Tannins in Perennial Legume and Forb Functional Forages

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Abstract
Feed is the greatest input cost for cattle producers. The studies summarized here employed non-bloating, tannin-containing irrigated perennial legume pastures or hay of legumes or a hydrolysable tannin-containing forb that were grown in the Mountain West USA, with non-tannin legume, grass, or feedlot treatments for comparison. Cattle grazing legume pastures or fed legume or forb hays had greater intake, gain and nitrogen retention, and in some cases, reduced enteric methane emissions compared with grass pastures or hay, and methane emissions were not different from feedlot-fed cattle.

Introduction
There is great potential value in the use of tannin-containing legumes and non-legume forbs based on studies carried out in the Mountain West. The forages we have studied include birdsfoot trefoil (*Lotus corniculatus*), sainfoin (*Onobrychis viciifolia*), and the non-legume forb small burnet (*Sanguisorba minor*). The non-bloating legume cicer milkvetch (*Astragalus cicer*) and the bloat-causing legume alfalfa (*Medicago sativa*) have been used as control non-tannin-containing forages. The pH of most soils in the Mountain West is slightly to moderately alkaline, and growing season temperatures fluctuate between daytime highs of 30°C or more to night-time lows near 15°C. The lack of growing season precipitation means that irrigation is required for optimal plant growth, and also results in relatively high photosynthetically active radiation that is augmented by elevation. The elevation at our study sites is close to 1400 m, and mid-summer total clear sky shortwave radiation is about 32 MJ/m^2/d. These conditions result in excellent forage yield and exceptional forage nutritive value characteristics (MacAdam and Griggs, 2006).

Birdsfoot Trefoil
Because perennial ryegrass is not drought-tolerant, pastures seeded to mixtures of white clover and perennial ryegrass in the Mountain West lose perennial ryegrass to drought during long sunny autumns because irrigation water is withdrawn at the end of the summer. White clover fills the resulting gaps in pastures, and can greatly elevate the incidence of pasture bloat. To address bloat concerns, we carried out grazing and clipping studies of binary grass-legume mixtures that included the non-bloating legume birdsfoot trefoil (MacAdam and Griggs 2006). Tannin-containing legumes have been extensively studied in New Zealand, and birdsfoot trefoil was found to be especially beneficial for ruminants (Waghorn et al. 1987; Waghorn 2008). While birdsfoot trefoil is not persistent in the humid Midwest due to root and crown rot diseases (Wen et al. 2002), it thrives in the dry atmosphere of the Mountain West (Beuselinck et al. 2005). Birdsfoot trefoil is also reputed to be invasive in the midwestern USA but under season-long rotational stocking there was no evidence that birdsfoot trefoil increases as a portion of binary grass-legume mixtures in the western USA (MacAdam and Griggs 2006).

In a study of 14 birdsfoot trefoil cultivars carried out in four USA locations (Michigan, Utah, West Virginia, and Wisconsin; Grabber et al. 2014), yield in Utah was double the mean of all locations, crude protein concentration was greater than the mean, and neutral detergent fiber and condensed tannin concentrations were less than the mean. The revelation from this study, which included a diversity of germplasms, was the persistence of birdsfoot trefoil cultivars, which maintained stands for eight years, and the yield, which was consistently two-thirds that of the two alfalfa check cultivars (MacAdam and Griggs 2014b). The other notable result was the elevated concentration of non-fiber carbohydrates in the birdsfoot trefoil cultivars (37 to 41%) relative to the two alfalfa cultivars in the study (29 to 33%) (MacAdam and Griggs 2014a).

