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Original Research

Effect of Soaking on Nitrate Concentrations in Teff Hay

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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 one of six soaking treatments: no soaking (control), cold water immersion for 10 seconds, warm water immersion for 10 seconds, cold water soaking for 1 hour, warm water soaking for 1 hour, and cold water soaking for 8 hours. 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 ($P < .05$), means were separated by Tukey's honest significant difference test. Soaking for 1 or 8 hours decreased water-soluble carbohydrate ($P < .0001$). Phosphorus, potassium, sodium, and zinc concentrations were reduced by soaking for 1 hour or longer (P, K, Na: $P < .0001$; Zn: $P = .003$). Nitrate concentrations were reduced ($P < 0.05$) to safe levels for horses (≤0.5%) by soaking hay for 1 hour or longer but soaking also reduced the concentrations of some nutrients in the hay. Minimal differences were observed between cold and warm soaking temperatures.

1. Introduction

The practice of soaking long stem hay before feeding is used to reduce respirable dust and mold particles [1,2]. Soaking also leaches nutrients from hay and thus affects its nutrient value [3–5]. 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 (WSCs) and nonstructural carbohydrates (NSCs). 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 that 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].

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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 nonruminants, 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 nonpregnant 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%–0.99% dry matter [DM] basis) [12]. In addition, it has been suggested that 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 use 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 used teff hay that contained high concentrations of nitrate. The aim of the experiment was to investigate the effects of soaking on nitrate concentrations and 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 CP concentration (29.5%; 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% (5,075 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 seconds (cold immersion), soaked in warm water for 10 seconds (warm immersion), soaked in cold water for 1 hour (cold 1 hour), soaked in warm water for 1 hour (warm 1 hour), and soaked in cold water for 8 hours (cold 8 hours). 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 $15 \pm 0.3^{\circ}C$ (mean $\pm$ standard error) for cold water and $39 \pm 0.1^{\circ}C$ 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 hours), the samples were then dried in a forced air oven at 60°C for 36 hours.

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 (ESCs), 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 $6.25 \times$ 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 $P \leq .05$. 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