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Preference by horses for bedding pellets made from switchgrass (*Panicum virgatum*) straw[1]

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ABSTRACT

The bedding system used for stalled horses can affect their health. This study examined the saponin concentration in switchgrass (*Panicum virgatum*) straw, and bedding pellets made from switchgrass straw. Further, this study determined the palatability of bedding pellets made from switchgrass straw and pine wood to horses. Saponins have been implicated in photosensitization in livestock, including horses. The average concentrations of the saponins dichotomin and protodioscin in switchgrass bales were 0.37 and 0.19 μg/g, respectively. The concentration of dichotomin decreased 38% from bales to the final pellets from a combination of environmental factors and dilution with other plant material. In the initial 8-d trial, horses were tested to determine their preference for (1) switchgrass straw bedding pellets, (2) Omolene horse feed, (3) alfalfa hay cubes, and (4) Equine Pine Pellet bedding. In a second 4-d trial, barley straw was substituted for Omolene. On each test day, the fasted horses were given 30 min to consume the feeds and bedding pellets. Horses preferred both alfalfa cubes and Omolene (consumption ≥99% of offered) and rejected ($P < 0.05$) both switchgrass bedding pellets and pine bedding pellets (≤0.6% of offered). When barley straw was substituted for Omolene in the second 4-d period, horses consumed a small amount of straw (6% of offered), and again horses rejected ($P < 0.05$; ≤0.2% of offered) both switchgrass and pine bedding pellets. This study suggests that the risk of intoxication from horses ingesting bedding pellets made with switchgrass straw is very low.

Key words: switchgrass, *Panicum virgatum*, horse, bedding, preference

INTRODUCTION

Many horses spend a major portion of their lives housed in stalls (Hotchkiss et al., 2007a; Parker et al., 2008; Leme et al., 2014). The housing system and bedding of horses may affect horse health, including such aspects as consumption of bedding and respiratory disorders from inhalation of dust, biological aerosols (Hotchkiss et al., 2007b; Fleming et al., 2008a), and biogenic gases (Ward et al., 2001; Fleming et al., 2008b, 2009). Stalled horses require up to 9 kg/d of bedding (Westendorf and Krogmann, 2004). Chopped or pelleted straw is the most common bedding used for stalled horses worldwide (Kusch 2013, 2014). Wood shavings are also widely used as bedding because of low cost, high absorbency, and low palatability (Chamberlain et al., 2004; Molnar and Wright, 2006).

One potential component of pelleted biomass for horse bedding is switchgrass (*Panicum virgatum*). Switchgrass straw, harvested when the plant is dormant, is highly absorbent, can be grown on marginal land, and requires little nutritional inputs and maintenance (Cherney and Cherney, 2011; ATCC, 2015; Mitchell et al., 2016). Switchgrass straw has recently been commercially marketed as bedding for horses (Ernst Biomass LLC, Meadville, PA).

Glycosidic steroidal saponins have been found in switchgrass (Lee et al., 2001, 2009), and saponins have been implicated as one cause of hepatogenous photosensitization in livestock grazing *Panicum virgatum* pastures (Lee et al., 2001; Stegelmeier et al., 2007). Ingestion of switchgrass or other *Panicum* spp. has been linked to hepatogenous photosensitization in lambs (Puoli et al., 1992) and horses (Lee et al., 2001; Stegelmeier, 2002; Johnson et al., 2006). The major glycosidic steroidal saponins in switchgrass were determined to be dichotomin, protodioscin, and a structurally related glycosidic steroidal saponin identified as saponin B (Lee et al., 2009).

The objectives of this study were to (1) determine the concentration of saponins in switchgrass straw and bedding pellets made from switchgrass straw and (2) determine the preference by horses for switchgrass straw pellets made for bedding to determine the potential risk of intoxication in horses exposed to this bedding material.

