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The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis

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Abstract

Tannins may reduce rumen degradability of protein, increase the proportion of feed protein reaching the lower digestive tract for enzymatic digestion and thereby increase the efficiency of protein utilization. The objective was to assess the effects of different types and levels of tannins on rumen *in vitro* gas production and its kinetics, *in vitro* true degradability (IVTD) and rumen degradability of protein (IVRDP), and microbial protein synthesis by incubating alfalfa (*Medicago sativa* L.) hay in buffered rumen fluid. Alfalfa was incubated in buffered rumen fluid with and without the addition of different levels of gallic acid (GA), quebracho tannin (QT), or tannic acid (TA). Tannins at the lower inclusion levels had minimal effects on fermentation products compared to the higher levels. Addition of QT and TA reduced ammonium-N (NH_4^+-N) concentration. Addition of QT at 20, 40, and 60 g/kg DM decreased NH_4^+-N by 2, 7, and 12% compared with control whereas addition of TA reduced NH_4^+-N by 5, 6, and 12% when added at 20, 40 and 60 g/kg DM, respectively. In experiment 2, addition of QT at 50, 100, and 150 g/kg DM, resulted in reduction of NH_4^+-N by 12, 30, and 51%, respectively, compared with the control. Addition of TA at 50, 100, and 150 g/kd DM reduced NH_4^+-N by 14, 26,

Abbreviations: ADF, acid detergent fiber; APR, acetate to propionate ratio; EMPS, efficiency of microbial growth; GA, gallic acid; IVRDP, *in vitro* rumen degradability of protein; IVTD, *in vitro* true degradability; N, nitrogen; aNDF, neutral detergent fiber; NH_4^+-N, ammonium nitrogen; OM, organic matter; QT, quebracho tannin; SCFA, short chain fatty acids; TA, tannic acid

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and 47% compared with control. Inclusion of QT at 50, 100, 150 DM reduced IVRDP by 13, 30, and 36% compared with control whereas at these levels of inclusion, TA resulted in reduction of IVRDP by 14, 25, and 48%. Rate of gas production decreased (P<0.001), while asymptotic gas production increased (P<0.0001) with increasing level of GA and TA. Quebracho tannin decreased (P<0.0001) both the rate and asymptotic of gas production. Gallic acid had a positive effect on fermentation as indicated by increased gas production and total short chain fatty acids (SCFA) production. Quebracho tannin decreased 24 h gas production, IVTD, and total SCFA production. Acetate to propionate ratio increased with the addition of GA and but decreased when QT was added. Addition of tannins did not markedly increase total purines but numerical values tended to be higher in the presence of tannins compared with the control. Efficiency of microbial growth was lower in the presence of GA and unaltered by TA, but higher in the presence of QT compared with the control. The effect of tannins on rumen fermentation and protein degradation varied with type and level of tannins. *In vivo* studies will be conducted to validate the *in vitro* results.

Keywords: Tannins; Gallic acid; Quebracho tannin; Tannic acid; *In vitro* gas production; Alfalfa

1. Introduction

Tannins are naturally occurring plant secondary compounds that are present in many species commonly consumed by ruminants. Tannins are generally defined as water soluble polymeric phenolics that precipitate proteins (Haslam and Lilley, 1988). They are broadly classified into hydrolysable and condensed tannins. Hydrolysable tannins are gallic acid and ellagic acid esters of a core molecule that consists of polyols including sugars and phenolics (*e.g.* catechin), whereas condensed tannins consist of oligomers of flavan-3-ols and related flavanol residues, which produce anthocyanidins on acid degradation (Reed, 1995). Condensed tannins affect nutrient supply to the animal by complexing with digestive enzymes, and dietary and endogenous proteins (Barry and Manley, 1984; Barry and Manley, 1986). By contrast, hydrolysable tannins are smaller molecules and may be hydrolysed in the digestive tract (McLeod, 1974).

A unique chemical property of tannins is their affinity to bind to feed proteins and thereby reduce excessive breakdown of protein in rumen (Getachew et al., 2000a) and increase availability of high quality protein for absorption in the lower gut of ruminants (Waghorn et al., 1987). In addition to protecting feed proteins from rumen degradation, tannins also play significant roles in the prevention of bloat in ruminants (Tanner et al., 1995), suppressing intestinal parasites (Min and Hart, 2003) and increasing amino acid absorption (Waghorn et al., 1987).

Leguminous forages such as alfalfa are typically high in nitrogen (N) content ranging from 2.74 to 3.06 g N/kg DM (Tremblay et al., 2002) and are important sources of dietary N for high producing lactating dairy cows (Getachew et al., 2006). However, the rapid and excessive degradation of protein during rumen fermentation can reduce the efficiency of N utilization in ruminants (Broderick and Clayton, 1992; Van Horn et al., 1996). Major consequences of reduced N utilization are increased cost of supplemental protein to compensate for the high ruminal losses of forage protein and excessive N excretion in urine that