 15 and from two of the horses on October 15, 6 months after being removed from the contaminated pasture, to determine Se concentrations. Hair samples were collected by clipping the medial part of the mane approximately 45−50 cm from the withers of each horse and from the base of the tail. The ends of the hair were aligned and tied together with a rubber band. At the laboratory, the hair was by cleaned by rinsing and mechanically rubbing the hair together in five to seven changes of hot water and then rinsing using ultrapure water. The hair was patted dry with Kimwipes and allowed to completely air-dry. The hair was cut into 2.5 cm segments, weighed (most samples contained 150−300 mg), and placed into a 30 mL Oak Ridge Teflon digestion tube (Nalge Nunc International, Rochester, NY, USA) using techniques previously described.[8,9] The segments were numbered sequentially starting with the most recent growth (closest to the skin) being number 1. Terminal mane and tail samples that did not have enough mass were not analyzed. Briefly, 0.8 mL of nitric acid (Fisher Scientific, Pittsburgh, PA, USA) was added to the digestion tube, and the cap was placed on

Special Issue: Poisonous Plant Symposium, Inner Mongolia

Received: February 18, 2014
Revised: May 14, 2014
Accepted: May 15, 2014
Published: May 15, 2014

the tube but not tightened completely. The tube was placed on the heat block (90 °C) for a minimum of 2 h or until all of the hair sample was digested. Following digestion the contents of the tube were transferred to a clean 10 mL graduated cylinder, and the sample volume was increased to 1 mL using nitric acid. Then 0.5 mL of digested sample was transferred to a 15 mL polypropylene, colorless, metal-free centrifuge tube containing 9.5 mL of ultrapure water. Appropriate dilutions were made when necessary, and after vortexing, the samples were analyzed using an ELAN 6000 inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer, Shelton, CT, USA) at the Utah Veterinary Diagnostic laboratory at Utah State University. Quantification of Se was performed by the standard addition method, using a four-point standard curve. A quality control sample (in similar matrix) was analyzed after every five samples, and analysis was considered acceptable if the Se concentration of the quality control sample fell within ±5% of the standard/reference value for the quality control.

Plant Digests. Plant samples of aster (*Symphyotrichum ascendens*), sedge (*Cyperaceae* spp.), and water cress (*Nasturtium* spp.) as well as a mixture of grasses were collected from various locations in the pasture, dried, and ground with a Wiley mill to pass through a 0.5 mm screen. The ground plant material (0.50 g) was placed into a labeled 30 mL Oak Ridge Teflon digestion tube (Nalge Nunc International). Trace metal grade nitric acid (9.0 mL) (Fisher Scientific) was added to the digestion tubes. The tubes were then heated at 90 °C for 2 h with the caps loose on the tubes. After digestion, tubes were allowed to cool and contents were quantum satis to 10 mL and then transferred to another trace metal-free tube. Five hundred microliters of sample was transferred into another trace metal-free tube containing 9.5 mL of ultrapure water, to make up a 5% nitric acid matrix. After vortexing, the samples were analyzed using an ELAN 6000 ICP-MS (PerkinElmer) at the Utah Veterinary Diagnostic Laboratory at Utah State University. Quantitation and quality control were performed as described above.

RESULTS

Three of the four horses that grazed on a Se-contaminated pasture were chronically poisoned by Se. The diagnosis of chronic Se poisoning was made in May, 6 months after the horses were removed from the pasture. ICP-MS analysis of forage samples from various locations in the pasture, collected during the following year, demonstrated that the forages which contained >5 μg/g Se were found within 7 m of the stream in the pasture. None of the six mixed forages that were collected from other locations in the pasture, that were >7 m from the stream, contained >1.31 μg/g Se. Some of the forages growing in the stream and in the meadow portion of the pasture contained up to 127.4 μg/g Se (dry weight) and are reported in Table 1. Water samples were also collected from the stream at various times the following year. On May 18 the Se concentration in the stream was 0.82 μg/L and by August 30 the Se concentrations had dropped to 0.06 μg/L (Table 1).