[1] The mention of trade or manufacturer names is made for information only and does not imply an endorsement, recommendation, or exclusion by USDA-ARS.
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MATERIALS AND METHODS

Plant Material, Pelleting, Chemistry, and Feed Analysis

The switchgrass variety Cave-in-Rock (USDA-NRCS, 2011) was mowed and baled into 1.2 × 1.8 m round bales on April 27, 2014, to May 6, 2014, after the plant had been dormant for approximately 7 mo in a field near Guys Mills, Pennsylvania (41°37′48.20″N, 79°58′39.20″W). The net-wrapped round bales were stored uncovered at ambient temperatures for 16 mo at the edge of the switchgrass field until 2 d before processing. While in the field, the bales were set in rows one bale high with the bales touching end to end, with approximately 15 cm between rows.

Big bluestem (*Andropogon gerardii*) was grown and used to mix with the switchgrass to make what the biomass industry terms as "feedstock" for the pelleting process. Big bluestem was mowed on September 29, 2014, and baled on October 17, 2014, into 1.2 × 1.8 m round bales after the plant had been dormant for approximately 1 mo from a field near Meadville, Pennsylvania (41°38′29.18″N, 80°09′05.23″W). The net wrapped round bales were stored for 9 mo at the edge of the big bluestem fields until 2 wk before processing. While in the fields, the bales were set in rows one bale high with the bales touching end to end, with 15 cm between rows.

Switchgrass Pellet-Making Process. On the day of pelleting, the baled switchgrass and big bluestem were ground through a Haybuster H-1100 tub grinder (DuraTech Industries International Inc. Jamestown, ND) to approximately 1- to 10-cm lengths in a 50:50 (wt:wt) ratio, creating the feedstock for the pellet-making process. The chopped switchgrass–big bluestem feedstock was pushed into an automated walking-floor bin that metered the plant material into the pellet mill via a chain drag and conveyor belt. After discharge from the pellet mill, the pellets were cooled and bagged.

The dryer temperature (the air stream temperature where the material is introduced) ranged from 139 to 186°C and averaged 160°C during the hour of collection. The temperature at the outlet end of the dryer ranged from 45 to 54°C and averaged 50°C. The temperature of the pellets immediately after exiting the pellet mill ranged from 74 to 85°C.

The residence time of the switchgrass–big bluestem feedstock through the process was determined to be approximately 6 min by applying Hi-Light Spray Indicator Dye (BASF Co., Florham Park, NJ) to 15 kg of the chopped switchgrass–big bluestem feedstock, introducing it into the pellet-making process, then timing the appearance of dye-colored pellets at the discharge chute of the pellet mill.

Sampling Switchgrass and Bedding Pellets. Switchgrass bale core samples (49 ± 12 g) were obtained by sampling 10 switchgrass bales from the side of the bale using a Best Harvest Drill-Type Hay Probe Bale Sampler (1.3-cm bore; Best Harvest Inc., Bay City, MI). The bale core samples do not directly correspond with the feedstock and pellet samples enumerated below. However, these samples were from bales from the same field and harvest as the feedstock and pellet samples.

Ten feedstock samples (50:50 wt:wt; switchgrass:big bluestem; 0.51 ± 0.14 kg) were taken over a 1-h period at 6-min intervals by holding the sample collection bag directly in the stream of material being dumped from the conveyor belt into the pellet mill. Ten pellet samples (136 ± 20 g) were taken over a 1-h period at 6-min intervals by sampling the pellets immediately after they exited the pellet mill discharge chute. Each pellet sample was collected 6 min after a feedstock sample; thus, the 10 pellet samples approximately correspond to the feedstock samples with the same sampling numbers.

All samples were stored in open brown paper bags. The samples were allowed to sit in a humidity- and temperature-regulated warehouse for 3 d before shipping. Before shipping, each bag was stapled closed. The switchgrass, feedstock, and pellet samples were shipped to the Poisonous Plant Research Laboratory, Logan, Utah, where they were oven dried for 24 h at 40°C and ground to pass through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and then a Cyclotec 1093 sample mill (Tecator, Hoganas, Sweden).