A 2-year-long study was initiated to determine if the gain of beef calves on irrigated birdsfoot trefoil pastures was influenced by the presence of condensed tannins, as was shown in New Zealand with sheep (Douglas et al., 1995). With irrigated cicer milkvetch pastures serving as the control non-tannin-containing legume, we found mean gains of 1.3 to 1.5 kg/d on birdsfoot trefoil and 1.2 kg/d on cicer milkvetch (MacAdam et al. 2011). A 3-year-long study with groups of yearling calves rotationally stocking monoculture legume pastures for 12 weeks during the growing season resulted in average daily gains of 1.3 kg/d on a total mixed feedlot ration control, 0.9 kg/d on birdsfoot trefoil pastures, 0.7 kg/d on cicer milkvetch pastures, and 0.4 kg/d on grass pastures. In this study, the feedlot ration, birdsfoot trefoil, cicer milkvetch and grass pastures had non-fiber concentrations of 43, 40, 38 and 19%, respectively and the birdsfoot trefoil pastures had a condensed tannin concentration of 1.24% (Pitcher et al. 2022). Cattle from the feedlot, birdsfoot trefoil and grass pasture treatments were slaughtered at 18 months of age and their ribeye steaks compared by consumer sensory panels (Chail et al. 2016). The tenderness, juiciness and overall approval of ribeye steaks from birdsfoot trefoil- and feedlot-finished cattle did not differ and were greater than for steaks from grass-finished cattle. On the other hand, the omega-6 to omega-3 fatty acid ratio was greater for feedlot-finished cattle (5.7) than for birdsfoot trefoil- (2.4) or grass-finished cattle (3.4); lower ratios are considered more healthy than higher values. This study demonstrated that steaks of cattle finished on birdsfoot trefoil pastures were liked by consumers as well as grain-finished cattle but had fatty acid profiles comparable to grass-finished cattle. A reduction in pastoral flavor in the meat of sheep grazing birdsfoot trefoil pastures compared with sheep grazing perennial ryegrass-white clover pastures was attributed by Schreurs et al. (2008) to the condensed tannins found in birdsfoot trefoil.

In a 5-year-long series of studies of beef calves, heifers and mature cows, methane emissions as a function of intake for cattle on rotationally stocked irrigated monoculture birdsfoot trefoil and cicer milkvetch pastures were not different from methane emissions on a total mixed ration, and were consistently less than for cattle on rotationally stocked irrigated grass pastures (MacAdam et al. 2022). Dietary nitrogen intake on the legume pastures was greater than on grass pastures or the total mixed ration, but nitrogen retention was no different for cattle on legume pastures and the total mixed ration diet, and greater than for cattle on legume than on grass pastures. In this study, the condensed tannin concentration of birdsfoot trefoil pastures averaged 1.7%, which was sufficient to prevent bloat but it did not reduce methane emissions more for cattle on birdsfoot trefoil than on cicer milkvetch pastures.

In a study of Holstein dairy cattle grazing irrigated birdsfoot trefoil or grass pastures, milk production was 20 and 17% greater on birdsfoot trefoil pastures in the first and second years after pasture establishment. In cheddar cheese made from the milk of birdsfoot trefoil- and grass-fed cows, the concentration of conjugated linoleic acid was elevated compared with cheese made from the milk of total mixed ration-fed cows (MacAdam et al. 2015). The omega-3 fatty acid concentration was significantly greater in the cheese of birdsfoot trefoil-fed than that of grass-fed cows, and both were greater than the omega-3 fatty acid concentration in cheese made from the milk of total mixed ration-fed cows. In a drylot study of dairy cows fed a total mixed ration where legume hay constituted 42% of the diet, replacing alfalfa hay with birdsfoot trefoil hay resulted in 0.51% condensed tannin in the ration and led to greater microbial protein yield and increased milk nitrogen, resulting in improved nitrogen use efficiency (Christensen et al. 2015). Substituting birdsfoot trefoil for alfalfa hay also increased the non-fiber carbohydrate concentration of the ration from 31 to 34%.

