Title: Effect of soaking on nitrate concentrations in teff hay

Authors: Tayler L. Hansen, M.S., Ashley L. Fowler, M.S., Laura A. Strasinger, B.S., Brittany E. Harlow, Ph.D., Susan H. Hayes, M.S., and Laurie M. Lawrence, Ph.D.

Affiliations: Department of Animal and Food Sciences, University of Kentucky, Lexington KY
40546-0215

Corresponding author:

Laurie Lawrence, Ph.D.

Department of Animal and Food Sciences

614 Garrigus

University of Kentucky

Lexington KY 40546-0215

Llawrenc@uky.edu

859-257-7509

Abstract

Soaking can affect respirable dust particles and the concentrations of some nutrients in alfalfa and cool season grass hays. However, the effect of soaking on nitrates in hay has not been reported. The aim of this study was to evaluate the effects of soaking on teff hay, a warm season annual grass, that contained high concentrations of nitrate ($>2.0\%$). Six bales of teff hay were used for the study. Six 0.5 kg samples were taken from each bale and assigned to 1 of 6 soaking treatments; no soaking (control), cold water immersion for 10 s, warm water immersion for 10 s, cold water soaking for 1 h, warm water soaking for 1 h, and cold water soaking for 8 h. After soaking, hay was dried, ground and submitted to a commercial laboratory for analysis. Data were analyzed by analysis of variance as a block design. When the main effect of treatment was significant ($P < 0.05$) means were separated by Tukey's honest significant difference test. Soaking for 1 or 8 h decreased WSC ($P < 0.0001$). Phosphorus, potassium, sodium, and zinc concentrations were reduced by soaking for 1 h or longer (P, K, Na: $P < 0.0001$; Zn: $P = 0.003$). Nitrate concentrations were reduced ($P < 0.0001$) to safe levels for horses ($\leq 0.5\%$) by soaking hay for 1 h or longer but soaking also reduced the concentrations of some nutrients in the hay. Minimal differences were observed between cold and warm soaking temperatures.

KEY WORDS: horse, minerals, warm season grass

1. Introduction

The practice of soaking long stem hay prior to feeding is used to reduce respirable dust and mold particles [1, 2]. Soaking also leaches nutrients from hay and thus affects its nutrient value [3, 4, 5]. A number of studies have examined the effect of soaking on alfalfa and cool season grasses, but the effect of soaking on warm season grass hays has received less attention.

Compared to cool season grasses, warm season grasses are characteristically higher in neutral detergent fiber (NDF) and hemicellulose and lower in water soluble carbohydrates (WSC) and nonstructural carbohydrates (NSC). Traditional warm season grasses used for horse hay include bermudagrass and bahia grass, however another warm season grass, teff (*Eragrostis tef* (Zucc.)), has entered the equine hay market in recent years. A feeding study found teff harvested in early maturity was acceptable to horses, and the authors suggested that the low NSC concentrations might make teff useful for horses with certain endocrine or metabolic disorders such as laminitis, equine metabolic syndrome, or polysaccharide storage myopathy [6].

When grown under conditions of low soil fertility warm season grasses tend to have low to moderate crude protein (CP) concentrations. However, with abundant nitrogen fertilization, warm season grasses can contain relatively high levels of CP and under certain circumstances can accumulate nitrate [7]. Nitrate accumulation can occur in teff that has been heavily fertilized with nitrogen and sporadically under lower fertility when growth stressed [8].

Animals may be negatively affected by high nitrate feeds when their gastrointestinal bacteria convert nitrate to nitrite [9]. Absorbed nitrite results in methemoglobinemia and impairs oxygen transport [9]. Nitrate concentrations above 1% are considered highly dangerous in cattle diets but negative effects may be observed at nitrate concentrations below 0.5% [7]. In non-ruminants, nitrates may be absorbed from the foregut, thus reducing the potential for bacterial conversion of nitrate to nitrite. Consequently, horses are generally considered more tolerant of nitrate ingestion than cattle [9, 10]. Forages containing up to 1.85% nitrate elevated blood nitrate concentrations but did not produce any clinically adverse effects in mature non-pregnant mares [11]. However, safety studies have not been performed in pregnant mares or in growing horses, and nitrate toxicity was suspected as a cause of death and abortion in mares consuming high

nitrate forages (range of 0.4 to 9.92% DM basis) [12]. In addition, it has been suggested nitrates may disrupt thyroid metabolism in horses, particularly in association with low dietary iodine levels [13].

