

EFFECTS OF CONJUGATED TANNINS ON FORAGE ENSILING AND *IN VITRO* RUMINAL FERMENTATION

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ABSTRACT: Alfalfa is widely used in rations of dairy cows in the Western United States. Protein in alfalfa is highly degradable in the rumen; in addition alfalfa protein can be degraded via the ensiling process. Condensed tannins have the ability to bind to protein forming a complex that resists ruminal degradation. Pecan shells contain 1% tannins and the internal material contains 26.4%. In 1999, NM and West TX produced about 31.7 million kg of in-shell pecan of which 55% was waste which is a potential feedstuff for ruminants. Information on the effects of condensed tannins from pecan shells on digestibility of protein and amino acids is limited. In this study, pecan shells (PS) and tannic acid (TA) were supplied at 2% PS, 3% PS, 2% TA, and 3% TA (DM) to *in vitro* fermentation of high quality alfalfa (HQ), low quality alfalfa (LQ), alfalfa silage (AS), soybean meal (SBM), and corn. Also, alfalfa was ensiled with 5% PS, 10% PS, and 3% TA. Samples were incubated for 0, 24, and 48h. Treatments had no effects ($P>0.05$) on pH at end of fermentation. Addition of PS and TA to HQ, LQ, and AS had no effect ($P>0.05$) on ADF digestibility and concentrations of acetate, butyrate, propionate, valerate, total VFA, total isoacids or acetate: propionate ratio of *in vitro* buffer concentrations. Addition of PS and TA to HQ, LQ, and AS lowered ($P<0.01$) CP of residual forage after incubation, IVDMD ($P<0.01$), NH_4 ($P<0.05$), isobutyrate ($P<0.02$), isovalerate ($P<0.02$), and lactate ($P<0.01$) concentrations. Adding PS and TA to SBM and corn lowered NH_4 concentrations ($P<0.01$), IVDMD ($P<0.01$) and CP ($P<0.05$) of the residual forage, also altered acetate, butyrate, isovalerate, and propionate concentrations ($P<0.05$). Ensiling alfalfa with PS and TA lowered ($P<0.05$) NH_4 concentrations and CP but had no effect ($P>0.05$) on IVDMD of silage and did not change concentrations of total or individual VFA or acetate: propionate. Adding PS and TA altered *in vitro* fermentations of HQ, LQ, AS, SBM, and corn. Ensiling alfalfa with PS and TA changed the fermentation characteristics of nitrogen fractions of the silage.

Keywords: tannins, pecan shell, rumen fermentation

Introduction

One way to increase animal performance is feeding a diet that meets the needs of the animal. In ruminants, protein is second only to energy in importance for achieving high performance. Unfortunately, most dietary protein is degraded in the rumen which can reduce its nutritive value by producing excess ammonia nitrogen which is not utilized by the animal and is excreted in urine. Forages provide ruminant animals with protein needed to meet its requirements. Rapid protein degradation in alfalfa

is considered one of the limiting nutritional factors. Efficient forage protein utilization occurs when rumen ammonia does not accumulate. When alfalfa is ensiled, plant proteases degrade 50% of alfalfa protein and microorganisms degrade 50% of remaining protein (Brodrick et al., 1990). A potential method to improve nitrogen efficiency is to reduce urinary nitrogen loss. High rate of alfalfa protein being converted to ammonia during silage fermentation results in a reduction of protein and an increase in NPN. This can cause excess rumen ammonia in relation to microbial growth rate (Vagnoni and Brodrick, 1997). Tannin binds protein forming a tannin-protein complex. This complex is slowly degraded in the rumen, and goes to the small intestine as rumen undegradable protein (Hagerman and Butler, 1981; Siebert et al., 1996). Pecan contains condensed tannins that are found in hulls. Pecan shells contain 1% tannins and hulls contain 26.4% tannins (Woodroof, 1967). Therefore a study was conducted to determine the effects of tannins either from pecan shells (PS) or tannic acid (TA) on forage ensiling and *in vitro* ruminal fermentation of several common dairy feedstuff.