Sainfoin, other Legumes, and Non-legume Forbs

While the value of condensed tannins in preventing bloat on pure legume pastures should not be under-valued, the tannins produced by individual plant species are unique, highlighted by the tremendous chemical and structural variability in this group of polyphenols, also reflected in their vast differences in bioactivity (Mueller-Harvey 2006; Pichersky et al. 2011; Salminen 2018). For example, the condensed tannin from purple prairie clover (*Dalea purpurea*) has been found to reduce the growth rate of *Escherichia coli* O157:H7 (Wang et al. 2013). The condensed tannin concentration of sainfoin (2.7%) fed to sheep shifted more nitrogen excretion from the urine to the feces compared with sheep fed non-tannin-containing alfalfa, birdsfoot trefoil with 1.3% condensed tannins, or a combination of alfalfa and birdsfoot trefoil with 0.6% condensed tannins (Lagrange and Villalba 2019). The polyphenol theaflavin has been found to complex with the alkaloid caffeine (Charlton et al. 2000), and sheep fed sainfoin with a 2.9% condensed tannin concentration consumed more endophyte-infected tall fescue (*Schedonorus arundinaceus*) than sheep fed non-tannin-containing cicer milkvetch (Villalba et al. 2016). In the same study, sainfoin-fed sheep consuming endophyte-infected tall fescue also had lower rectal temperatures per gram of feed ingested than cicer milkvetch-fed sheep. In a study comparing the responses of beef heifers on alfalfa, birdsfoot trefoil or sainfoin pastures or on all 2- and 3-way pasture combinations (Lagrange et al., 2020), average daily gains were 40% greater in the first year of the study on birdsfoot trefoil and sainfoin pastures (1.7 and 5.9% condensed tannin, respectively) than for heifers on alfalfa pastures. Blood urea and urinary nitrogen concentrations were reduced and fecal nitrogen concentrations were greater for birdsfoot trefoil- and sainfoin-fed heifers than alfalfa-fed heifers. Heifers grazing mixtures of birdsfoot trefoil and sainfoin had the least blood urea and urinary nitrogen concentrations, suggesting associative effects.

Lower concentrations of condensed tannin can be measured in the hay of legumes than in fresh forage of the same species from pastures. In a study of beef heifers and mature cows fed diets consisting of hay of a single species supplemented only with mineralized salt blocks, birdsfoot trefoil and sainfoin hays contained 0.6 and 2.5% condensed tannins, respectively, while hay of the non-legume forb small burnet (*Sanguisorba minor*) contained 4.5% hydrolysable tannins (Stewart et al. 2019). The study also included diets of alfalfa, cicer milkvetch and meadow bromegrass (*Bromus biebersteinii*) hays. Tannin-containing hay diets resulted in less urinary and blood urea nitrogen. Mature cows fed the non-tannin cicer milkvetch and tannin-containing birdsfoot trefoil retained 50% of their nitrogen intake, and heifers fed these hays as well as those fed sainfoin hay retained 40 percent of the nitrogen they were fed. Of the nitrogen fed to cows and heifers on a diet of small burnet hay, 75 to 80% was found in their feces while only 10% of fed nitrogen was retained. Mature cows on a small burnet diet emitted less methane than cows on grass and legume diets other than alfalfa, and methane emissions from heifers were less for those fed small burnet and all legume hays than for those fed grass hay. While the hydrolysable tannin concentration of small burnet reduced methane in some cattle, the reduction in nitrogen retention on a diet consisting solely of small burnet hay was excessive.

The value of condensed tannins from sericea lespedeza (*Lespedeza cuneata*) in reducing parasitic nematodes in small ruminants is well-established, and a meta-analysis also demonstrated a 38% reduction in methane emissions compared with control diets (Pech-Cervantes et al. 2021); the condensed tannin concentration of sericea lespedeza can range from 5 to 18% (Mueller-Harvey 2006). However, digestibility and dry matter intake were negatively affected when sericea lespedeza constituted more than 60% of the diet. Combining relatively small amounts of hydrolysable and condensed tannins can effectively reduce ruminant enteric methane emissions while still improving production (Aboagye et al. 2018).

Conclusions

There is a strong case to be made for improving the agronomic traits of tannin-containing legumes with positive effects on ruminant health, gain and meat quality, such as the rate of germination and establishment of birdsfoot trefoil. The tannin concentration of birdsfoot trefoil is sufficient for the prevention of bloat, there is a positive effect of birdsfoot trefoil tannins on ruminant production, and the elevated intake of rapidly digested legumes can significantly reduce methane emissions compared with grass-based diets. There is also evidence that tannins can improve the intake of endophyte-infected tall fescue and mitigate its negative effects. Selection or management strategies that reduce the woodiness of sericea lespedeza could improve its adaption for grazing and use in mixtures, potentially reducing the negative effects of endophyte-infected tall fescue. Releasing cultivars of alfalfa and white clover with foliar tannin expression, which already express tannins in seed coats and inflorescences, respectively (Hancock et al., 2012) would allow these legumes species to be grazed in pure stands. Work in the Mountain West USA has demonstrated that perennial temperate legumes have the capacity to accumulate non-fiber carbohydrates to levels found in citrus or beet pulp, resulting in levels of nitrogen retention achieved on concentrate-based diets (Villalba et al. 2021) and providing the energy often deficient in pasture-based diets. Tannins from many temperate legumes can benefit ruminants and the environment, but allowing nutritive-dense legumes to be grazed without risk of bloat is one of their most important traits.