Ideally, high nitrate forages should be avoided in equine feeding programs. However, horse owners may not realize they have acquired a forage with high nitrate concentrations until the forage is analyzed, which could be several months after purchase and delivery. Consequently, there may be a desire or need to utilize the high nitrate forage for economic reasons or when other forages are not available. Because nitrate is a water-soluble compound, we hypothesized nitrates could be reduced in forage with a short period of soaking. In this study we utilized teff hay that contained high concentrations of nitrate. The aim of the experiment was to investigate the effects of soaking on nitrate concentrations and in nutrient composition of high nitrate teff hay.

2. Material and methods

2.1 High Nitrate Hay

Teff grown in Central Kentucky was used in the experiment. Approximately 150 bales of hay had been stored in a barn for several months before it was sampled for chemical composition. Samples were obtained from randomly selected bales using a hay probe. The forage analysis revealed the hay had an unusually high crude protein (CP) concentration (29.5%; dry matter (DM) basis). In previous reports, the CP concentration of teff hay was below 20% [6, 14, 15]. Because of the high CP concentration and the previous report of nitrate accumulation in teff [8], the hay sample was submitted to a commercial forage testing laboratory (Dairy One, Ithaca,

NY) for nitrate analysis. The nitrate concentration (DM basis) was reported to be 2.25% (5075 ppm nitrate-nitrogen DM basis).

2.2 Soaking Procedure

Six bales (approximately 20 kg each) of the teff hay were randomly selected from the lot of hay described above. The bales were opened and six aliquots (approximately 0.5 kg each) were taken from each bale. The six aliquots from each bale were randomly assigned to one of six soaking treatments; not soaked (Control), soaked in cold water for 10 s (Cold Immersion), soaked in warm water for 10 s (Warm Immersion), soaked in cold water for 1 h (Cold 1 h), soaked in warm water for 1 h (Warm 1 h), and soaked in cold water for 8 h (Cold 8 h). Weighed hay samples were placed in mesh nylon bags with 4 mm holes. Each hay sample was individually soaked in 7 L of tap water in a 38 L capacity muck tub for the specified hay soaking treatment. Tap water had a mean temperature of $15 \pm 0.3^{\circ}\text{C}$ (mean $\pm$ SE) for cold water and $39 \pm 0.1^{\circ}\text{C}$ for the warm water.

After soaking, hay was air-dried on a wire rack in the nylon mesh bags. Two box fans were used to circulate air and to facilitate drying. Once the air drying was complete (approximately 48 h) the samples were then dried in a forced air oven at 60°C for 36 h.

After drying, samples were ground with a Wiley mill to pass through a 1 mm screen. A 100 g subsample was sent to a commercial laboratory (Dairy One Inc., Ithaca, NY) for analysis of nitrate, CP, NDF, acid detergent fiber (ADF), starch, WSC, ethanol soluble carbohydrates (ESC), Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo. Nitrate concentrations were determined by the RQflex reflectometer method. Crude protein was determined as $6.25 \times \text{N}\%$ according to Association of Official Agricultural Chemists method 990.03. Fiber concentrations were determined using the filter bag technique of ANKOM Technology. Starch concentrations were

determined using an YSI 2700 select biochemistry analyzer. Water soluble carbohydrates and ESC were determined according to methods described by Hall et al. [16]. Nonstructural carbohydrates were calculated as WSC plus starch concentrations for each subsample. Mineral concentrations were determined using inductively coupled plasma spectrometry after acid digestion.

2.3 Statistical Analysis

Data were analyzed as a block design using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The main effect was soaking treatment and the block was bale. Statistical differences were defined as $P \leq 0.05$. When the main effect of soaking treatment was significant, means were separated using Tukey's honest significant difference test.