None of the horses had alopecia of mane, tail, or body hair. The predominant lesions in the three horses that grazed the pasture for the entire previous grazing season were hoof lesions (Figure 1). Mane and tail samples were collected approximately 6 months after the horses were removed from the pasture, the ends were aligned, and the samples were segmented into 2.5 cm segments and analyzed for Se content. The lengths of the mane and tail samples collected from each horse are reported in Table 2. The Se concentrations in mane samples ranged from 0.7 to 27.6 μg/g Se and showed an increase and decrease in Se concentrations for each of the horses sampled (Figure 2A). The Se concentrations in the tail samples ranged from 0.6 to 47.6 μg/g Se, and for each of the longer tail samples the Se concentrations followed a cyclic pattern (Figure 2B).

Table 1. Selenium Concentrations of Forages and Water in the Selenium-Contaminated Pasture Where Horses Were Poisoned[a]

plants in pasture	collection date	Se (μg/g)
sedge from meadow	May 18	60.82
watercress from meadow	May 18	127.40
sedge from meadow	June 10	30.64
watercress from meadow	June 10	91.13
aster, 7 m from stream	June 10	14.29
grass, >7 m from stream	June 10	<1.31
water in pasture	collection date	Se (μg/L)
stream	May 18	0.82
stream	June 10	0.33
stream	July 29	0.09
stream	Aug 30	0.06
stream	Sept 29	0.04
stream	Oct 28	0.04

[a]All plant values are the Se concentration in μg/g on a dry weight basis of a composite sample of 5−7 plants.

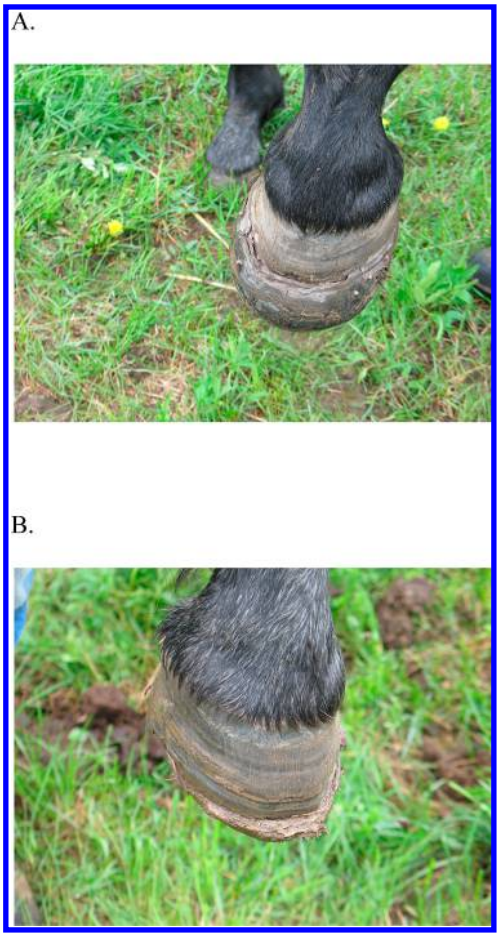

Figure 1. Photographs of (A) left front and (B) right front hooves of a horse chronically poisoned on selenium 7 months after being removed from the pasture containing high-selenium forage and water. The horse had been shod 6 weeks previously and had sloughed part of the right front hoof 1 day prior to this photograph being taken.

Horse 1 developed severe hoof lesions (Figure 1). Clinically his condition deteriorated and he became lame and was fitted with pads to help relieve the pain. However, within 4 months of his last exposure he became reluctant to bear weight on both

dx.doi.org/10.1021/jf500861p | J. Agric. Food Chem. 2014, 62, 7393−7397

Table 2. Description of Horses Chronically Poisoned on Selenium and the Length of Their Mane and Tail Samples Collected and Analyzed for Selenium Content

			first sampling (May 15)		second sampling (Oct 15)	
horse	description	time in contaminated pasture	length of mane (cm)	length of tail (cm)	length of mane (cm)	length of tail (cm)
1	5-year-old gelding	~May 15 to Nov 15 for 3 years	24.5	68.5	NA	NA
2	9-year-old mare	~May 15 to Nov 15 for 3 years	35.5	87.6	35.5	101.6
3	11-year-old mare	~May 15 to Nov 15 for 3 years	20.3	33.0	38.1	91.4
4	15-year-old gelding	Sept 1 to Nov 15 for 1 year	30.4	69.8	NA	NA