Sample Extraction for Saponin Analysis. Extraction of switchgrass, feedstock, and pellet material for saponin analysis was done by weighing 100 mg of ground switchgrass plant material into a 13-mL, screw-top test tube equipped with Teflon lined caps (Pierce, Rockford, IL). Methanol (MeOH, 5 mL) was added to each test tube, and each test tube was placed in a mechanical shaker for 30 min and then centrifuged at 2,500 × *g* for 10 min at 20°C to separate the plant residue and MeOH extract. The MeOH extract was transferred to a clean 20-mL test tube. The switchgrass residue was extracted 2 more times with 5 mL of MeOH for 30 min and all MeOH extracts combined for a total of 15 mL. A 1.0-mL aliquot was transferred to a 7-mL vial and evaporated to dryness on a heat block at 65°C under a gentle flow of nitrogen. The dried aliquot was then reconstituted in 1.0 mL of 0.1% formic acid:acetonitrile (90:10). The sample was then passed through a 0.20-μm syringe filter (National Scientific, Rockwood, TN), and 0.5 mL was then transferred to a 1-mL autosample vial for analysis.

Chemical Analysis for Saponin. Samples were injected (25 μL) onto a Betasil C-8 reversed phase column (100 × 2.1 mm i.d.; Thermo Electron Corporation, Waltham, MA) protected by a guard column of the same phase. The saponins were eluted from the column with an isocratic flow (0.500 mL/min.) of 72:28 (0.1% formic acid:acetonitrile) mobile phase. The total HPLC run time was 3.0 min. Flow from the column was connected directly to a Thermo Finnigan (San Jose, CA) LCQ ion trap mass spectrometer via an electrospray ionization source. Full scan mass data were collected for a mass range of 300 to

1,300 amu. Tandem mass spectrometry product ion spectra were collected after isolation of a selected precursor ion (±5 amu) and the relative collision energy manually adjusted to observe significant fragmentation of the selected ion. Steroidal saponin concentrations were quantitated against a protodioscin (ChromaDex, Irvine, CA) 6-point standard curve, over the range of 0.156 to 5.0 ng/mL prepared by serial dilution in 0.1% formic acid:acetonitrile (90:10). Peak areas of the individual saponins dichotomin, protodioscin, and saponin B were determined from reconstructed ion chromatograms of the respective MH^+-H_2O ions (m/z = 1,177, 1,031, and 885).

Feed Analysis. Subsamples of all feeds were taken on a daily basis throughout the experimental periods; composited; ground to pass a 1-mm screen in a Wiley mill; and analyzed for DM, CP (N × 6.25; LECO FP-528 Nitrogen Analyzer, LECO Corp., St. Joseph, MI), and NDF (ANKOM Fiber Analyzer system, ANKOM Technology, Macedon, NY). The NDF procedure was modified by addition of heat-stable amylase (Sigma Chemical, St. Louis, MO). All analyses are reported on a DM basis.

Animals, Husbandry, and Treatments

All procedures were conducted under veterinary supervision, approved by the Utah State University IACUC (protocol # 2565), and adhered to the ASAB/ABS (2012) guidelines for the use of animals in research. Four healthy horses 4 to 10 yr old (3 geldings and 1 mare; 410 kg of BW) were adapted for 7 d to 4 × 4 m indoor pens, with wood chips as bedding. Barn temperature was kept at 8°C; an alfalfa–grass hay mixture was the basal diet and was fed at 2.5% of BW in a feed bunk within each pen. Water was provided ad libitum.

Training and Exposure to Test Items. There were 4 test items used in the initial study: (1) switchgrass straw bedding pellets (Ernst Biomass LLC); (2) Omolene 400 Complete Advantage horse feed (Purina Animal Nutrition, Gray Summit, MO); (3) alfalfa hay cubes (Chamberlain Ranch, Challis, ID), and (4) Equine Pine Pellet bedding (Tractor Supply Co., Brentwood, TN). The Equine Pine pellets were made from "southern yellow pine," which is a mix of *Pinus taeda* (loblolly), *Pinus palustris* (longleaf), *Pinus echinata* (shortleaf), and *Pinus elliottii* (slash). In the second portion of the study, Omolene 400 was replaced by locally grown barley straw (*Hordeum vulgare*; 4–10 cm particle length).