3. Results and Discussion

3.1 Effect of Soaking on Nitrate Concentrations

The concentrations of nitrate and selected nutrients in the control samples obtained from each bale are shown in Table 1. Nitrate concentration (DM basis) in the control samples averaged 2.55%. Four of the six bales contained between 2.0 and 3.0% nitrate, one bale contained less than 2.0% nitrate and one bale contained more than 4.0% nitrate. The six bales of hay were obtained from different locations in the stack of approximately 150 bales, thus the variation likely represents variability in soil fertility or moisture in different parts of the field where the hay was grown [7].

We hypothesized that soaking high-nitrate hay would reduce nitrate concentrations. Previous studies have shown the duration of soaking and/or the temperature of the soak water

can influence the removal of water soluble components in hay [3, 4]. Thus we compared two soaking temperatures and three soaking durations in regard to their effect on nitrate concentrations (Figure 1). Brief immersion has been shown to reduce respirable particles in hay [1] but did not reduce ($P > 0.05$) nitrate concentrations in the teff hay in this study. However, the Cold 1 h, Warm 1 h and Cold 8 h soaking treatments reduced nitrate concentrations ($P < 0.05$) compared to control. Both 1 h soaking protocols reduced nitrate concentrations to approximately 20% of control values, with mean concentrations of $0.49 \pm 0.07\%$ in Cold 1 h and $0.38 \pm 0.05\%$ in Warm 1 h. Although the hay soaked in warm water for 1 h had numerically lower nitrate concentrations than the hay soaked in cold water for 1 h, the treatments were not significantly different ($P > 0.05$). Similarly, soaking the hay for 8 h produced a small numerical decrease in nitrate concentrations compared to the 1 h soak, but nitrate concentrations on a DM basis were not different between the 1 or 8 h treatments ($P > 0.05$). The nitrate concentrations in all samples soaked for 1 or 8 h would have been in the acceptable range for horses [9, 10] and as a component of the total ration for many ruminants [7]. In this study, the hay was not agitated during soaking and the soak water was not changed during the soaking period. It is unknown whether agitating and/or changing or replacing the soak water at some point during the soaking period would have resulted in even lower nitrate concentrations.

3.2 Effect of Soaking on Protein and Mineral Concentrations

Crude protein concentrations were affected by soaking treatment (Table 2; $P < 0.0001$). Previous studies have not found a decrease in CP concentrations in cool season grass hays after 1 h of soaking in cool or warm water [3, 4]. However, in this study the Cold 1 h, Warm 1 h and Cold 8 h treatments all resulted in decreased CP concentrations. The magnitude of the decrease

in CP concentrations in the teff hay used in this study was consistent with the change in nitrate in the hay, suggesting that the decrement in CP concentration was related to the leaching of nitrate-nitrogen from the hay, not a loss in amino acids from the hay.

Soaking did not affect the concentrations of Ca, Mg, Fe, Cu or Mn (data not shown) but did affect P ($P < 0.0001$), Na ($P < 0.0001$), Zn ($P = 0.003$), and K ($P < 0.0001$) concentrations (Table 2). Soaking hay for 1 h or more decreased K concentrations by greater than 60%, which could have implications for horses affected by hyperkalemic periodic paralysis (HYPP). Feeding diets containing more than 1.9% K increased the muscular symptoms associated with HYPP, whereas symptoms were not observed in horses fed a diet containing 1.1% K [17]. Grass hays are preferred over legume hays for horses with HYPP because they are expected to contain a lower concentration of K [18]. The initial K concentration in this teff hay was 3.02%, and the K content of teff hay was previously reported to range between 1.6 and 2.2% [6]. Consequently, it appears teff hay can contain concentrations of K that would be considered undesirable for HYPP-affected horses. However, soaking the hay for 1 h reduced the K concentration to less than 1%, which would render the hay more acceptable for HYPP-affected horses.