Materials and Methods

Rumen fluid was obtained from a 500 kg Angus cow fed *ad libitum* high quality alfalfa hay diet and fitted with ruminal cannula. Rumen fluid was incubated in a water bath at 39°C for 30 min with continuous bubbling of CO_2 allowing the feed particles to rise to the top of the flask. Particle free rumen fluid was added (ratio 1:5 by volume) to a buffer that was prepared as described by Russell and Martin (1984). Forty mL of each salt was mixed with 0.60 g cestein hydrochloride, 1 mL of 1% resazorine solution and 875 mL of deionized water. Media was autoclaved, cooled under CO_2 bubbling, and 100 mL of 8% Na_2CO_3 (boiled under CO_2) was added. Fifty mL of the total solution were anaerobically added to each bottle containing 0.5 g of feed substrate. Bottles were incubated in a water bath at 39°C for 0 h, 24 h, and 48 h. All feed substrates were dried and ground to pass through 2 mm. Substrate samples were analyzed for dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber. Pecan shells were analyzed for N and condensed tannins (May and Galyean, 1996). After all fermentations pH was measured directly.

Experiment I. Alfalfa silage (AS) and high quality alfalfa (HQ) were prepared as described above and either pecan shells (PS) (0%, 1%, 2%, 3%, and 4%) or tannic acid (TA) (0%, 1%, 2%, 3%, and 4%) added. Samples were incubated for 0 h and 24 h and centrifuged (Sorvall Refrigerated Centrifuge Model RT 6000, Kendro

Laboratory Products, Newtown, CT; 10,000 x g for 15 min at 4°C) and stored for ammonia analysis.

Experiment II. Five substrates, Alfalfa silage (AS), high quality alfalfa (HQ), low quality alfalfa (LQ), corn, and soybean meal (SBM) were prepared as previously described and incubated for 0 h, 24 h, and 48 h. Pecan shells (PS) or tannic acid (TA) were added at 0%, 2%, and 3% of substrate weight. After incubation, samples were centrifuged. Supernatant was transferred to polypropylene tubes and stored at -20°C for later. Supernatant was analyzed for ammonia following a procedure modified for micro analysis as described by Brodrick and Kang, (1980), using 96 wells microplate reader (MRX HD, Dynex Laboratories, Chantilly, VA). Supernatant was analyzed for volatile fatty acids (VFA) by gas chromatography (Star 3400, Varian, Walnut Creek, CA). Supernatant was prepared for VFA analysis by mixing 500 µL of supernatant with 100 µL of metaphosphoric acid (May and Galyean, 1996). In vitro dry matter digestibility (IVDMD) was determined by filtering the residue through a Buckner funnel, with #1 Whaterman filter paper dried and the difference in weight (before incubation and after incubation) was calculated to estimate IVDMD. The residue was then analyzed for N and crude protein was estimated.

Experiment III. Alfalfa silage (AS) samples were previously ensiled by packing in laboratory scale bucket silos with no additive, 5% PS, 10% PS, and 3% TA. After removal from the silo, samples were dried and in vitro fermentations were performed with no additional TA or PS. Samples were incubated for 0 h, 24 h, and 48 h. Samples were analyzed for pH, VFA, IVDMD, and ammonia

Results and Discussion

Experiment I. Tannin doses (0-4% DM) were within a range in which condensed tannins are hypothesized to promote ruminal escape protein (Barry et al., 1986). Alfalfa silage and HQ treated with PS and TA had no effect on ammonia concentrations ($P > 0.05$). The pH averaged 6.7 ± 0.08 for HQ and AS after 24 h of fermentation. Adding PS and TA did not reduce ($P > 0.05$) ammonia concentration. However, numerically lower values were observed for 2 and 3% PS and TA. Therefore, these concentrations were used in experiment 2. Lower ammonia concentrations can indicate less protein was degraded in the rumen or increases utilization of ammonia pool by rumen microbes.