Horses were initially naïve to all the test feeds with one exception; the horses had been exposed earlier in life to barley straw bedding. After a 7-d adaptation period to the pen environment alone, the horses were exposed over 8 d to the test foods. Each day began at 0800 h. After an overnight fast, 200 g of each test food was offered in the hay bunk for 15 min/d in a balanced order, and the refusals were weighed at the end of the period.

To train the horses in the procedures, galvanized hanging horse feeders (79 cm wide, 81 cm tall, 40 cm of depth) were placed equidistant from one another in the horse pens, attached to the side of the pens. After each daily exposure to the test foods during this 8-d period, horses were trained to investigate each feeder by placing a handful of whole oats into each feeder for 10 min. The horses rapidly learned to consume all of the oats in each feeder and then move to the next feeder with minimal delay. After exposure to all test foods and after offering oats in the feeders, the horses were fed hay at 2% of BW. Any hay not eaten by 1700 h was removed for the night.

Preference Trials. The preference trials followed the methods of Pfister et al. (2013) and were conducted for 12 d. In the first trial (8 d), the horses were fasted overnight and then at 0730 h offered 500 g each of the switchgrass straw bedding pellet, the pine bedding pellet, alfalfa cubes, and Omolene 400 horse feed for 30 min. Additional feed was not added if an individual offering (e.g., Omolene) was consumed within the 30-min time limit. Refusals were weighed at the end of the time period. At 0830 h the horses were fed alfalfa hay at 2.5% of BW for 8 h, and then excess feed was removed.

To remove any position bias, each test feed was moved to a new position (i.e., a different hanging horse feeder numbered 1, 2, 3, and 4) within the pen each day, and the trial lasted for 8 d (i.e., 2 rotations around the pen). The trial was repeated with barley straw replacing the Omolene, and switchgrass straw bedding pellets, pine bedding pellets, and alfalfa cubes. The second trial lasted for 4 d (i.e., one rotation through the feeders).

Statistical Analysis

The fixed effects of position, day, treatment (i.e., different test feeds), and the day × treatment interaction on proportion consumed (% of offered) was assessed using a generalized linear mixed model with a β distribution, a logit link, and Laplace estimation. Pen (i.e., different animals) was a random effects blocking factor. Pairwise comparisons among treatment means within a given day were adjusted for family-wise Type I error ($\alpha = 0.05$) using the Tukey-Kramer method. The analysis was accomplished using the GLIMMIX procedure in SAS/STAT 12.1 (SAS Institute Inc., Cary, NC). Correlation and regression analysis with diagnostic plots was done on dichotomin concentrations in switchgrass bales, feedstock, and pellets. One dichotomin observation in the pellet data was clearly an outlier (Cook's distance >0.4) and was deleted.

RESULTS AND DISCUSSION

Saponin Concentration of the Switchgrass Bales, Feedstock, and Pellets

Dichotomin, protodioscin, and saponin B were each quantified against a 6-point standard curve using protodioscin as the calibration standard. Dichotomin was the major saponin in the switchgrass bales, feedstock, and