3.3 Effect of soaking on carbohydrate concentrations

Forages low in ESC, WSC and NSC are often recommended for horses with certain metabolic or endocrine disorders such as laminitis and equine metabolic syndrome [18, 19]. The recommended upper limit for total NSC in the diet of horses with equine metabolic syndrome or laminitis is 10% [18, 19]. Nonstructural carbohydrate concentration has been calculated as the sum of WSC and starch. Starch concentrations were not reduced by any of the soaking treatments in this study ($P = 0.062$). However, WSC concentrations were decreased ($P < 0.0001$)

in Cold 1 h, Warm 1 h and Cold 8 treatments (Table 3). Previous studies reported the NSC concentrations in teff hay to be 12% or less [6, 15]. Prior to soaking, the concentration of total NSC in the teff hay used in this study was less than 10%. Soaking hay for 8 h decreased NSC concentration to less than 5% in all samples. Soaking hay also decreased ($P < 0.0001$) ESC concentration (Table 3). The concentration of ESC in hay subjected to the 8 h Cold treatment was less than 50% of the original ESC concentration in the control samples.

Soaking for 1 or 8 h resulted in an increase ($P < 0.05$) in ADF and NDF concentrations and an 8 to 10% decrease ($P < 0.05$) in the calculated digestible energy concentration. Decreasing digestible energy concentrations could be beneficial in feeding programs designed to produce weight loss in obese or insulin resistance horses. Relative feed value (RFV) was also decreased ($P < 0.05$) by soaking for 1 or 8 h. Designed as an index to compare different forages intended for cattle [20], an RFV of 100 represents the value for full bloom alfalfa hay [21]. The relevance of RFV to forage consumed by horses has not been well tested; however, it does provide another means of estimating the effect of soaking on forage quality. Soaking resulted in a marked reduction in mean RFV from 108.8 in control samples to 82.2 in Cold 8 h samples. Forages with low concentrations of NSC, digestible energy, and low RFV may be desirable for obese horses or horses with certain metabolic disorders. However, the Cold 1 h, Warm 1 h and Cold 8 h hays in this study would be less desirable for growing horses, broodmares, and performance horses that have increased nutrient and energy requirements.

4. Conclusions

Soaking high nitrate teff hay in water for either 1 or 8 h reduced nitrate concentrations from 2.55% to levels considered safe for horses. Therefore, soaking could be employed by horse owners to reduce nitrate levels when high nitrate hay is the only forage available. However, the

brief immersion of hay in either warm or cold water did not affect any of the variables measured in this study, including nitrate. Soaking for 1 or 8 h reduced NSC and K concentrations in the hay, and thus could be a useful management practice for horses with specific endocrine, metabolic, or muscular disorders such as laminitis, insulin resistance, polysaccharide storage myopathy, or hyperkalemic periodic paralysis. However, soaking also concentrated the fiber components of the hay and reduced the calculated digestible energy concentration and the RFV. Consequently, soaking should be employed when it is necessary to reduce specific components of the hay (such as nitrates, potassium, or NSC) but may not be desirable for hays with low nitrate concentrations that are intended for normal horses or horses with high nutrient needs.

Acknowledgements

This work was supported by the Kentucky Agricultural Experiment Station and the National Institute of Food and Agriculture (Hatch), and is published with approval of the Director of the Kentucky Agricultural Experiment Station as publication 16-07-004. The authors have no known conflict of interest.

References

- [1] Clements JM, Pirie RS. Respirable dust concentrations in equine stables. Part 2: The benefits of soaking hay and optimizing the environment in a neighbouring stable. *Res Vet Sci.* 2007;83:263-8.