Experiment II. The addition of TA (2 and 3%) to HQ decreased ($P < 0.05$) ammonia concentrations compared to control at 24 h and 48 h, and addition of PS was similar to control. For AS samples at 24 h, 3% PS and 3% TA increased ($P < 0.05$) ammonia concentrations and 2% PS increased ammonia concentrations at 48 h (Table 1). Addition of 3% TA to SBM decreased ($P < 0.05$) ammonia concentrations at 24 h and 48 h. Ammonia concentrations in corn fermentations were similar ($P > 0.05$) among treatments at 0, 24, and 48 h of fermentation (Table 1). Lowering ammonia concentrations with PS and TA could indicate tannins binding to protein forming an insoluble complex, decreasing protein degradability. Barry et al., (1986) reported that tannin-protein complex resist ruminal degradation and dissociated in the small intestine. The

IVDMD decreased ($P < 0.05$) with addition of 3% PS and 3% TA for 24 h and 48 h fermentation of LQ compared to control. The IVDMD decreased ($P < 0.05$) with 3% TA for HQ at 0 h fermentation. For AS, IVDMD decreased ($P < 0.05$) at 3% TA for both 24 h and 48 h fermentations compared to control. The SBM IVDMD decreased ($P < 0.05$) with 2% TA, 3% TA, and 3% PS at 24 h and 48 h compared to control (Table 1). Miller and Ehlke (1994) reported that condensed tannins reduced ruminal protein degradation with little reduction in dry matter digestibility. Tannins may decrease IVDMD because tannins are firmly bound to cell wall and cell protein forming a less digestible complex and decreasing digestibility (Reed, 1995). Addition of PS and TA to fermentation of HQ, LQ, and AS had a variety of effects on CP of the undigested fraction of forage ($P < 0.01$). The 48 h LQ fermentations were lower ($P < 0.05$) in CP for the 3% PS treatment. The CP at 24 h fermentation of HQ was higher ($P < 0.05$) with 2% TA and 3% TA compared to control. In 24 h fermentations of AS, addition of 2% PS lowered ($P < 0.05$) CP while CP increased ($P < 0.05$) in 48 h fermentation of AS with 2 and 3% TA (Table 1). Tannins protect protein from enzyme hydrolysis by binding to the protein or free amino acids and rendering them inaccessible for enzyme hydrolysis (Hagerman and Butler, 1981; Barry, 1989). In the current experiment, addition of TA or PS reduced in vitro degradation of SBM. This agrees with data presented by Makkar et al., (1995).

Experiment III. At 24 h of fermentation of AS, 10% PS and 3% Ta had lower ($P < 0.05$) ammonia concentrations than the control. At 48 h, the 10% PS treatments had the lowest ($P < 0.05$) ammonia concentrations and the 5% PS had the highest ($P < 0.05$) concentration (Table 2). At 48 h of fermentation the 5% PS and 10% PS were lower ($P < 0.05$) in CP while 3% TA was not different from the control. Ensiling alfalfa with PS and TA had no effects on IVDMD and did not change ($P > 0.05$) concentrations of total or individual VFA and acetate:propionate ratio (Table 2).

Implications

Addition of PS and TA to in vitro ruminal fermentation of forage and concentrate, even at low levels used in this study, altered ruminal fermentations. Changes in protein degradation, VFA concentrations, and IVDMD were seen. Addition of PS and TA to feed may reduce protein degradation in the silo or rumen. This reduction may be accompanied by a reduction in the dry matter and fiber digestibility when PS and TA are added to the diet. Tannins could be fed at low concentrations; this will protect protein from degradation while having minor effects on microbial fermentations. Pecan shells can be used as feed additives without adversely affecting ruminal fermentation or digesta kinetics. Further research is needed to define the use of pecan shells as a tannin source in feeding ruminant animals.