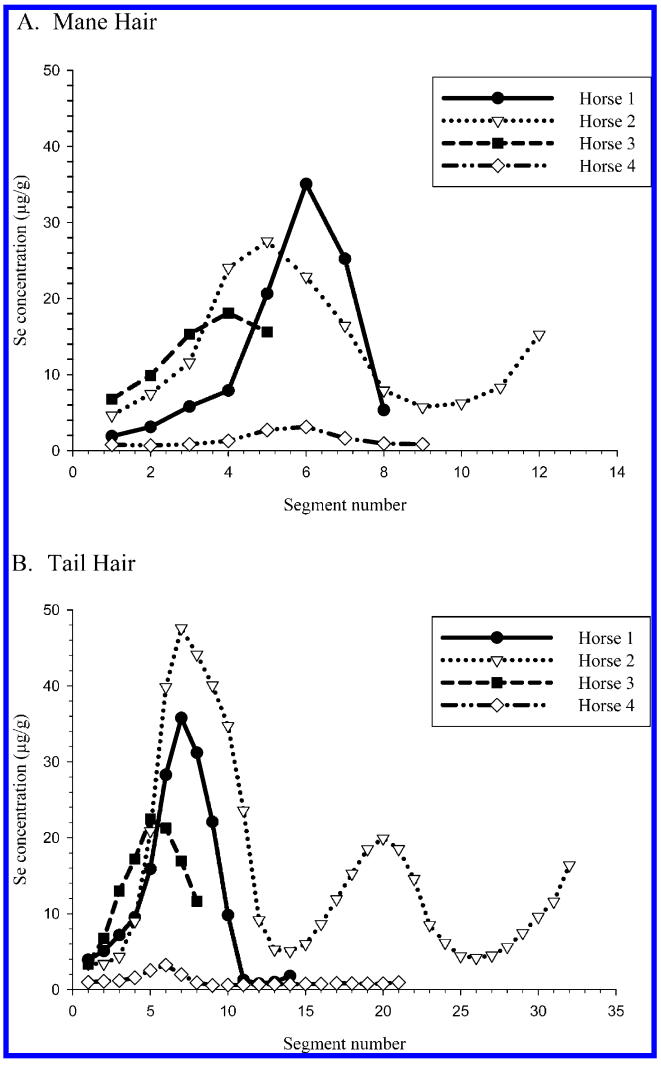

Figure 2. Selenium (Se) concentrations in (A) mane segments (2.5 cm) and (B) tail segments (2.5 cm) from four horses that grazed in a pasture with high-Se forages and water. The hair segments were numbered sequentially with the most recent growth (closest to skin) being number 1. Horses 1−3 grazed the pasture from May 15 to November 15 for three consecutive years. Horse 4 was in the pasture for 1.5 months during the third year only.

front feet. Seven months postexposure there was severe necrosis of the laminae of both front feet and the hoof wall sloughed away from underlying laminae up to the hyperplastic circular band of new growth that had developed around the hooves postexposure. The peak Se concentrations in the tail of horse 1 were from segment seven and were 35.8 μg/g Se. The mane of horse 1 was only 24.5 cm long, and the peak Se concentration in his mane was in segment six and contained 34.2 μg/g Se. Horse 1 required nearly 18 months to recover as he was rested as the hoof laminae healed and regrew. He was returned to normal work, although the prognosis of complete recovery with normal hoof strength and growth is questionable.

Horse 2 had hoof lesions that included vertical cracks in the hoof walls and prominent circular bands below the coronary band. The horse periodically exhibited minor signs of lameness but was able to be used for light ranch work. The mane of horse 2 was divided into 12 segments that could be analyzed, and the Se concentration of each segment plotted as a curve showed the complete increase and decrease of Se concentrations over one grazing season and the decrease of Se from a second grazing season (Figure 2A). The tail of horse 2 was 87.6 cm long and had increased Se concentrations during two grazing seasons that were flanked by lower Se concentrations marking the growth during months without high-Se exposure (Figure 2B). Horse 2 also had the highest peak Se concentration found in any of the hair from the horses with segment seven of the tail containing 47.6 μg/g Se.

Horse 3 had the shortest mane and tail samples but the cyclic pattern was the same as observed in horses 1 and 2. The peak Se concentrations in the mane and tail were both in segment five and were 18.1 and 22.5 μg/g Se, respectively. Horse 3 had minor vertical hoof wall cracks with some circular laminae disruption below the coronary band. She did not become lame at any time and was therefore able to be used for normal work on the ranch.

