Pine needle effects on in vivo and in vitro digestibility of crested wheatgrass

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Abstract

In vitro and in vivo digestion trials with lambs were conducted to determine effects of ponderosa pine needles (PN; Pinus ponderosa Laws.) on digestibility of crested wheatgrass (CW; Agropyron desertorum [Link] Schultes) hay. Pine needles contained shikimic acid (15-28 mg/g) and several monomeric phenolics (p-hydroxy benzoid acid, caffeic acid, p-coumaric acid, ferulic acid) and flavonoids. Tannin concentration exceeded assay limits (>10%) and terpenes were not found, probably due to the drying procedure. In the in vitro trial, needles were mixed with CW in 10% increments from 0% to 100%. In the in vivo trial, PN were fed to lambs as follows: (1) 0%, (2) 12.5%, (3) 25%, and (4) 50%, with the remainder of the diet as CW. In vitro organic matter digestibility (IVOMD) was regressed on level of PN in the substrate. As the proportion of PN increased, IVOMD declined cubicly (P<0.01). The IVOMD values ranged from 54% for 100% CW to 24% for 100% PN. In vivo digestibility of organic matter, neutral detergent fiber and acid detergent fiber declined linearly (P<0.01) as PN were increased from 0% to 50% of the diet. Apparent crude protein digestibility and N retention by lambs declined linearly (P=0.02 and P<0.01, respectively) and urinary N increased cubicly (P<0.01) as dietary PN increased from 0% to 50%. We concluded that PN reduce in vitro and in vivo nutrient digestibility, reduced N retention by lambs, and effects were detectable even at low levels.

Key Words: Pinus ponderosa, plant secondary compounds, sheep toxic effects, poisonous plants, nitrogen retention


Pine needles contain a number of secondary phytochemicals such as phenolics, terpenes, and tannins (Smith 1964, Hanover 1966). Levels of secondary compounds and forage digestibility are negatively correlated (Jung and Fahey 1981, Akin 1982, Jung 1985); phenolics also negatively impact rumen microbial populations (Akin 1982, Chesson et al. 1982).

Despite the negative effects of pine needles on cattle reproduction and possible negative influences of pine needles on forage digestibility, there is little information available on effects of pine needles on digestion and metabolism of ruminants. The objectives of this study were to determine effects of pine needles on digestibility in vitro and in vivo and on N retention in lambs. Our hypothesis was that pine needles would reduce forage digestibility and N retention.

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Materials and Methods

Digestibility Trials

In vitro and in vivo digestion trials were conducted to determine effects of PN on digestibility of crested wheatgrass (CW: Agropyron desertorum [Link] Schultes) hay. Mature (full seedhead) CW hay was harvested from a 13-ha site 1 km west of Miles City, Mont. Crested wheatgrass was selected because it is often grazed and/or fed as a hay on sites with ponderosa pine where cattle are wintered. Green PN on branches from trees were collected in the spring and fall at a site about 20 km east of Miles City, Mont., and were air dried in a room at 20° C. After drying, PN were stripped by hand from branches. Spring PN were fed with CW to lambs (average weight = 26 kg) in a conventional digestion trial. Treatments were diets of (1) 100% CW, (2) 87.5% CW and 12.5% PN, (3) 75% CW and 25% PN, and (4) 50% CW and 50% PN. Before feeding, CW and PN were ground through a hammermill with a 2.5-mm screen. Sixteen crossbred (primarily Targhee × Suffolk × Hampshire) wether lambs were utilized in the digestion trial. The trial consisted of 2 periods with 8 lambs per period (2 lambs/treatment). Lambs were fed experimental diets for a 12-day adjustment period, followed by a 7-day total collection of feces and urine. Lambs were fed at 0700 and 1600 daily (90% of their ad libitum intake during 12-day adjustment period) during the collection period. Feces and urine were collected daily, and composited within each period for chemical analysis. In a few instances, there were some ors from PN or CW during the collection period. Ors were collected and composited across days for each lamb.

Dry matter (DM), ash, and N in feed, ors and fecal samples and N content of urine were determined by standard methods (AOAC 1984). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined in feed and fecal samples by the procedure of Goering and Van Soest (1970).