References

Aboagye, I.A., Oba, M., Castillo, A.R., Koenig, K.M., Iwaasa, A.D., and Beauchemin, K.A. 2018. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet. *J. Anim. Sci*., 96: 5276–5286.

Beuselinck, P.R., Brummer, E.C., Viands, D.K., Asay, K.H., Smith, R.R., Steiner, J.J., and Brauer, D.K. 2005. Genotype and environment affect rhizome growth of birdsfoot trefoil. *Crop Sci.,* 45: 1736–1740.

Chail, A., Legako, J.F., Pitcher, L.R., Griggs, T.C., Ward, R.E., Martini, S., and MacAdam, J.W. 2016. Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef. *J. Anim. Sci.,* 94: 2184–2197.

Charlton, A.J., Davis, A.L., Jones, D.P., Lewis, J.R., Davies, A.P., Haslam, E., and Williamson, M.P. 2000. The self-association of the black tea polyphenol theaflavin and its complexation with caffeine. *J. Chem. Soc., Perkin Trans.,* 2: 317–322.

Christensen, R.G., Yang, S.Y., Eun, J.-S., Young, A.J., Hall, J.O., and MacAdam, J.W. 2015. Effects of feeding birdsfoot trefoil hay on neutral detergent fiber digestion, nitrogen utilization efficiency, and lactational performance by dairy cows. *J. Dairy Sci.,* 98: 7982–7992.

Douglas, G.B., Wang, Y., Waghorn, G.C., Barry, T.N., Purchas, R.W. Foote, A.G., and Wilson, G.F. 1995. Liveweight gain and wool production of sheep grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). *N. Z. J. Agric. Res*., 38: 95-104.

Grabber, J., Riday, H., Cassida, K., Griggs, T., Min, D.-H., and MacAdam, J.W. 2014. Yield, morphological characteristics, and chemical composition of European and Mediterranean-derived birdsfoot trefoil cultivars grown in the colder continental USA. *Crop Sci.,* 54: 1893-1901.

Hancock, K.R., Collette, V., Fraser, K., Greig, M., Xue, H., Richardson, K., Jones, C., and Rasmussen, S. 2012. Expression of the R2R3-MYB transcription factor TaMYB14 from *Trifolium arvense* activates proanthocyanidin biosynthesis in the legumes *Trifolium repens* and *Medicago sativa*. *Plant Physiol*., 159: 1204–1220.

Lagrange, S., and Villalba, J.J. 2019. Tannin-containing legumes and forage diversity influence foraging behavior, diet digestibility, and nitrogen excretion by lambs. *J. Anim. Sci*., 97: 3994–4009.

Lagrange, S., Beauchemin, K.A., MacAdam, J., and Villalba, J.J. 2020. Grazing diverse combinations of tanniferous and non-tanniferous legumes: Implications for beef cattle performance and environmental impact. *Sci. Total Environ.*, 746: 140788.

MacAdam, J.W., and Griggs, T.C. 2006. Performance of birdsfoot trefoil, white clover, and other legume-grass mixtures under irrigation in the Intermountain West U.S.A. In A. Popay (ed.) *Proc. N. Z. Grassl. Assoc*., 64: 355-359.

MacAdam, J.W., and Griggs, T.C. 2014a. Irrigated birdsfoot trefoil variety trial: forage nutritive value. AG/Forages/ 2013–02pr. Utah State Univ. Coop. Ext. Serv., Logan.

MacAdam, J.W., and Griggs, T.C. 2014b. Irrigated birdsfoot trefoil variety trial: forage yields; AG/Forages/2013–01pr. Utah State Univ. Coop. Ext. Serv., Logan.