Horse 4 was in the pasture for approximately 45 days during one grazing season from the first of October until the middle of November. The horse did not develop hoof lesions, nor did it become lame at any time. The peak Se concentrations in the mane and tail of horse 4 were both in segments six and were 3.1 and 3.2 μg/g Se, respectively.

A second collection of tail and mane samples was made from horses 2 and 3 at 5 months after the first hair collections. The hair samples were segmented in the same manner as the hair from the first collection, and the Se concentrations were determined and are shown in Figures 3 and 4, respectively.

DISCUSSION

The horses were diagnosed with chronic Se poisoning on the basis of hoof lesions and mane and tail Se concentrations. On the basis of the cyclic pattern that was observed in the longer tail samples and forage and water analysis, it was determined that the forage and water during 6 months of each year were likely the toxic sources of Se. Mineral supplements were eliminated as a possible source of excessive Se because the horses were on the same mineral supplement on a year-round basis. The Se concentrations in streams contaminated by Se in the mining area are generally elevated during the spring months when runoff is high; thus, forages growing in or near the water source can also contain elevated Se concentrations.

The lesions observed in this case are typical of those reported for chronic selenosis in horses.[4,6] Horses that lose hair will eventually grow their mane and tail to normal lengths over a

dx.doi.org/10.1021/jf500861p | J. Agric. Food Chem. 2014, 62, 7393−7397

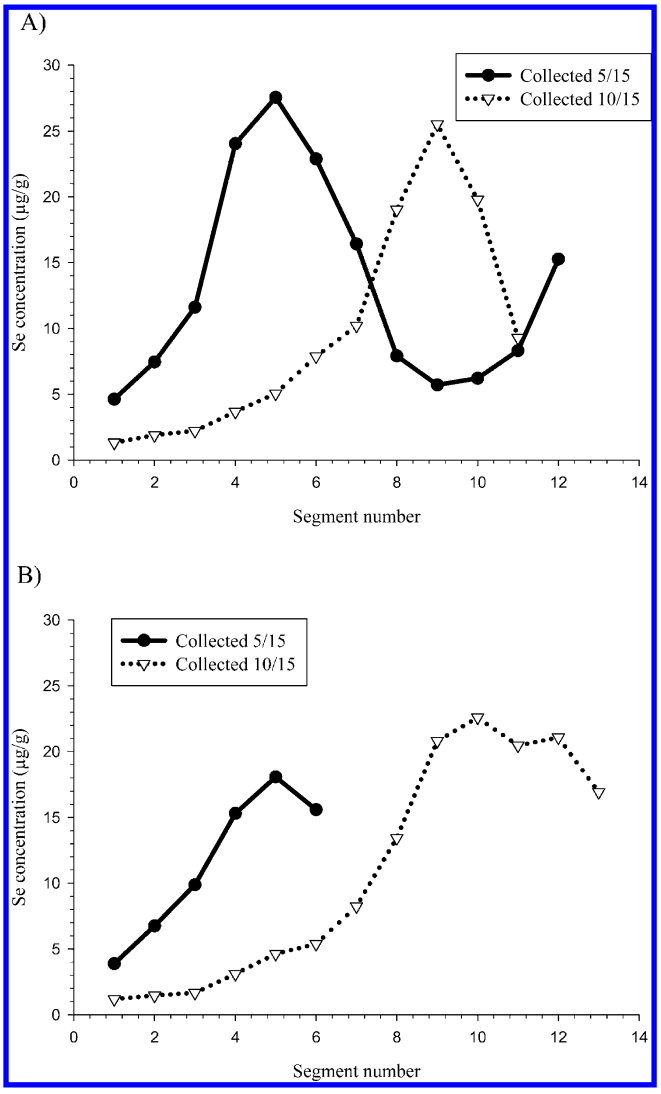

Figure 3. Selenium (Se) concentrations in mane segments (2.5 cm) from horse 2 (A) and horse 3 (B) that grazed in a pasture with high-Se forages and water. The hair segments were numbered sequentially with the most recent growth (closest to the skin) being number 1. The samples were collected 6 and 11 months postremoval from the pasture.