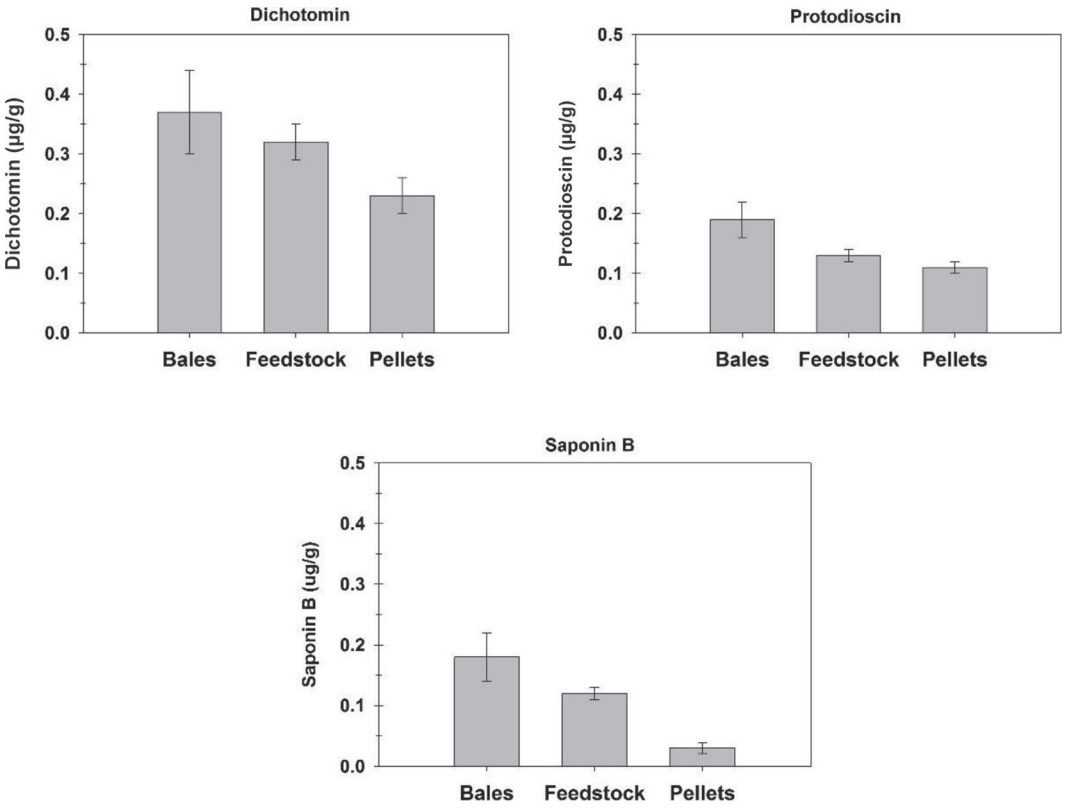

Figure 1. Steroidal saponin concentration (µg/g ± SEM, n = 10) in switchgrass (*Panicum virgatum*) bales, feedstock, and bedding pellets. Three steroidal saponins (dichotomin, protodioscin, and saponin B) were present in switchgrass plant material. Big bluestem (*Andropogon gerardii*) was mixed 50:50 (wt:wt) with the switchgrass bales to form the feedstock material. The exact identity of saponin B is known, but no name was provided (Watanabe et al., 1983). Thus, in the current study it is called saponin B.

pellets. The average concentration of dichotomin, protodioscin, and saponin B in the switchgrass bales was 0.37, 0.19, and 0.18 µg/g, respectively (Figure 1). The concentration of dichotomin decreased 38% from bales to the final pellets. There was no correlation ($r < 0.07$, $P > 0.83$, $n = 10$) between the dichotomin concentration in bales and feedstock, or bales and pellets, but there was a high correlation ($r = 0.95$, $P = 0.0001$) between the dichotomin concentration in feedstock and pellets.

Pelleting processes have been shown to alter the nutritional composition of forages (Goering et al., 1973; Shelford et al., 1980), and saponins are reported to be thermal sensitive (Shi et al., 2004; Brady et al., 2007). It was of interest in this study to assess possible quantitative changes in saponin concentration as a result of the pellet-making process (Güçlü-Üstündağ and Mazza, 2007). Studies with saponin-containing quinoa has shown reductions of >80% with processing at 180°C (Ridout et al., 1991; Gee et al., 1993). Legumes that contain saponins (e.g., fava beans) have shown variable reductions (7–53%) in saponin concentrations from cooking (Shi et al., 2004), although autoclaving removes 60 to 80% of the saponins from beans (Shi et al., 2004). Oat saponins were stable up to 100°C, whereas heating at 140°C resulted in partial degradation (Önning et al., 1994). Even though the initial drying temperature in the processing of the switchgrass straw averaged 160°C, there was no apparent reduction in saponin concentrations from the pelleting process, only from the dilution of the switchgrass straw with big bluestem grass.