Table 1. Effect of pecan shell (PS) and tannic acid (TA) on ammonia concentrations (NH₄, mM), in vitro dry matter digestibility (IVDMD %), and crude protein (CP %) of *In Vitro* mixed culture fermentations of forages and concentrates.

| Sample ² | Time ¹ | | | | | | | | | | | | SE(n=2) | | | | |
|---------------------|--------------------|--------------------|--------------------|---------------------|--------------------|---------------------|----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|--------------------|-------|--|
| | 0 h | | | | 24 h | | | | 48 h | | | | | | | | |
| | Con ³ | 2% PS | 3% PS | 2% TA | 3% TA | Con | 2% PS | 3% PS | 2% TA | 3% TA | Con | 2% PS | | 3% PS | 2% TA | 3% TA | |
| LQ | | | | | | | | | | | | | | | | | |
| NH ₄ | 4.85 | 4.80 | 4.87 | 4.66 | 4.73 | 5.34 | 5.59 | 5.77 | 5.26 | 6.01 | 5.33 | 5.48 | 5.69 | 4.73 | 5.20 | 0.54 | |
| CP% | 2.49 | 2.59 | 2.50 | 2.46 | 2.42 | 3.19 | 3.28 | 3.81 | 3.29 | 3.20 | 3.76 ^a | 3.42 ^{ab} | 2.57 ^b | 3.67 ^{ab} | 3.58 ^{ab} | 0.40 | |
| IVDMD% | 37.19 | 37.83 | 37.43 | 37.63 | 38.90 | 42.50 ^a | 40.54 ^{abc} | 36.14 ^{bc} | 41.14 ^{ab} | 35.35 ^c | 55.87 ^a | 51.55 ^{ab} | 49.84 ^b | 50.59 ^{ab} | 43.93 ^c | 1.96 | |
| HQ | | | | | | | | | | | | | | | | | |
| NH ₄ | 4.97 | 5.15 | 4.66 | 4.88 | 4.95 | 12.93 ^a | 13.30 ^a | 12.23 ^a | 10.00 ^b | 9.46 ^b | 15.27 ^a | 14.63 ^a | 14.47 ^a | 12.48 ^b | 12.62 ^b | 0.54 | |
| CP% | 6.81 ^c | 7.21 ^{bc} | 7.54 ^{bc} | 8.28 ^{ab} | 8.91 ^a | 5.47 ^b | 5.47 ^b | 5.39 ^b | 7.50 ^a | 7.71 ^a | 3.87 ^b | 4.63 ^b | 4.64 ^b | 7.50 ^a | 7.35 ^a | 0.40 | |
| IVDMD% | 60.47 ^a | 56.94 ^a | 59.98 ^a | 56.70 ^{ab} | 53.30 ^b | 60.55 ^a | 60.10 ^{ab} | 55.32 ^{ab} | 54.93 ^b | 55.33 ^{ab} | 62.08 ^a | 60.60 ^{ab} | 56.60 ^b | 56.18 ^b | 56.60 ^b | 1.96 | |
| AS | | | | | | | | | | | | | | | | | |
| NH ₄ | 4.56 | 4.89 | 4.38 | 5.08 | 4.72 | 11.10 ^{bc} | 12.45 ^{ab} | 13.17 ^a | 10.42 ^c | 13.14 ^a | 13.87 ^{bc} | 15.78 ^a | 14.94 ^{ab} | 14.58 ^{abc} | 13.31 ^c | 0.054 | |
| Crude Protein% | 6.92 | 6.76 | 6.44 | 7.00 | 6.47 | 8.46 ^a | 7.14 ^b | 7.76 ^{ab} | 8.27 ^a | 8.25 ^{ab} | 5.43 ^b | 4.85 ^b | 5.01 ^b | 7.05 ^a | 7.38 ^a | 0.40 | |
| IVDMD% | 52.54 | 50.47 | 50.97 | 51.81 | 53.95 | 54.63 ^a | 52.32 ^{ab} | 50.31 ^{ab} | 54.66 ^a | 48.78 ^b | 62.15 ^a | 60.17 ^{ab} | 58.73 ^{ab} | 58.28 ^{ab} | 56.57 ^b | 1.96 | |
| SBM | | | | | | | | | | | | | | | | | |
| NH ₄ | 4.53 | 4.38 | 4.56 | 4.40 | 4.33 | 14.94 ^a | 14.23 ^{ab} | 15.31 ^a | 13.57 ^{ab} | 12.82 ^b | 39.26 ^a | 38.11 ^a | 37.57 ^a | 37.78 ^a | 29.77 ^b | 0.67 | |
| IVDMD% | 64.15 ^a | 62.93 ^a | 62.78 ^a | 55.81 ^b | 54.77 ^b | 85.29 ^a | 82.03 ^{ab} | 79.21 ^{bc} | 76.22 ^c | 70.46 ^d | 94.63 ^a | 92.12 ^{ab} | 89.31 ^b | 87.68 ^{bc} | 83.78 ^c | 1.67 | |
| Corn | | | | | | | | | | | | | | | | | |
| NH ₄ | 3.99 | 4.55 | 4.33 | 4.19 | 4.61 | 4.10 | 4.25 | 4.08 | 4.38 | 3.03 | 5.06 | 5.19 | 4.65 | 4.31 | 3.87 | 0.67 | |
| IVDMD% | 25.17 | 26.35 | 27.39 | 24.98 | 22.93 | 85.56 ^a | 83.34 ^{ab} | 82.56 ^{ab} | 78.75 ^{bc} | 75.48 ^c | 90.22 ^a | 87.27 ^{ab} | 85.50 ^b | 85.60 ^{ab} | 83.37 ^b | 1.67 | |