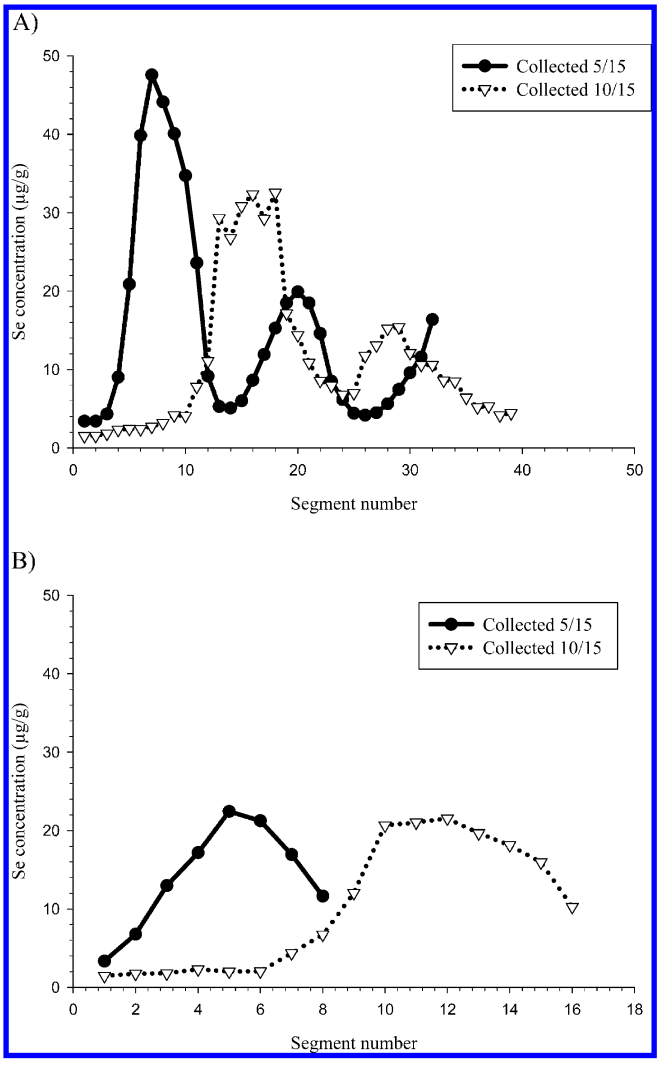

Figure 4. Selenium (Se) concentrations in tail segments (2.5 cm) from horse 2 (A) and horse 3 (B) that grazed in a pasture with high-Se forages and water. The hair segments were numbered sequentially with the most recent growth (closest to the skin) being number 1. The samples were collected 6 and 11 months postremoval from the pasture.

period of 1−2 years. However, horses that suffer severe hoof lesions and slough their hoofs often suffer from severe laminitis and may need to be euthanized to alleviate their suffering.

To avoid similar problems in the future the recommendation was made to use the pasture as a holding pasture for yearling steers for durations of 1−3 days when steers are received and shipped. The ranchers were told to avoid using the pasture for horses for any length of time because horses are more susceptible to Se toxicosis than many other livestock species.

Selenium accumulates in the hair of horses when exposed to forage with high Se content, and the concentrations of Se in the forage have been previously correlated with Se concentration in the mane and tail.[4] Hair analysis has been used to track human exposure to toxic metals.[10] Previous studies have demonstrated that there is a continual growth in the permanent hair of equine mane and tail. These studies indicate a relatively constant rate of growth of manes and tails over periods of time up to 12 months.[11,12] Dunnet and Lees[12] reported that tail and mane (medial) grew at 0.792 and 0.746 mm/day, respectively. In our study the horses were in the contaminated pasture for approximately 6 months each year. The distance between the peak Se concentrations and lowest Se concentration in each hair sample was very symmetrical, indicating that the growth rate of the hair was relatively the same over the time period during which the hair grew. Our data also support previous studies[12] that tail hair grows more rapidly than mane hair as the distance between the peak Se concentrations and the closest low Se concentration before concentration increased again was generally one segment less.

Often horses chronically poisoned on Se will have alopecia of the mane and tail. The reason for alopecia in some cases and not others is unknown. However, we speculate that it may be due to the forms and amounts of Se to which the horses are exposed. It may also be due to the Se and nutritional status of the horse at the time of the exposure.