Steroidal saponins have been found in several *Panicum* species, including the variety Cave-in-Rock switchgrass (Lee et al., 2009), and have been suggested as the primary agents causing hepatogenous photosensitization in grazing livestock (Patamalai et al., 1990; Holland et al., 1991; Miles et al., 1992; Munday et al., 1993). The highest concentration of steroidal saponins in Cave-in-Rock switchgrass is found in the leaves; concentrations in stem material are much lower (Lee et al., 2009). The concentration of saponins in the Cave-in-Rock switchgrass bales in the current study was much lower than the concentration of the saponins reported earlier (Lee et al., 2009). Lee et al. (2009) found that the saponin concentration for Cave-in-Rock switchgrass grown in Nebraska was 2,400, 0, and 400 µg/g for dichotomin, protodioscin, and saponin B, respectively. Even though the switchgrass variety in the current study is the same as reported by Lee et al. (2009), the Cave-in-Rock switchgrass from the Lee et al. (2009) study was grown in Nebraska, and special care was taken in hand sampling leaf and stem material, drying the samples, and in sample storage. For the current study, the switchgrass was dormant in the field in Pennsylvania for 7 mo before harvest. The field was then harvested mechani-

cally and baled, and the bales were stored outside under ambient conditions for 16 mo. Lima et al. (2015) reported a reduction in protodioscin during the hay making process in *Brachiaria brizantha* and *Brachiaria decumbens* of 46 and 48%, respectively. It seems likely that the location, harvest, and storage conditions contributed greatly to the low saponin concentrations in the switchgrass straw used to make the bedding pellets. The saponin concentration in the pellets was reduced even more through dilution as the feedstock was made by mixing switchgrass and big bluestem grass.

Nutrient Content of Feeds and Bedding Pellets

The NDF and CP content of the various feeds and bedding pellets offered to horses is given in Table 1. As expected, the rank order for nutritional quality was Omolene ≈ alfalfa cubes > straw > switchgrass pellets > pine pellets. Both Omolene and alfalfa cubes exceeded their guaranteed analysis for CP (12 and 15%, respectively).

Horse Preference for Feeds and Bedding Pellets

Horses preferred ($P < 0.05$) both alfalfa cubes and Omolene and largely rejected both the switchgrass straw bedding pellets and the pine bedding pellets during the first trial period ($P < 0.05$; Table 2). When barley straw was substituted for Omolene in the second 4-d period, alfalfa cubes were still highly preferred ($P < 0.05$) by horses, whereas a small amount of barley straw was selected by the horses ($P < 0.05$). As in the first trial period, horses rejected ($P < 0.05$) both the switchgrass straw bedding pellets and the pine bedding pellets (Table 2).

Straw is typically used as bedding for lactating mares because of its low potential toxicity when compared with wood shavings or sawdust, which may contain resins or heavy metals (Ward et al., 2000). However, the ingestion of large quantities of straw bedding may increase the risk of colic (Gonçalves et al., 2002). Another *Panicum* species, *Panicum maximum*, has been planted on 10 million ha in Brazil; this *Panicum* species appears to cause potentially fatal colic in horses and mules, but the toxin is unknown (Cerqueira et al., 2009; Schons et al., 2012).

Horses typically eat numerous small meals at frequent intervals (Ralston, 1984), for up to 18 h/d, because horses evolved to forage constantly (Goodwin, 2002). Confinement to stalls may induce behavioral stress in horses (Kiley-Worthington, 1990; Heleski et al., 2002) and lead to development of aberrant behaviors such as excess consumption of bedding (Cooper and Mason, 1998; Goodwin et al., 2002). Typically, at the termination of meals, stalled horses sniff or nibble available bedding (Ralston, 1986; Cooper et al., 2005). Some horses are categorized as "bed eaters" (Cooper et al., 2005); there may be a link between bedding type and some abnormal behaviors (McGreevy et al., 1995). Bedding-directed activities (i.e., sniffing, nosing, or eating bedding) occupied almost 30% of stalled horse activity time during some portions of the day (Coo-

Table 1. Nutritional composition (% of DM) of the test feeds[1] (i.e., composite samples) offered to horses during 2 trial periods of 8 and 4 d, respectively