- [2] Couteil LL, Hinchcliff KW. Non-infectious diseases of the lower respiratory tract. In:
Hinchcliff KW, Kaneps AJ, Geor RJ, editors. Equine Sports Medicine and Surgery.
Philadelphia PA: Saunders Elsevier; 2004. p. 613-56.
- [3] Martinson KL, M. Hathaway M, Jung H, Scheaffer C. The effect of soaking on protein and mineral loss in orchardgrass and alfalfa hay. J Equine Vet Sci. 2012;32:776-82.
- [4] Longland AC, Barfoot C, Harris PA. Effect of period, water temperature and agitation on loss of water soluble carbohydrates and protein from grass hay: implications for equine feeding management. Vet Rec. 2014. doi:10.1136/vr.101820.
- [5] Mack SJ, Dugdale AH, Argo CMcG, Morgan RA, McGowan CM. Impact of water-soaking on the nutrient composition of UK hays. Vet Rec. 2014;174:452 doi: 10.1136/vr.102074.
- [6] Staniar WB, Brussard JR, Repard NM, Hall MH, Burk AO. Voluntary intake and digestibility of teff hay fed to horses. J Anim Sci. 2010;88:3296-303.
- [7] Nicholson SS. Southeastern plants toxic to ruminants. Vet Clin Food Anim. 2011;27:447-58.
- [8] Hurder KA. Nitrogen Rate and Source Effects on Biomass Yield of Teff Grown for Livestock Feed in the Mid-Atlantic Region. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg VA. 2011.
- [9] National Research Council. Nutrient Requirements of Horses. National Academies of Science, Washington DC. 3007.
- [10] Lewis, L.D. Equine Clinical Nutrition. Williams and Wilkens. Media, PA. 1995.
- [11] Burwash L, Ralston B, Olson B. Effect of high nitrate hay on mature idle horses. In:
Proceedings of the 19th Equine Science Society Symposium; Tuscon AZ; 2005. p. 174-9.
- [12] Oruc HH, Akkoc A, Uzunoglu I, Kennerman E. Nitrate poisoning in horses associated with ingestion of forage and alfalfa. J Equine Vet Sci. 2010;30:159-62.

- [13] Coenen M. Macro and trace elements in equine nutrition. In: Geor RJ, Harris PA, Coenen M, editors. Equine Applied and Clinical Nutrition. Philadelphia PA: Saunders Elsevier; 2013. p. 190-228.
- [14] McCown S, Brummer M, Hayes S, Olson G, Smith Jr. SR, Lawrence L. Acceptability of teff hay by horses. J Equine Vet Sci. 2012;32:327-31.
- [15] Rodiek AV, Jones BE. Voluntary intake of four hay types by horses. J Equine Vet Sci. 2012;32:579-83.
- [16] Hall MB, Hoover WB, Jennings JP, Miller TK. A method for partitioning neutral detergent soluble carbohydrates. J Food Sci Agric. 1999;79:2079-86.
- [17] Reynolds JA, Potter GD, Greene LW, Wu G, Carter GK, Martin MT, et al. Genetic-diet interactions in the hyperkalemic periodic paralysis syndrome in Quarter Horses fed varying amounts of potassium: Part II-Symptoms of HYPP. J Equine Vet Sci. 1998;18:655-60.
- [18] Secombe CJ, Lester GD. The role of diet in the prevention and management of several equine diseases. Anim Feed Sci Tech. 2012;173:86-101.
- [19] Geor RJ, Harris PA. Obesity. In: Geor RJ, Harris PA, Coenen M, editors. Equine Applied and Clinical Nutrition. Philadelphia PA: Saunders Elsevier; 2013. p. 487-502.
- [20] Undersander DJ, Mertens DR, Thiex NJ. Forage analysis and procedures. Omaha NE: Natl Forage Testing Assoc; 1993.
- [21] Ball DM, Hoveland CS, Lacefield GD. Southern Forages. Lawrenceville GA: Graphic Communication Corp. 2002.