In cases in which Se-exposed horses do not have mane and tail alopecia, the data from this study demonstrate that hair samples can be taken 1−3 years postexposure to document exposure to seleniferous forages, diets, or contaminated water.

dx.doi.org/10.1021/jf500861p | J. Agric. Food Chem. 2014, 62, 7393−7397

Additionally, the data presented in Figures 3 and 4 in which two of the horses were sampled a second time, 5 months after the first sampling, demonstrate that the concentrations of the Se in the mane and tail are relatively stable. The plotted curves of Se concentration in the mane and tail from the second sampling of horses 2 and 3 contain the same pattern with nearly the same Se concentrations as the hair contained when the final samples were collected. However, the curves are shifted to the right as the hair has grown longer and the high Se-containing segments have moved farther down the hair samples. The sampling is repeatable because the Se has been incorporated into the hair.

Cases of chronic selenosis in horses continue to occur in horses grazing seleniferous forages or when forage produced on soils with high concentrations of available Se is harvested and fed to horses. The cost of poisoning can be high because some trained horses are very valuable and are often treated as companion animals. Some animals may need to be euthanized. However, in some cases such as this one, even though the animal is not functional for an extended period of time because of lameness, the poisoned horse may be able to recover and be used for normal work.

In conclusion, sampling and segmenting of equine tail and mane can be a valuable tool to document or determine exposure to excessive Se in the diet for up to 3 years postexposure in some cases. Additionally, this study demonstrates that the concentrations of Se in mane and tail hair remain relatively stable over time.

AUTHOR INFORMATION

Corresponding Author

*(T.Z.D.) Phone: (435) 752-2941. Fax: (435) 753-5681. E-mail: zane.davis@ars.usda.gov.

Funding

This research was supported by USDA/ARS and the Utah Agriculture Experiment Station, Utah State University.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Katie Lott, Mary Hubbard, and Harold Winger for their assistance with this research.

REFERENCES

(1) Asano, K.; Suzuki, K.; Chiba, M.; Sera, K.; Asano, R.; Sakai, T. Twenty-eight element concentrations in mane hair samples of adult riding horses determined by particle-induced x-ray emission. *Biol. Trace Elem. Res.* **2005**, *107*, 135−140.

(2) Crinion, R. A.; O'Conner, J. P. Selenium intoxication in horses. *Ir. Vet. J.* **1978**, *32*, 81−86.

(3) McLauglin, J. G.; Cullen, J. Clinical cases of chronic selenosis in horses. *Ir. Vet. J.* **1986**, *40*, 136−138.

(4) Witte, S. T.; Will, L. A.; Olsen, C. R.; Kinker, J. A.; Miller-Graber, P. Chronic selenosis in horses fed locally produced alfalfa hay. *J. Am. Vet. Med. Assoc.* **1993**, *202*, 406−409.

(5) Martin, J. L. Selenium compounds in nature and medicine. In *Organic Selenium Compounds: Their Chemistry and Biology*; Klayman, D. L., Gunther, W. H. H., Eds.; Wiley: New York, 1973; pp 663−691.

(6) Kingsbury, J. M. Selenium. In *Poisonous Plants of the United States and Canada*; McElroy, W. D., Swanson, C. P., Eds.; Prentice-Hall: Englewood Cliffs, NJ, USA, 1964; pp 44−50.

(7) Raisbeck, M. F. Selenosis. *Vet. Clin. North Am. Food Anim. Pract.* **2000**, *16*, 465−480.

(8) Tiwary, A. K.; Stegelemeier, B. L.; Panter, K. E.; James, L. F.; Hall, J. O. Comparative toxicosis of sodium selenite and selenomethionine in lambs. *J. Vet. Diagn. Invest.* **2006**, *18*, 61−70.

(9) Davis, T. Z.; Stegelmeier, B. L.; Panter, K. E.; Cook, D.; Gardner, D. R.; Hall, J. O. Toxicokinetics and pathology of plant-associated acute selenium toxicosis in steers. *J. Vet. Diagn. Invest.* **2012**, *24*, 319−327.

(10) Chatt, A.; Katz, S. A. *Hair Analysis: Applications in the Biomedical and Environmental Sciences*; VCH Publications: New York, 1989; pp 14−16.

(11) Popot, M. A.; Stojiljkovic, N.; Garcia, P.; Richard, C. A.; Bonnaire, Y.; Tabet, J. C. Additional studies on the detection of drugs in horse hair. In *14th International Conference of Racing Analysts and Veterinarians*; R&W Publications: Orlando, FL, USA, 2002.

(12) Dunnett, M.; Lees, P. Trace element, toxin and drug elimination in hair with particular reference to the horse. *Res. Vet. Sci.* **2003**, *75*, 89−101.