¹Data were collected after 3 periods of fermentation: 0 h, 24 h, and 48 h.

²Five substrates were used: low quality alfalfa (LQ), high quality alfalfa(HQ), alfalfa silage (AS), soybean meal (SBM) and corn.

³Treatments were control, 2% PS, 3% PS, 2% TA, and 3% TA.

^{abcd}Means within a row with different subscript differ ($P < 0.05$).

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Table 2. Effects of Pecan shell (PS) and tannic acid (TA) ensiled with alfalfa on alfalfa silage (AS) ammonia concentrations (NH₄, mM), crude protein (CP), and in vitro dry matter digestibility (IVDMD).

| Sample ² | Time ¹ | | | | | | | | | | | | SE (n=2) | |
|---------------------|-------------------|----------|-----------|----------|--------------------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------------|--|
| | 0 h | | | | 24 h | | | | 48 h | | | | | |
| | Con ³ | 5% PS | 10% PS | 3% TA | Con | 5% PS | 10% PS | 3% TA | Con | 5% PS | 10% PS | 3% TA | | |
| AS | | | | | | | | | | | | | | |
| NH ₄ | 5.46 | 5.68 | 5.62 | 5.64 | 12.34 ^a | 11.94 ^{ab} | 11.29 ^b | 11.62 ^b | 13.48 ^b | 14.44 ^a | 12.71 ^c | 13.69 ^b | 0.23 | |
| CP | 6.64 | 6.21 | 5.99 | 6.07 | 7.41 | 5.83 | 5.90 | 7.68 | 8.60 ^a | 5.84 ^b | 5.90 ^b | 7.83 ^{ab} | 0.75 | |
| IVDMD | 51.39 | 49.32 | 48.02 | 49.22 | 62.68 | 59.81 | 57.18 | 58.22 | 57.80 | 61.45 | 60.54 | 60.22 | 1.56 | |

¹Data were collected after 3 periods of fermentation: 0 h, 24 h, and 48 h.

²One substrate was used: Alfalfa silage (AS).

³Treatments were control, 5% PS, 10% PS, and 3% TA.

^{abc}Means within a row with different subscript differ ($P < 0.05$).