Table 1: Initial composition of material sampled from six bales of teff hay¹

Item	Bale 1	Bale 2	Bale 3	Bale 4	Bale 5	Bale 6	Mean	SD
CP (%)	26.3	27.4	28.0	28.5	28.9	29.6	28.12	1.17
Nitrate (%)	2.05	2.22	2.51	2.78	1.56	4.20	2.55	0.91
Nitrate-nitrogen (ppm)	4636	5021	5672	6270	3518	9498	5769	2053
NDF (%)	61.1	55.8	60.5	54.0	57.3	55.5	57.37	2.86
ADF (%)	28.4	26.3	30.1	26.9	29.2	28.2	28.18	1.41
Starch (%)	0.2	0.2	<0.1	0.3	0.3	0.1	0.22	0.08
WSC (%)	8.6	11.5	6.3	6.4	7.7	7.0	7.92	1.95
ESC (%)	4.7	7.0	3.6	5.9	7.5	5.4	5.68	1.45
Ca (%)	0.60	0.54	0.49	0.62	0.64	0.70	0.60	0.07
P (%)	0.33	0.35	0.33	0.38	0.38	0.46	0.37	0.05
Ca:P	1.8	1.5	1.5	1.6	1.7	1.5	1.60	0.13
K (%)	2.36	3.04	2.97	2.65	2.23	4.89	3.02	0.97
Na (%)	0.042	0.051	0.022	0.028	0.028	0.011	0.03	0.01
Zn (ppm)	41	31	43	35	25	40	35.83	6.88
Cu (ppm)	12	7	11	7	7	8	8.67	2.25

¹ Dry matter basis. Analyses performed by Dairy One, Ithaca NY.

Table 2: Effect of soaking time and temperature on concentrations¹ (DM basis) of crude protein (CP) and selected minerals (n = 6; true means).

	Control	Cold Immersion	Warm Immersion	Cold 1 h	Warm 1 h	Cold 8 h	Pooled S.E.	P-Value²
CP (%)	28.1 ^a	28.3 ^a	27.0 ^a	26.3 ^b	25.2 ^{bc}	25.1 ^c	0.26	0.0001
K (%)	3.02 ^a	2.72 ^a	2.63 ^a	0.92 ^b	0.77 ^b	0.59 ^b	0.15	0.0001
P (%)	0.37 ^a	0.36 ^a	0.37 ^a	0.27 ^b	0.22 ^c	0.18 ^c	0.01	0.0001
Na(%)	0.030 ^a	0.028 ^a	0.028 ^a	0.015 ^b	0.012 ^b	0.010 ^b	0.002	0.0001
Zn (%)	35.8 ^a	35.8 ^a	37.0 ^a	34.5 ^{a,b}	33.5 ^{a,b}	31.2 ^b	2.15	0.003

¹Analyses conducted by Dairy One, Ithaca NY.

² P-value for main effect of treatment

^{a,b,c} Means with common letters are not different ($P > 0.05$)

Table 3: Effect of soaking time and temperature on concentrations¹ (DM basis) of neutral detergent fiber (NDF), digestible energy (DE), relative feed value (RFV), water soluble carbohydrates (WSC), ethanol soluble carbohydrates and starch (n = 6; true means).

	Control	Cold Immersion	Warm Immersion	Cold 1 h	Warm 1 h	Cold 8 h	Pooled S.E.	P-Value ²
NDF (%)	57.4 ^a	58.8 ^a	58.2 ^a	66.4 ^b	68.5 ^{bc}	70.4 ^c	0.58	0.0001
DE (Mcal/kg)	2.09 ^a	2.05 ^a	2.07 ^a	1.92 ^b	1.89 ^b	1.88 ^b	0.01	0.0001
RFV	108.8 ^a	104.5 ^a	105.7 ^a	88.8 ^b	85.5 ^{bc}	82.2 ^c	1.05	0.0001
WSC (%)	7.92 ^a	7.25 ^a	6.62 ^a	4.77 ^b	3.88 ^b	2.58 ^b	0.3	0.0001
ESC (%)	5.68 ^a	5.32 ^a	5.07 ^{ab}	3.95 ^{ab}	3.37 ^b	2.43 ^b	0.4	0.0001
Starch (%)	0.22	0.38	0.27	0.28	0.25	0.18	0.05	0.062
NSC (%)	8.13 ^a	7.63 ^a	6.88 ^a	5.05 ^b	4.13 ^b	2.77 ^c	0.22	0.0001

¹Analyses conducted by Dairy One, Ithaca NY.

² P-value for main effect of treatment

^{a,b,c} Means with common letters are not different ($P > 0.05$)

Figure Legend

Figure 1: Effect of soaking time and temperature on nitrate concentration (% , DM basis) in teff hay (n = 6; true mean $\pm$ SE). Means with common letters are not different ($P > 0.05$)

:][i fY%