Period and test feed	NDF	CP[2]
8-d trial		
Omolene 400	33.0	17.3
Alfalfa pellets	40.3	19.9
Switchgrass pellets	80.1	5.5
Pine pellets	87.2	2.4
4-d trial		
Straw	70.1	9.7
Alfalfa pellets	36.6	19.2
Switchgrass pellets	81.3	4.8
Pine pellets	88.1	1.5

[1]Test feeds were (1) switchgrass straw bedding pellets (Ernst Biomass LLC, Meadville, PA); (2) Omolene 400 Complete Advantage horse feed (Purina Animal Nutrition, Gray Summit, MO); (3) alfalfa hay cubes (Chamberlain Ranch, Challis, ID), and (4) Equine Pine Pellet bedding (Tractor Supply Co., Brentwood, TN).

[2]N × 6.25.

Table 2. Mean consumption (% of offered ± SEM) of feeds and bedding pellets offered to horses during preference trials during 2 periods

Period[1]	Feed or bedding offered[2]	Mean % consumed ± SEM
1	Alfalfa cubes	100 ± 0.0[a]
	Omolene 400	99.5 ± 0.06[a]
	Switchgrass straw pellets	0.6 ± 0.2[b]
	Pine pellets	0.1 ± 0.06[b]
2	Alfalfa cubes	100 ± 0.0[a]
	Barley straw	6.2 ± 2.7[b]
	Switchgrass straw pellets	0.2 ± 0.13[c]
	Pine pellets	0.0 ± 0.0[c]

[a–c]Means during the same period followed by different superscript letters are different ($P < 0.05$).

[1]The first period was 8 d, and the second period was 4 d.

[2]Alfalfa cubes were commercially made from alfalfa hay (Chamberlain Ranch, Challis, ID); Omolene 400 is a commercially prepared horse feed (Omolene 400 Complete Advantage horse feed, Purina Animal Nutrition, Gray Summit, MO); switchgrass bedding pellets are commercially made from switchgrass (*Panicum virgatum*) straw for livestock bedding (Ernst Biomass LLC, Meadville, PA); pine bedding pellets are commercially made from pine tree (*Pinus* spp.) sawdust for livestock bedding (Equine Pine Pellet bedding, Tractor Supply Co., Brentwood, TN). Barley straw was coarsely chopped straw locally grown near Logan, Utah.

per et al., 2005). Stalled horses spent about 50% more time in bedding-directed activity (15%) when bedded with straw than with wood shavings or straw pellets (Werhahn et al., 2010), although Cooper et al. (2005) did not find a difference in bedding-directed behavior attributable to bedding type (i.e., wood shavings, paper, or straw). Cooper et al. (2005) reported that a higher feeding frequency reduced bedding-directed behavior. Horses may consume excess amounts of straw bedding when housed in a limited-forage environment (Goodwin et al., 2002). In some circumstances, it may be advantageous for horses to be bedded on unpalatable material. In the current study, neither the switchgrass straw bedding pellets nor the pine bedding pellets were palatable to horses, even though the horses had been fasted overnight. Consumption of bedding is problematic if it results in decreased health, welfare, or nutrition, including toxicity or intestinal impaction. The risk of excess consumption of switchgrass straw bedding pellets appears to be low. Further, straw-based pellets are advantageous in that they have a high water binding capacity, and the use of straw-based pellets can also improve air quality (Fleming et al., 2008a) and reduce ammonia concentrations within the bedding material (Fleming et al., 2008b).

IMPLICATIONS

Horses consumed little or no switchgrass straw or pine bedding pellets in the preference trials. Switchgrass straw bedding pellets contained very low, but measureable, concentrations of 3 steroidal saponins that are potentially toxic. In previous work, switchgrass that contained much higher concentrations of steroidal saponins was given as the sole feed for horses for 90 d and did not produce any signs of poisoning (Stegelmeier et al., 2007). This study suggests that the risk of intoxication in horses being bedded with switchgrass straw bedding pellets is very low.

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