Intake of DM, ash, N, ADF, and ADF was adjusted for content in ors. Digestion coefficients for DM, organic matter, NDF, ADF, and the apparent digestion coefficient for crude protein were calculated as described by Schneider and Platt (1975). Spring- and fall-collected PN were mixed in varying proportions with CW in an in vitro digestion study. The CW for both in vitro trials was a subsample of CW used in the in vivo trial. Before in vitro digestion, PN and CW were ground to pass a 1-mm screen in a Wiley mill. In independent trials (i.e., different dates) using spring and fall PN, substrate was also varied from 100% CW% to 80% CW by increments. To more precisely define PN effects at low levels, the PN:CW combination with a modified (Cochran et al. 1986) 2-stage procedure was used (80% PN and 20% CW). Before digestion, CW and PN were dried using Na2SO4, and the internal standard vanillic acid was added (0.25 mg). This was blown dry in a 9-ml aliquot of 80/20 methanol/water (20 ml). A 9-ml aliquot was blown dry, taken up in pyridine, and 0.5 mg erythritol, the internal standard, was added. The compounds in solution were derivitized with trimethylchlorosilane and hexamethyldisilazane. A 5 μl aliquot of the supernatant was injected into a Hewlett-Packard capillary gas chromatograph equipped with a 100% methyl polysiloxane, 25-m column. Nitrogen was the carrier gas at 0.5 ml/min. Injector temperature was 250° C, detector temperature was 260° C, and initial temperature was 100° C for 1 min. Compounds were identified by co-chromatography using known standards. Data for these compounds, as well as all other compounds, were expressed as mg/g dry weight of tissue.

Tannins as Analyzed by Astringency

Because the ecologically important property of tannins has been suggested to be astringency (i.e., protein-complexing activity), tannin content was analyzed by the astringency method (Horner et al. 1987). Air dried, ground plant tissue (300 mg) was extracted in 8 ml 100% methanol. This was concentrated to 4 ml under N at 46° C. Water was added, the extract was washed with 8 ml hexane, and blown dry at 46° C. Methanol/HCl (0.5 ul) was added to the extract, the extract was heated to 100° C for 30 min, and then 1 ml water was added. This was extracted with 1.5 ml ethyl acetate, dried using Na2SO4, and the internal standard vanillic acid was added (0.25 mg). This was blown dry as above, derivitized in 100 μl dimethylformamide and d100 μl bis-trifluoroacetamide plus 1% trimethylchlorosilane, and 5 μl were injected in a Hewlett-Packard capillary gas chromatograph (GC). The GC was equipped with a Hewlett-Packard ultra 2.5% phenyl/5% methylsilicone column. 25-m long, and 1D 0.32 mm. The temperature regime is that described for carbohydrates. Compounds were identified by co-chromatography using known standards.

Results

Pine Needle Chemistry

Pine needles contained several carbohydrates; galactose was the most prevalent (Table 1). Two acids, shikimic and citric acid, were present in PN tissue (Table 1). An alcohol, inositol, was also found in needles. The spring and fall pine needles had large numerical differences in levels of several carbohydrates and shikimic acid.

We found several monomeric phenolic acids in PN tissue; flavonoids were also present in needles (Table 1). Terpenes were not detected in PN tissue, probably due to volatilization during air-drying plant material. Tannins were present in large quantities (i.e., >10%), exceeding detection limits of the astringency technique used. Fall and spring needles differed quantitatively in levels of several phenolic and flavonoid compounds; the biological signifi-
The cubic relationships for digestibility of any nutrient measured. In vivo digestibility of DM, OM, NDF, and ADF declined linearly (P < 0.01) with increasing levels of PN in the diet. However, there was a consistent trend (P = 0.02) occurred between dietary PN proportion and crude protein digestibility. Apparent protein digestibility was reduced by more than 90% as PN were increased from 0% to 50% of the diet.

Dry matter intake significantly affected urinary N excretion and N retention by lambs; therefore, urinary N and N retention were adjusted to a common level of intake. Dry matter intake was 17.0, 16.6, 16.0, and 12.0 g/kg of body weight for diets containing 0%, 12.5%, 25%, and 50% PN, respectively. The relationship between N excreted in the urine and dietary PN percentage was cubic (P = 0.02). Excretion of N in the urine declined slightly as PN percentage was increased from 0% to 25%. A large increase in urinary N excretion occurred between the 25% and 50% levels of PN.

Nitrogen retention was negative for lambs on all treatments (Table 3). The relationship between N retained and level of PN consumption was cubic (P < 0.01). Nitrogen retention declined slightly between the 0% and 12.5% levels of PN, then increased slightly as PN were increased from 12.5% to 25% of the diet. A large reduction in N retention occurred between the 25% and 50% PN levels.