MacAdam, J.W., Hunt, S.R., Griggs, T.C., Christensen, R., Eun, J.-S., Ward, R.E., and McMahon, D.J. 2015. Enhanced forage intake and milk production on birdsfoot trefoil pastures in the western US. *Proc. 2015 Org. Agric. Res. Symp*., 25–26 Feb. 2015, La Crosse, WI.

MacAdam, J.W., Pitcher, L.R., Bolletta, A.I., Guevara Ballesteros, R.D., Beauchemin, K.A., Dai, X., and Villalba, J.J. 2022. Increased nitrogen retention and reduced methane emissions of beef cattle grazing legume vs. grass irrigated pastures in the Mountain West USA. *Agronomy*, 12: 304.

MacAdam, J.W., Ward, R.E., Griggs, T.C., Min, B.-R., and Aiken, G.E. 2011. Average daily gain and blood fatty acid composition of cattle grazing the nonbloating legumes birdsfoot trefoil and cicer milkvetch in the Mountain West. *Prof. Anim. Sci*., 27: 574–583.

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric., 86: 2010-2037.

Pech-Cervantes, A.A., Terrill, T.H., Ogunade, I.M., and Estrada-Reyes, Z.M. 2021. Meta-analysis of the effects of dietary inclusion of sericea lespedeza (*Lespedeza cuneata*) forage on performance, digestibility, and rumen fermentation of small ruminants. *Livestock Sci*., 253: 104707.

Pichersky, E., and Lewinsohn, E. 2011. Convergent evolution in plant specialized metabolism. *Annu. Rev. Plant Biol.* 62: 549– 662.

Pitcher, L.R., MacAdam, J.W., Ward, R.E., Han, K.-J., Griggs, T.C., and Dai, X. 2022. Beef steer performance on irrigated monoculture legume pastures compared with grass- and concentrate-fed steers. *Animals*, 12: 1017.

Salminen, J.P. 2018. Two-dimensional tannin fingerprints by liquid chromatography tandem mass spectrometry offer a new dimension to plant tannin analyses and help to visualize the tannin diversity in plants. *J. Agric. Food Chem*. 66: 9162-9171.

Schreurs, N.M., Lane, G.A., Tavendale, M.H., Barry, T.N., and McNabb, W.C. 2008. Pastoral flavor in meat products from ruminant fed fresh forages and its amelioration by forage condensed tannins. *Anim. Feed Sci. Technol*., 146: 193–221.

Stewart, E.K., Beauchemin, K.A., Dai, X., MacAdam, J.W., Christensen, R.G., and Villalba, J.J. 2019. Effect of tannin-containing hays on enteric methane emissions and nitrogen partitioning in beef cattle. *J. Anim. Sci*., 97: 3286–3299.

Villalba, J.J., Ates, S., and MacAdam, J.W. 2021. Non-fiber carbohydrates in forages and their influence on beef production systems. *Front. Sust. Food Syst.,* 5:566338.

Villalba, J.J., Spackman, C. Goff, B.M., Klotz, J.L., Griggs, T., and MacAdam, J.W. 2016. Interaction between a tannin-containing legume and endophyte-infected tall fescue seed on lambs' feeding behavior and physiology. *J. Anim. Sci*., 94: 845–857.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production–progress and challenges. *Anim. Feed Sci. Technol*. 147: 116–139.

Waghorn, G.C., Ulyatt, M.J., John, A., and Fisher, M.T. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *Br. J. Nutr*., 57: 115–126.

Wang, Y.L., Jin, L., Ominski, K.H., He, M., Xu, Z., Krause, D.O., Acharya, S.N., Wittenberg, K.M., Liu, X.L., Stanford, K., and McAllister, T.A. 2013. Screening of condensed tannins from Canadian prairie forages for anti–*Escherichia coli* O157:H7 with an emphasis on purple prairie clover (*Dalea purpurea* Vent). *J. Food Prot*., 76: 560–567.

Wen, L., Kallenbach, R.L., Williams, J.E., Roberts, C.A., Beuselinck, P.R., McGraw, R.L., and Benedict, H.R. 2002. Performance of steers grazing rhizomatous and non-rhizomatous birdsfoot trefoil in pure stands and in tall fescue mixtures. *J. Anim. Sci*., 80: 1970–1976.