### Table 2. Nutrient composition of crested wheatgrass (CW) and pine needles (PN) used in digestion trials

<table>
<thead>
<tr>
<th>Item</th>
<th>CW</th>
<th>Spring PN</th>
<th>Fall PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>91.7</td>
<td>92.7</td>
<td>92.2</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>95.0</td>
<td>96.5</td>
<td>97.6</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>5.9</td>
<td>7.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Neutral detergent fiber (%)</td>
<td>70.5</td>
<td>54.8</td>
<td>49.9</td>
</tr>
<tr>
<td>Acid detergent fiber (%)</td>
<td>40.6</td>
<td>42.3</td>
<td>36.9</td>
</tr>
</tbody>
</table>

1. Dry matter basis.
2. Pine needles used in an in vitro and in vivo trial.

### Table 3. Effects of pine needles on digestibility of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP), and on urinary N excretion (UN) and N retained (NR) by lambs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent of pine needles in the diet</th>
<th>Significance of orthogonal polynomial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>DM (%)</td>
<td>58.7</td>
<td>50.6</td>
</tr>
<tr>
<td>OM (%)</td>
<td>60.6</td>
<td>55.7</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>58.8</td>
<td>49.4</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>52.4</td>
<td>39.8</td>
</tr>
<tr>
<td>CP (%)</td>
<td>43.9</td>
<td>37.9</td>
</tr>
<tr>
<td>UN (g/day)</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>NR (g/day)</td>
<td>-0.1</td>
<td>-1.3</td>
</tr>
</tbody>
</table>

1. Digestibility coefficients are on a dry matter basis.
2. Remainer of diet was crested wheatgrass hay.
3. L = linear, Q = quadratic, C = cubic, N = 4.
4. EMS = Error mean squares.
Inhibitory effect of PN on digestibility added in smaller increments produced a similar relationship as when PN were added in larger amounts (Fig. 1).

Discussion

Pine needles contained a number of secondary phytochemicals that have been shown to depress forage digestibility. For example, Jung (1985) and Cherney et al. (1989) found that benzoic, cinnamic (e.g., p-coumaric and ferulic acid), and caffeic acids depressed digestion of cellulose. Flavonol glycosides (e.g., kaempferol) are also of interest (Strack et al. 1988) because these compounds may limit degradation of plant material in ruminants (Huang et al. 1986). The predominant acid in PN tissue, shikimic acid, is a major precursor of cinnamic acid and lignin in plant tissue (Goodwin and Mercer 1972).

There are numerous published reports that indicate tannins have negative impacts on digestibility (Kumar and Singh 1984). The high level of tannins found in our study may have inhibited microbial breakdown of plant cell wall (Cooper and Owen-Smith 1985). However, it appears unlikely that dietary tannins inhibit digestion through inhibition of digestive enzymes (Bernays et al. 1989). Under in vivo conditions, dietary tannins are generally not accessible to digestive enzymes (Butler et al. 1984, Mehansho et al. 1983). Tannins have been shown to be much more deleterious under in vitro conditions (Blytt et al. 1988).

Isolation of crude extracts and individual compounds and testing their effects in an in vitro system will be required in future work to determine which compound(s) is most inhibiting to forage digestion.

The reduction of digestibility observed for dietary components examined in this study is consistent with findings for other plant species containing various phytochemicals (Barry and Manley 1986, Jung 1985, Palo 1985, Burratt et al. 1984). Organic matter digestibility declined as PN percentage increased with both in vitro and in vivo digestion. Surprisingly, the impacts of PN on digestibility traits were observed at as little as 2% PN in vitro and had a marked effect at 12.5% of the diet with lambs.

The effects of PN were greater for in vitro than for in vivo digestion, and the difference became greater as level of PN increased. Our findings suggest that toxic effects of PN metabolism accumulate at higher levels in in vitro compared to in vivo systems, accentuating PN effects in vitro.

Although PN reduced digestibility of all nutrients in the in vivo trial, the most striking effect was on digestibility of crude protein. The negative effects of PN on digestibility are at least partially due to effects of phenolics or other secondary compounds on rumen microbial populations (Wiedmeier, unpublished data); other studies have noted that low molecular weight phenolics limit microbial degradation of structural carbohydrates (Akin 1982, Chesson et al. 1982).

We found a large inhibition of protein digestibility with PN inclusion in diets. Research at our laboratory (Pfister, unpublished data) with cattle consuming pine needles has shown that PN levels about 15% of the diet reduce ammonia-N concentrations below levels adequate for ruminal function, which would further reduce forage digestibility (Slyter et al. 1979, Ørskov 1982).

Nitrogen retention was negative for all levels of PN because of low levels of intake and concentrations of crude protein in the diet. The high negative N balance at the 50% level of dietary PN is explained by the 3.9% apparent crude protein digestibility and the 8.4 g N/day excreted in the urine. At some point between the 25% and 50% level of PN, increasing the level of dietary PN increases N passing through the feces and urine and profoundly reduces N retention. Ingestion of browse material commonly results in poor N retention in ruminant livestock. Holechek et al. (1990) fed 6 shrub diets varying in phenol content to goats, and found that all diets produced a negative N balance.

From relationships between PN consumption and digestibility coefficients and N retention observed in this study, we conclude that PN at low levels adversely affect in vitro and in vivo forage digestibility and N retention. Because of the adverse effects of PN...
on digestibility and N retention, PN may have negative impacts on the nutritional status of cattle (i.e., loss of live weight), in addition to abortifacient effects.

Literature Cited


